DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR ACRYLONITRILE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

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Peer reviewers for the comment draft of the Toxicological Profile for Acrylonitrile were:

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Comments provided by Peer Reviewer #1

ATSDR Charge Questions and Responses and Reviewer Comments

Chapter 1

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT 1: The section pertaining to the cancer effects in humans does not adequately reflect the heterogeneity and quality of the literature. The current assessment relies heavily on two published meta-analyses that assign equal weight to existing published studies, ignoring crucial differences in study design, samples size and number of observed events, exposure assessment, association measures assessed (SMR vs HR/RR), and assessment of confounding. Without properly considering these factors, and others, the conclusions about the summary of the literature regarding acrylonitrile exposure and cancer in human is inadequate. Specific details are provided below re: Chapter 2.

Minor comment regarding respiratory effects: Additional evidence from human studies on non-malignant respiratory effects could be added from Koutros et al. 2019. Suggested details have been added as track-changes in Section 2.4 on pg. 49-50 and should be updated in other sections related to respiratory effects.

Minor comment regarding overview: Suggest an acknowledgement that workers can be exposed during the production or use of acrylonitrile (occupational setting). See pg.1, Section 1.1.

RESPONSE: *The discussion of cancer effects in Section 1.2 has been revised to delete the results of the meta-analysis studies:*

> In general, these studies have not reported increased risk of cancer associated with acrylonitrile occupational exposure.

The results of the Koutros et al. (2019) study was added to Section 1.2:

> …a longer-term study of workers found an increased risk of deaths from pneumonitis (Koutros et al. 2019).

The text in Section 1.1 was revised to include occupational exposure scenarios:

> Workers involved in the production of acrylic fibers, resins, and chemical intermediates may be exposed to higher levels of acrylonitrile.

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT 2: The effects observed in animals may be relevant to humans. Studies in animals help to provide biologically plausible links and mechanistic insights between exposure and health effects. These are needed to supplement human studies (where exposure can't be carefully controlled/manipulated) and help identify the biological pathways that might be altered (even if there are some differences noted only in animals) leading to the various disease states. Data from the animal and human literature are complementary in understanding the implications on health.

RESPONSE: *No response needed.*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain.

COMMENT 3: The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain.

COMMENT 4: The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose.

COMMENT 5: The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT 6: The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 7: The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

Chapter 2

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT 8: The section pertaining to the cancer effects in humans does not adequately reflect the heterogeneity and quality of the literature. The two cited meta-analyses should be eliminated from the review. The first one is out of date; it includes several studies for which there has been extended follow-up with newer data available. The second has several methodological flaws that misrepresent the published literature. Aside from the scientific flaws, it should be noted that both reviews (Collins and

Acquavella 1998 and Alexander et al. 2021) have been funded by the acrylonitrile industry and these conflicts of interest are noted in those publications.

My suggestion would be to remove the meta-analyses and to provide additional context about the individual contributing studies (perhaps expanding Table 2-6). Re-analyses should not be considered as these don't provide any additional data beyond the original publication. For example, Marsh et al. 2001 is a 're-examination' of the Blair et al. 1998 study; this 're-examination' includes alternate SMRs (external comparisons of cancer rates) rather than comparisons of exposed versus unexposed workers (superior internal comparison where hazard ratios can be estimated). Extended mortality follow-ups should be included, and the most recent follow-up should be used for assessment of effects (while still referencing the prior reports). Some of the studies of overlapping populations are commented in Table 2-4. It's important to understand these are not independent.

It may be more informative to briefly cite the literature on other cancer site in text (as there is little published data on these) and then to focus on lung cancer, the site where most of the evidence is available.

Other cancer sites: prostate Oberg 1985, Chen 1988; bladder Thiess 1980, Delzell 1982, Kiesselbach 1979, Koutros 2019; brain/central nervous system Swaen 1998; and lymphohematopoietic Thiess 1980, Swaen 1998 cancers

Lung Cancer: Inclusion of the individual studies noted in Alexander et al. 2021 for an expanded Table 2-6 would be recommended along with additional context to assess the contribution of each study. For example:

Ref,study type	Total Pop Size	#of Lung, Bronchus, Trachea	Exposure Assessment	External Comparison (SMR)	Internal Comparison (HR/RR/OR)	Adj. for co-exposures	Adj for Smoking
Koutros et al. 2019 (update of Blair et al 1998), retrospective cohort	25,460	N=808	Expert Assessment; Quantitative estimates 8-hour TWA byjob/department/facility by time period	0.87 (0.81, 0.93)	Q5 Cumulative exposure HR=1.43 (1.13, 1.18)	Yes	Yes
Scelo et al 2014; Case-control	5,979	N=2861; 39 exposed cases	Expert Assessment; lifetime occupational histories and specialized questionnaires		Ever OR=2.2 (1.11-4.36); T3 Cumulative exposure OR=2.87 (0.85-9.66)	Yes	Yes
Mastrangelo 1993; retrospective cohort	671	N=2	Company records; qualitative high/low	2 Observed, 2.61 Expected	N/A	DMA only	No

When the above is complete, more weight should be put on studies that had sufficient numbers of lung cancer cases/death, had quantitative estimates of exposure, those using internal comparison to assess risk, and those adjusting for co-exposure to other chemicals and for smoking status. Studies relying on only SMRs are limited in their inference as these only compare cancer rates with external populations, which is a noted limitation in studies of workers who are healthier than the general population (healthy worker survivor bias). In addition, studies with small numbers of observed cases are limited in their power to detect effects and may provide an uninformative null finding.

Ultimately, when the above review of individual study data is complete, the totality of the evidence for cancer (in humans) is still limited but there is suggestive evidence for an association with lung cancer based on the totality of the carcinogenicity literature, including the results from the largest existing cohort study of acrylonitrile workers where risks were observed with increasing exposure (compared to unexposed workers) after multivariate adjustment for smoking and co-exposures to other chemicals (Koutros et al. 2019).

Is there a reason that many of the elements in Appendix C (risk of bias, confidence, hazard identification conclusions) do not consider cancer? Was this not deemed as a sensitive outcome? NTP, EPA and IARC assessments (as noted in the profile) all indicate that acrylonitrile is likely to be a human carcinogen. These assessments (along with the published literature) suggest enough evidence exists to consider cancer as a health effect outcome of concern. Although most of the comments above focus on human studies, which are more limited, there is a preponderance of evidence from animal studies indicating the carcinogenicity of acrylonitrile.

RESPONSE: *The discussion of the epidemiological studies in Section 2.19 has been revised. The table summarizing key epidemiological studies (formerly Table 2-6, now Table 2-4) was expanded to include the larger studies, including the Scélo et al. (2014) and Mastrangelo et al. (1993) studies cited in the Reviewer's table. For studies that have follow-ups, only the most recent study was included; the original studies are noted. ATSDR disagrees with the Reviewer that the results of the meta-analyses should not be included in the toxicological profile because they were funded by industry. The conclusions of the meta-analyses investigators have been retained; however, the table (Table 2-5) listing the results of the Alexander et al. (2021) study was deleted from the profile.*

It is noted that ATSDR does not conduct a weight-of-evidence evaluation of the carcinogenicity data. Rather, the discussion is limited to reporting study results.

The systematic review in Appendix C is limited to noncancer endpoints since the purpose is to support MRL derivation (MRLs are not based on noncancer endpoints).

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT 9: See comments above in #1

RESPONSE: *See Response to Comment 8.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

Chapter 3

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Are the biomarkers of exposure specific for the substance? Please explain.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Are the biomarkers of effect specific for the substance? Please explain.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

Chapter 4

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Is information provided on the various forms of the substance? Please explain.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

Chapter 5

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

Chapter 6

QUESTION: Do you know of other studies that may fill a data gap? Please provide any relevant references.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Do you agree with the identified data needs? Please explain.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Are the data needs presented in a neutral, non-judgmental fashion? Please note any bias in the text.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

Chapter 7

QUESTION: Are you aware of any additional regulations or guidelines that should be included? Please provide citations.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Are there any that should be removed? Please explain.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

Annotated Comments on the Toxicological Profile

COMMENT 10: Regarding the text in the second bullet in Section 1.1, the Reviewer suggested the following addition: Exposure to acrylonitrile has also been characterized in the occupation setting among workers involved in the production of acrylic fibers, resins, and chemical intermediates.

RESPONSE: *The following bullet was added to Section 1.1:*

- Workers involved in the production of acrylic fibers, resins, and chemical intermediates may be exposed to higher levels of acrylonitrile.

COMMENT 11: Regarding the discussion of cancer effects in Section 1.2, the Reviewer commented: See comments in Chapter 2.

RESPONSE: *The discussion of the cancer epidemiological data in Section 1.2 has been revised:*

In general, these studies have not reported increased risk of cancer associated with acrylonitrile occupational exposure.

COMMENT 12: Regarding Section 2.4, the Reviewer suggested adding the following paragraph: In a cohort of 25,460 workers employed at eight U.S. facilities producing or using acrylonitrile (Koutros et al, 2019), increasing exposure to acrylonitrile (derived from work histories, plant records and monitoring data) was significantly associated with death from a subset of non-malignant respiratory diseases characterized by a heterogeneous group of interstitial lung diseases (ICD codes 'Pneumonitis due to solids and liquids'). The observed increased risks were unchanged with additional adjustment for other co-exposures (including to acetic anhydride and phthalic anhydride) which have previously been linked to this outcome. A small number of total were observed (of n=27 deaths) but a positive, monotonic exposure-response was observed for all exposure metrics including cumulative (ppm-years, p-trend=0.007), average (ppm, p-trend=0.07) and duration (years, p-trend =0.01) of exposure to acrylonitrile.

RESPONSE: *The results of the Koutros et al. (2019) study were added to Section 2.4:*

A mortality study conducted by Koutros et al. (2019) found an increased risk of deaths from pneumonitis in workers with exposures higher than the median level (>3.12 ppm-years cumulative exposure and duration of exposure of >14.5 years).

COMMENT 13: Regarding the following statement in Section 2.19—Most of these studies share several limitations including either the lack of monitoring information or limited monitoring data from which exposure was estimated, lack of control for simultaneous exposure to other chemicals, and no or limited information on smoking—the Reviewer commented: This is true, yet use of the meta-analysis for the ultimate conclusion puts each study on equal footing. The study design, exposure assessment, association measures assessed (SMR vs HR), and assessment of confounding will and should greatly impact the weight of evidence assigned to each study. This is not reflected in the below review of the cancer studies and the ultimate conclusions.

RESPONSE: *ATSDR did not provide a critique of the meta-analysis studies, nor did the Agency conduct a weight-of-evidence analysis of the epidemiological cancer studies. The purpose of the referenced statement was to inform the reader of the general study limitations.*

COMMENT 14: Regarding the Table 2-4 list of studies included in the Collins and Acquavella (1998) meta-analysis, the Reviewer commented on the Blair et al. (1998) study: Koutros et al 2019 is an extended follow-up of the same population in Blair et al.

RESPONSE: *ATSDR notes that this is a list of the studies included in the Collins and Acquavella (1998) meta-analysis. It is outside of the scope of the profile for ATSDR to comment on the studies selected by the investigators.*

COMMENT 15: Regarding the Table 2-4 list of studies included in the Collins and Acquavella (1998) meta-analysis, the Reviewer commented on the Collins et al. (1989) study: This study population is part of Koutros et al 2019 and should not be considered independent.

RESPONSE: *The purpose of Table 2-5 (formerly Table 2-4) is to list the studies included in the Collins and Acquavella (1998) meta-analysis. It is outside of the scope of the profile to critique the studies included in the analysis.*

COMMENT 16: Regarding the Table 2-4 list of studies included in the Collins and Acquavella (1998) meta-analysis, the Reviewer commented on the Wood et al. (1998) study: O'Berg 1985, Chen 1987, Wood 1998 = Symons 2008

RESPONSE: *ATSDR notes that the Symons et al. (2008) study was published after the Collins and Acquavella (1998) meta-analysis.*

COMMENT 17: Regarding the Table 2-4 list of studies included in the Alexander et al. (2021) meta-analysis, the Reviewer added Benn and Osborne (1998).

RESPONSE: *The Benn and Osborne (1998) study was added to Table 2-5 (formerly Table 2-4).*

COMMENT 18: Regarding Table 2-5, the Reviewer commented: Suggest deleting. These meta-analyses mix effect measures and the inference that can be made. SMRs should not be considered equivalent to HRs/RR/ORs.

RESPONSE: *ATSDR notes that Table 2-5 was deleted from the profile.*

COMMENT 19: The Reviewer suggested the following revision (marked in red) to Section 2.19: NTP (2001) noted that a similar mechanism of carcinogenicity in rats and mice have been reported for other compounds such as 1,3-butadiene, vinyl chloride, benzene, and ethylene oxide, which are epoxides or are metabolized to mutagenic epoxide intermediates.

RESPONSE: *The suggested revision was made in Section 2.19:*

NTP (2001) noted that a similar mechanism of carcinogenicity in rats and mice has been reported for other compounds such as 1,3-butadiene, vinyl chloride, benzene, and ethylene oxide, which are epoxides or are metabolized to mutagenic epoxide intermediates.

COMMENT 20: Regarding Table 2-6, the Reviewer commented: Unclear why onl y these studies were selected. See suggestions for modification in Charge question document.

RESPONSE: *As noted in the Response to Comment 8, ATSDR has revised this table to include the larger studies evaluating cancer endpoints. The text in Section 2.19 has been revised to include information on the study selection:*

Summaries of the findings of eight of the larger studies are presented in Table 2-4. Several of these studies are updates of older studies, only the most recent examination is included in the table. Lung cancer was the most well studied cancer endpoint.

COMMENT 21: Regarding the Marsh et al. (1999) study summarized in Table 2-6, the Reviewer commented: Included in Koutros/Blair; not independent, delete.

RESPONSE: *The Marsh et al. (1999) study was deleted from Table 2-4 (formerly Table 2-6).*

COMMENT 22: Regarding the Marsh et al. (2001) study summarized in Table 2-6, the Reviewer commented: Delete. This is not an independent study.

RESPONSE: *The Marsh et al. (2001) study was deleted from Table 2-4 (formerly Table 2-6).*

Comments provided by Peer Reviewer #2

General Comment

This reviewer's expertise and research experience are in the fields of biostatistics and epidemiology. As most of the Chapters (or significant parts of Chapters) of this Tox Profile deal with the scientific areas of toxicology, pharmacology, biochemistry, and the like, they fall outside my areas of expertise. Thus, my comments in those sections of the Tox Profile are more general in nature. I provide specific, more detailed comments in the sections involving my areas of expertise.

ATSDR Charge Questions and Responses and Reviewer Comments

Chapter 1

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT 1: I agree with the summary of the "cancer effects" provided on page 4. The other noncancer effects involve scientific areas outside of my area of expertise (see General Comment above).

RESPONSE: *No response needed.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT 2: Many of the effects observed in animals have been observed in humans as noted in Chapter 2. I cannot adequately answer the question regarding effects only observed in animals as this involves scientific area outside of my areas of expertise.

RESPONSE: *No response needed.*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain.

COMMENT 3: No comments. See General Comment above.

RESPONSE: *No response needed.*

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain.

COMMENT 4: No comments. See General Comment above.

RESPONSE: *No response needed.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose.

The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT 5: No comments. See General Comment above.

RESPONSE: *No response needed.*

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 6: No comments. See General Comment above.

RESPONSE: *No response needed.*

Chapter 2

GENERAL COMMENT: I have provided comments directly in the draft toxicology profile regarding the presentation of certain Figures and Tables.

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT 7: With the exception of providing more information regarding the strengths and weaknesses of the epidemiology studies and on results of exposure-response evaluations (see below comments), I found the conclusions of Chapter 2 to adequately reflect the findings from the epidemiological studies.

RESPONSE: *No response needed.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT 8: The epidemiology studies described in chapter 2 included no review of study strengths or limitations as noted above. The studies summarized in table 2.6 included only reference, study type, and population, exposure, outcome evaluated and result. There was also no discussion of these studies in the text other than noting that they were published after the last meta-analysis done by Collins nd

Acquavella in 1998 and that they did not find consistent evidence of an increased cancer mortality risk among acrylonitrile workers.

RESPONSE: *It is beyond the scope of the profile to provide an in-depth discussion of individual epidemiological studies. Table 2-4 (formerly Table 2-6) is consistent with ATSDR guidance on epidemiological study summary table tables as described in the latest version of ATSDR's "*Guidance for the Preparation of Toxicological Profiles*."*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT 9: No comments. See General Comment above.

RESPONSE: *No response needed.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT 10: No comments. See General Comment above.

RESPONSE: *No response needed.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

COMMENT 11: No comments on the adequacy of the animal experiments reported in the toxicological profile (see General Comment above). Regarding the epidemiology studies, no information was provided on the exposure-response relationships evaluated in the various studies. Table 2.6 includes some information on exposure levels and some information about results relative to certain categories of exposure, but nothing is said about overall exposure-response relationships or lack thereof. It would be helpful if Table 2.6 included another column that summarized any findings related to a quantitative exposure- response relationship. This is important because in some studies even though there may be an overall excess of risk for a given cancer, the exposure-response relationship is not present therefore arguing against a causal association.

RESPONSE: *See Response to Comment 8.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT 12: Similar to the first reanalysis of the NCI AN cohort published by Marsh et al. in 1999 (and shown here in Table 2.6), Marsh and Kruchten have submitted a paper for publication that describes a reanalysis of the most recent update of the NCI AN cohort study (Koutros et al. 2019). Some reviewer comments have been received and it is likely that the editor will recommend revise and resubmit. Thus,

the paper could be accepted for publication in the first quarter of 2023. If so, it should be included in the Toxicological Profile in Chapter 2 and Table 2.6. The results of the reanalysis show no evidence of elevated risks for lung cancer or cancers of any site to an even greater extent than the conclusions of the NCI update. Unlike the NCI study, the Marsh and Kruchten reanalysis shows cancer risks based on external mortality comparisons, accounts for possible confounding by smoking at the total cohort level and shows study site-specific data.

RESPONSE: *The Marsh and Kruchten paper has not been published; ATSDR will evaluate this study for future inclusion in the toxicological profile.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT 13: No comments. See General Comment above.

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RESPONSE: *No response needed.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT 14: No comments. See General Comment above.

RESPONSE: *No response needed.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT 15: No comments. See General Comment above.

RESPONSE: *No response needed.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT 16: No comments. See General Comment above.

RESPONSE: *No response needed.*

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT 17: No comments related to the toxicological data (see General Comment above). However, regarding the epidemiological data presented in the toxicological profile, this reviewer feels

that the appropriate conclusions were drawn from that body of literature regarding cancer risks among humans exposed to acrylonitrile. However, as noted in other responses, these conclusions would be better supported by providing a summary of the strengths and weaknesses of each study and information regarding any quantitative exposure-response relationships evaluated in these studies.

RESPONSE: *As noted in responses to previous comments, it is beyond the scope of the profile to discuss the strengths and weaknesses of individual studies. This is in accordance with the latest version of ATSDR's "*Guidance for the Preparation of Toxicological Profiles*." As noted in previous responses, ATSDR does not conduct a weight-of-evidence evaluation of the carcinogenicity data. Rather, the discussion is limited to reporting study results. General weaknesses of the epidemiological studies are discussed in the text of Section 2.19.*

Chapter 3

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT 18: No comments. See General Comment above.

RESPONSE: *No response needed.*

QUESTION: Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

COMMENT 19: No comments. See General Comment above.

RESPONSE: *No response needed.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT 20: No comments. See General Comment above.

RESPONSE: *No response needed.*

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT 21: No comments. See General Comment above.

RESPONSE: *No response needed.*

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT 22: No comments. See General Comment above.

RESPONSE: *No response needed.*

QUESTION: Are the biomarkers of exposure specific for the substance? Please explain.

COMMENT 23: No comments. See General Comment above.

RESPONSE: *No response needed.*

QUESTION: Are the biomarkers of effect specific for the substance? Please explain.

COMMENT 24: No comments. See General Comment above.

RESPONSE: *No response needed.*

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT 25: The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT 26: The Reviewer did not provide a response to this question.

RESPONSE: *No response needed.*

Chapter 4

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT 27: No comments. See General Comment above.

RESPONSE: *No response needed.*

QUESTION: Is information provided on the various forms of the substance? Please explain.

COMMENT 28: No comments. See General Comment above.

RESPONSE: *No response needed.*

Chapter 5

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT 29: While most of the chapter relates to areas outside of my expertise, this chapter appears to present a complete overview of the areas of concern to ATSDR.

RESPONSE: *No response needed.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT 30: While most of the chapter relates to areas outside of my expertise, this chapter appears to present a complete overview of the areas of concern to ATSDR.

RESPONSE: *No response needed.*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT 31: While most of the chapter relates to areas outside of my expertise, this chapter appears to present a complete overview of the areas of concern to ATSDR.

RESPONSE: *No response needed.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT 32: While most of the chapter relates to areas outside of my expertise, this chapter appears to present a complete overview of the areas of concern to ATSDR.

RESPONSE: *No response needed.*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT 33: While most of the chapter relates to areas outside of my expertise, this chapter appears to present a complete overview of the areas of concern to ATSDR.

RESPONSE: *No response needed.*

Chapter 6

QUESTION: Do you know of other studies that may fill a data gap? Please provide any relevant references.

COMMENT 34: While most of the chapter relates to areas outside of my expertise, this chapter appears to present a complete overview of the areas of concern to ATSDR. I cannot recommend any additional citations.

RESPONSE: *No response needed.*

QUESTION: Do you agree with the identified data needs? Please explain.

COMMENT 35: While most of the chapter relates to areas outside of my expertise, this chapter appears to present a complete overview of the areas of concern to ATSDR. I cannot recommend any additional citations.

RESPONSE: *No response needed.*

QUESTION: Are the data needs presented in a neutral, non-judgmental fashion? Please note any bias in the text.

COMMENT 36: While most of the chapter relates to areas outside of my expertise, this chapter appears to present a complete overview of the areas of concern to ATSDR. I cannot recommend any additional citations.

RESPONSE: *No response needed.*

Chapter 7

QUESTION: Are you aware of any additional regulations or guidelines that should be included? Please provide citations.

COMMENT 37: While most of the chapter relates to areas outside of my expertise, this chapter appears to present a complete overview of the areas of concern to ATSDR. I cannot recommend any additional citations.

RESPONSE: *No response needed.*

QUESTION: Are there any that should be removed? Please explain.

COMMENT 38: While most of the chapter relates to areas outside of my expertise, this chapter appears to present a complete overview of the areas of concern to ATSDR. I cannot recommend any additional citations.

RESPONSE: *No response needed.*

Appendices

QUESTION: Please provide any comments on the content, presentation, etc. of the included appendices.

COMMENT 39: While most of the appendices relate to areas outside of my expertise, they appear to be complete and clearly presented. I have no concerns or other comments.

RESPONSE: *No response needed.*

Annotated Comments on the Toxicological Profile

COMMENT 40: Regarding the Version History, the Reviewer commented: This appears to be incomplete as only one date is given?

RESPONSE: *The Version History is updated when the profile is finalized.*

COMMENT 41: Regarding the bulleted text in Section 1.2, the Reviewer commented: Why are cancer risks not noted per info provided on page 4?

RESPONSE: *Cancer was not included as a bullet since this endpoint did not undergo systematic review. As noted in an earlier response, the systematic review in Appendix C is limited to noncancer endpoints since the purpose is to support MRL derivation (MRLs are not based on noncancer endpoints). See ATSDR's "*Guidance for the Preparation of Toxicological Profiles*" for additional information. However, the text above the bullets was revised to include cancer:*

> As illustrated in Figures 1-1 and 1-2, the most sensitive effects appear to be nasal lesions following inhalation exposure, nonglandular stomach (i.e., forestomach) damage following oral exposure, neurological effects, and cancer. A systematic review of the noncancer endpoints resulted in the following hazard identification conclusions:

COMMENT 42: Regarding Figure 1-1, the Reviewer commented: Why are acute and chronic MRLs not shown at bottom of graph as in Fig 1-2, 1-3?

RESPONSE: *No inhalation MRLs were derived for acute and chronic duration exposures.*

COMMENT 43: Regarding the discussion of cancer effects in Section 1.2, the Reviewer commented: this should be included somehow in summary on page 1.

RESPONSE: *As noted in the Response to Comment 42, the text in Section 1.2 has been revised to include cancer.*

COMMENT 44: Regarding Figure 2-1, the Reviewer commented: Not clear what the %s in pie chart relate to relative to bar graphs? Animal or human or both combined?

RESPONSE: *The pie charts illustrate the percentage of studies by each exposure route or exposure duration. It represents both human and animal data.*

COMMENT 45: Regarding Figure 2-2, the Reviewer commented: The graphs should note that the symbols and nos. are defined in previous table.

RESPONSE: *There is a note in Section 2-1 referring the reader to the User's Guide in Appendix D for assistance in interpreting the LSE figures.*

COMMENT 46: Regarding Figure 2-2, the Reviewer commented: Not clear what the dashed line under BMCL hec refers to?

RESPONSE: *This is an anchor line connecting the MRL point of departure (in this case a $BMCL_{HEC}$) to the MRL value.*

COMMENT 47: Regarding Figure 2-3, the Reviewer commented: Same as previous comment. Not clear about meaning of dashed line. Should be in the legend.

RESPONSE: *See Response to Comment 46.*

Comments Provided by Peer Reviewer #3

General Comments

COMMENT 1: The authors of the draft profile have successfully undertaken and completed a challenging task in surveying the fairly abundant literature to identify key documents/reports and in presenting succinct interpretations of the toxicological data for acrylonitrile. The profile is well organized and balanced, and the text is written in a very readable fashion. It is also thorough without being a comprehensive criteria document. However, the draft profile has omitted consideration of a few relevant issues and reports that are discussed and referenced in this summary report.

RESPONSE: *No response needed.*

COMMENT 2: This reviewer has carefully gone through and studied the profile, responded to the charge questions where there was a lack of agreement, and offered constructive critiques regarding a few areas of differences in opinion or omission of several essential and/or significant reports. The suggested changes are focused primarily on issues presented in, or missing from, Chapters 1-3, while minor comments and suggested edits encompass the entire profile. The nature of this summary report is partly influenced by the training and experiences of this reviewer in the areas of chemical carcinogenesis/mutagenesis and experimental neuro-oncogenesis of nitrosoureas and epoxide and epoxide-forming compounds including ethylene oxide, acrylonitrile, 1,3-butadiene and its epoxide metabolites, and vinyl chloride mostly in rodent models. In addition, this reviewer was conducting research on the molecular dosimetry of acrylonitrile, ethylene oxide, and vinyl chloride at the Chemical Industry Institute of Toxicology (CIIT) during the years of a research program focused on the toxicokinetics and biomarkers of acrylonitrile exposure and mechanism of action of acrylonitrile as a rodent carcinogen. The CIIT research program resulted in numerous publications cited in the current toxicological profile for acrylonitrile. In addition, this reviewer is a veterinary pathologist who (i) was peripherally involved and participated in the necropsy of rats [and collection of tissues for DNA adduct studies (Walker et al 2020a PMID 32529823)] in the cancer bioassay of acrylonitrile conducted by Darell Bigner's group (Bigner et al 1986), (ii) has written several book chapters concerning the pathology of spontaneous and chemically-induced tumors of the central and peripheral nervous system in rodents, and (iii) has participated in panels deliberating the morphological classification of rodent brain tumors and the mode of action of chemical neuro-carcinogens including acrylonitrile (Haber and Patterson 2005 PMID 16270753). With this background, this reviewer has identified four primary areas where change or additional work is needed including (i) the incomplete consideration and updating of the classification of brain and spinal cord tumors in acrylonitrile-exposed versus control rats, (ii) the incomplete consideration, updating, and discussion of genotoxic effects and biomarkers of exposure and effect of acrylonitrile, (iii) a near complete lack of consideration of the possible mechanisms of action of acrylonitrile as a rodent carcinogen, and (iv) and an inadequate consideration of whether the carcinogenic "effects only observed in animals are likely to be of concern to humans". This reviewer reasonably anticipates that others with differing but interfacing backgrounds and expertise were selected as reviewers to achieve a more complete evaluation of these and other aspects of the draft toxicological profile.

RESPONSE: *ATSDR thanks the Reviewer for their comments on the toxicological profile.*

COMMENT 3: The most pressing issue for this reviewer is the level of attention given in the toxicological profile to the change over time (nearly 4 decades) in the morphological classification of primary brain and spinal cord tumors, based upon standard histopathology [typically using two

histological stains: hematoxylin and eosin (H&E)], across a substantial number of cancer bioassays of acrylonitrile in rats. This reviewer has spent considerable time addressing the issue of the morphologic diagnosis of the brain tumors in acrylonitrile-exposed rats because the molecular pathways from normal to neoplastic cells may differ significantly between differing glial cell neoplasms, including astrocytomas, microgliomas, and oligodendrogliomas. By consistently referring to a specific, potentially incorrect morphologic diagnosis, covert or overt support for particular neoplastic transformation events is maintained while excluding others. As discussed later, these circumstances then have ramifications for understanding the mechanisms of action of acrylonitrile as a neuro-carcinogen.

Prominently, the toxicological profile overlooked the results of recent robust immunohistochemical studies providing definitive evidence that the brain tumors in acrylonitrile-exposed Sprague-Dawley rats are malignant microgliomas (Kolenda-Roberts et al 2013 PMID 22821367), which confirmed the histopathology-based classification of "microgliomas" in the brain and spinal cord in a very early 2-year rat "drinking-water" study of acrylonitrile first reported in brief in the Federal Register (Occupational Safety and Health, 1978). From the late 1970s through 2002, several differential morphologic 'diagnoses' based upon standard histopathology examinations typically using H&E-stained sections have been applied to rat brain and spinal cord tumors including (in temporal order) microgliomas (Fed Reg 1978), astrocytomas (Quast et al 1980a, 1980b), brain tumors-difficult to classify (Bigner et al 1986), gliomas (labeled as oligodendrogliomas in photomicrographs) (Maltoni et al 1988), astrocytomas (Quast 2002); and "glial cell tumors" diagnosed as "astrocytomas (Johannsen and Levinskas 2002a, 2002b). Yet, the text of the toxicological profile consistently refers to acrylonitrile-induced brain and spinal cord tumors as "astrocytomas" based upon selective old and incomplete information.

To serve as a basis for forthcoming requests for changes in the text, and in some figures and tables, a history of the evolution in the morphologic classification or 'diagnosis' of both acrylonitrile-induced and spontaneous brain tumors in the rat is necessary. As noted by Darell Bigner (Bigner et al 1986), there were no reports "published in critically edited journals about the neuro-oncogenicity of acrylonitrile" in the earliest 2-year studies in rats. A brief summary of the putative central nervous system (CNS) tumors in an early "drinking water" study (called the "Dow Reports") of acrylonitrile in Sprague-Dawley rat was reported in the Federal Register (1978), with the brain and spinal cord tumors in exposed animals considered "microgliomas" by the study investigators. The "drinking water" study was reviewed by Dr. Cipriano Cueto, Jr. (the Acting Chief of the Toxicology Branch of the Carcinogenesis Testing Program in the Division of Cancer Cause and Prevention at the National Cancer Institute) who noted that the presence of microgliomas, due to their rare spontaneous occurrence, was highly important in implicating acrylonitrile as carcinogenic to rats of both sexes (Fed Reg 1978). Three other board-certified pathologists also examined the rat-tissue sections and noted the histopathological findings of microgliomas and tumors of the forestomach and Zymbal gland (Fed Reg 1978). In the same Federal Register report (1978), it was noted that Professor Cesare Maltoni had performed two 52-week studies of acrylonitrile, by inhalation or by gavage, in Sprague-Dawley rats and found increased incidences of several cancer types including Zymbal gland carcinomas, forestomach papillomas, and "encephalic brain tumors". However, in the view of expert reviewers, the study design of the 1-year studies "significantly reduces their sensitivity and limits their usefulness …OSHA finds that the Maltoni studies, when viewed in the context of the Dow reports, provide additional supportive evidence of the ability of acrylonitrile to induce cancer in animals". In a subsequent two-year drinking water study of acrylonitrile, Maltoni et al (1988) reported a significant increase in 'encephalic gliomas' in both male and female rats that were diagnosed as "oligodendrogliomas" based upon features shown in photomicrographs of H&E-stained sections. In contrast, Quast et al reported that acrylonitrile exposures of Sprague-Dawley rats in a 2-year inhalation study (1980a) and a drinking water study (1980b) were associated with significant increases in focal or multifocal glial cell tumors diagnosed as "astrocytomas" without providing any photomicrographs or descriptions of the morphological characteristics of these CNS tumors in the text. Furthermore, Quast et al (1980a) considered CNS gliosis, with and without perivascular cuffing, to be a

"primary treatment-related effect" that was later interpreted by Quast (2002) "to be a tumor precursor" (or "earlier stage" or pre-malignant lesion) related to the formation of brain tumors in acrylonitrile-treated rats.

Bigner et al (1986) reported increased incidences of brain tumors that were "undifferentiated morphologically" in acrylonitrile-exposed Fisher 344 (F344) rats at 18 months into a planned 2-year drinking water study. In spite of the superficial morphologic similarity to astrocytomas, the brain tumors did not exhibit staining for glial fibrillary acidic protein (GFAP) characteristic of astrocytomas, while prominent staining occurred in adjacent reactive and normal astrocytes in the same tissue sections. The authors also reported the absence of GFAP staining in two series of spontaneous rat brain tumors 'putatively' classified as astrocytomas in F344 and Sprague-Dawley rats. The lack of definitive evidence of astrocytic lineage or relatedness in the brain tumors from acrylonitrile-exposed F344 rats contrasts sharply with clear-cut evidence of astrocytic differentiation detected by GFAP and electron microscopy in brain tumors induced by ethylnitrosourea and methylnitrosourea or detected by the same methods in astrocytic tumors in humans (Bigner et al 1986). Furthermore, differences between acrylonitrile and other neuro-oncogenic agents (such as nitroso compounds and polycyclic aromatic hydrocarbons) include an apparent lack of transplacental activity (Swenberg 1982 6953800), a uniformity in the morphology of induced brain tumor from animal to animal, and the difficulty of transplantation and establishment of permanent cell lines in culture from acrylonitrile-induced brain tumors. Bigner et al (1986) did not pursue additional testing to differentiate between glial cell types in the spontaneous and acrylonitrile-induced rat brain tumors because available immunocytochemical techniques, other than GFAP and S-100 protein antibodies, were based upon human species-specific antibodies that were not applicable to rat tumors. **Notably, Bigner et al (1986) observed that 'gliosis' has been reported in brain sections of rats exposed to various neuro-oncogenic chemicals in long-term studies; however, gliosis is not regarded [by most experts in the field of experimental neuro-oncogenesis (see references in Bigner et al, 1986)] as a transitional stage in the development of gliomas.**

In 2002, results of three additional cancer bioassays of acrylonitrile in rats were published in back-to-back reports. In a 2-year oral study of acrylonitrile in Sprague-Dawley rats, Quast (2002) reported a significantly increased incidence of CNS tumors in treated animals that were diagnosed as "astrocytomas" based upon the morphologic appearance of microscopic tumors in the brain and spinal cord (albeit, no photomicrographs were provided in the report). Based upon distribution and morphology, the CNS tumors observed in these rats resembled those found in Sprague-Dawley rats exposed in earlier chronic inhalation and drinking water studies of acrylonitrile performed by the same group (Quast et al 1980a, 1980b). Furthermore, the morphologic characteristics of the acrylonitrile-induced CNS tumors described by Quast (2002) resembled those reported (and shown in photomicrographs) by Bigner et al (1986) for acrylonitrile-induced brain tumors in F334 rats to the extent that Quast (2002) noted that the tumors in both rat strains "appeared to be identical". In chronic studies of acrylonitrile administered to Sprague-Dawley rats in drinking water or by gavage, Johannssen and Levinskas (2002b) reported significant increases in brain and spinal cord tumors that were diagnosed as "astrocytomas" based upon morphologic appearance of microscopic tumors (albeit, no photomicrographs were provided in the report). Likewise, in a chronic study of acrylonitrile administered to F344 rats in drinking water, Johannsen and Levinskas (2002a) found significant increases in "glial cell tumors" diagnosed as "astrocytomas"; however, no microscopic descriptions or photomicrographs were included in the report.

In summary, there has been significant overlap in the morphologic characteristics described (Bigner et al 1986; Maltoni et al. 1988; Quast 2002; Johannssen and Levinskas 2002b), and illustrated in photomicrographs (Bigner et al 1986; Maltoni et al 1988), for acrylonitrile-induced CNS tumors, leading to several 'differential diagnoses' of malignant microglioma, brain tumor (difficult to classify), glioma/glial cell tumor, oligodendroglioma, and astrocytoma. It is widely recognized that pathologists and others trained in rodent histopathology, even while looking at the same slides, usually agree on the

morphological characteristics and differential diagnoses for CNS and peripheral nervous system tumors in H&E-stained sections, but often disagree on the final classification of a given tumor or tumor series [e.g., see the section on 'Diagnostic Problems' in Walker et al 1989, Peripheral nerve sheath tumors, rat. In: Monographs on pathology of laboratory animals (T.C. Jones, ad.), Vol. 6, The nervous system, International Life Sciences Institute, Springer-Verlag, N.Y., pp 143-153]. Since the report by Bigner et al (1986) that acrylonitrile-induced rat brain tumors or spontaneous brain tumors 'putatively' diagnosed as astrocytomas in control rats showed no evidence of neoplastic cells that reacted positively for GFAP – the histogenesis and cellular phenotype of both the induced and spontaneous tumors have been debated among those concerned about chemical-induced neuro-oncogenesis. Thus, when a panel of experts met in 2008 to conduct an independent review of an acrylonitrile risk assessment (Haber and Patterson, 2008), there was an agreement to avoid this debate by consistently referring to tumors in the CNS of acrylonitrile-treated rats as 'brain tumors'.

To further characterize the histogenesis, cellular phenotype, and appropriate classification of spontaneously occurring primary brain tumors and acrylonitrile-induced brain tumors in the rat, Kolenda-Roberts et al (2013) used a panel of immunohistochemistry stains (specific for the rat) to target rat brain cells and distinguish between astrocytes, microglia (or macrophages), oligodendrocytes, neurons, and proliferating cells of rat origin. As noted above, difficulties often accompany the interpretation of brain tumor induction in the rat for several reasons (Kolenda-Roberts et al 2013 and references therein): (i) brain tumors are rare in untreated rats and are most often given a putative diagnosis of 'astrocytoma' based upon H&E-stained sections (Bigner et al. 1986; Quast et al 1980a, 1980b; Quast 2002; Johannssen and Levinskas 2002b; reviewed in Ward and Rice 1992 PMID 6953796), (ii) small increases in the observed incidences of brain tumors over background in chronic cancer bioassays of chemicals in rats have created uncertainties and debate for decades, and (iii) and 'putative' astrocytomas in control and acrylonitrile-treated rats have proven to be uniformly negative to staining for GFAP, an astrocyte marker that has been useful for tumor characterization in man and in rats treated with nitroso compounds. The last observation has led to the postulate that spontaneous and acrylonitrile-induced rat brain tumors resembling astrocytes morphologically demonstrated poor cellular differentiation and astrocytic protein expression, unlike astrocytomas in humans and nitrosourea-induced astrocytomas in rats (Bigner et al 1986; Kolenda-Roberts et al 2013).

To address these issues, Kolenda-Roberts et al (2013) performed robust immunohistochemical examination of nine brain tumors from rats treated with acrylonitrile in a 2-year drinking water study plus 28 spontaneous rat brain tumors previously diagnosed as **14 astrocytomas**, eight gliomas/mixed gliomas, five oligodendrogliomas, and one case of severe gliosis. The resulting immunochemical-based studies of the spontaneous neoplasms clearly demonstrated that these 28 tumors from control rats were classified as 16 oligodendrogliomas, nine malignant microgliomas, and three mixed tumors with immunohistochemical characteristics of both oligodendrogliomas and malignant gliomas (or perhaps oligodendrogliomas invaded by microglial cells). The Kolenda-Roberts et al. (2013) report is the first to show that spontaneous rat brain tumors developing at a low incidence in aging rats are not astrocytomas, but are primarily oligodendrogliomas, malignant microgliomas, or perhaps a mixture of both, which supports the susceptibility of tumor development in these two cell types (but not in astrocytes) in the rat. Strikingly, the immunochemical staining profiles for all nine brain tumors from acrylonitrile-treated rats, previously diagnosed as astrocytomas (Quast 2002), were consistent with those of the spontaneous malignant microglial tumors except that there was a mild increase in subpopulations of GFAP+ and Olig2+ cells throughout the neoplasms. [Although not specified in the report by Kolenda-Roberts et al (2013), Dr. Jerry Hardisty, the senior author of the report, confirmed to this reviewer that the tissue sections evaluated in their immunohistochemistry studies were from acrylonitrile-exposed Sprague-Dawley rats from the bioassay of Quast et al (2002)]. **Unexpectedly, no astrocytomas (i.e., GFAP, S100 beta, and/or glutamine-synthetase positive neoplasms) were identified by immunohistochemical characterization among the sets spontaneous and acrylonitrile-induced brain**

tumors. Instead, oligodendrogliomas (57%) and malignant microgliomas (32%) were the most commonly observed tumors among spontaneous glial tumors and **100% of brain tumors from acrylonitrile-treated rats were identified as malignant microglial tumors**, **showing that "acrylonitrile-induced (brain) neoplasms are microglial/histiocytic in origin"** (Kolenda-Roberts et al 2013).

Thus, the results of the above immunohistochemistry profile strongly support the redesignation of acrylonitrile-induced CNS tumors in rats as 'malignant microgliomas' (or 'microglial cell tumors") and calls into question the relevance of these neoplasms to humans (discussed later). This reviewer has written a paragraph succinctly summarizing the sequence of events reviewed in the above three-plus pages for editing by ATSDR scientists and inclusion in the text of the toxicological profile under "*Cancer Effects*" (page 4) in acrylonitrile-exposed rats as the middle of three paragraphs in under this heading:

"While there has been significant agreement in the morphologic appearance of microscopic brain tumors from differing cancer bioassays in rats, several differential morphologic diagnoses (based upon standard histological findings) have been applied to brain and spinal cord tumors in acrylonitrile-treated rats, including microgliomas (Fed Reg 1978), astrocytomas (Quast et al 1980a, 1980b; Johannsen and Levinskas 2002a, 2002b), oligodendrogliomas (Maltoni et al 1988), and brain tumors-difficult to classify (Bigner et al 1986). Designating these brain tumors as astrocytomas also was based upon the observation that acrylonitrile-induced glial cell tumors closely resembled spontaneous brain tumors that occur at a low incidence in rats and are most often classified as 'astrocytomas' (Bigner et al 1986; Maltoni et al 1988; Quast 2002; Johannsen and Levinskas 2002b; Ward and Rice 1992 PMID 6953796). To further characterize the histogenesis, cellular phenotype, and appropriate classification of spontaneously occurring and acrylonitrile-induced glial cell tumors in the rat, Kolenda-Roberts et al (2013) used a panel of immunohistochemistry stains to distinguish between astrocytes, microglia, and oligodendrocytes. These immunohistochemical studies found no evidence of astrocytomas among the sets of spontaneous and acrylonitrile-induced brain tumors; on the contrary, oligodendrogliomas followed by microgliomas were more common as spontaneous tumors, while acrylonitrile-induced gliomas were microglial/histiocytic in origin."

Thus, the repeated mentions throughout the text that acrylonitrile induces 'astrocytomas' needs to be changed to read either 'glial cell tumors' (or 'malignant microgliomas' or 'microglial cell tumors'), where indicated below for each page and line under "Suggested Edits". The remaining question is how to handle the presentation of differential diagnoses for primary brain and spinal cord tumors induced by acrylonitrile in differing chronic studies in rats in, for example, Table 2-7. This reviewer suggests three possibilities to resolve this problem. (1) A recent review has handled this problem by listing the authors and characteristics of each cancer bioassay of acrylonitrile in rats and then added a footnote after each differential diagnosis (using an asterisk*) given for the chemically-induced CNS tumors. For example, for each differential diagnosis in the relevant tables in Kobets et al (2022), the authors have added the footnote noting that acrylonitrile-induced CNS tumors in the rat are "Now considered to be malignant microglial tumors" along with a numerical citation for Kolenda-Roberts et al (2013). Likewise, in a comprehensive review of "all currently available reports of acrylonitrile's genotoxicity and its potential mode of action, Albertini et al (2023, in press) note that acrylonitrile-induced CNS tumors in the rat now are deemed to be malignant microglial tumors. However, this reviewer has discussed the approach used by Kobets et al (2022) with other knowledgeable investigators and there remains a concern that a set of less-informed readers will ignore the footnotes if a similar approach as used in Table 2-7. For example, if a footnote is added to the first listing (and subsequent listings) under Central Nervous System [i.e., Brain glial cell astrocytomas (rats)[a] … Quast et al 1980a, with the footnote reading "[a] Now considered to be malignant microglioma tumors (Kolenda-Roberts et al 2013)"], how many readers will ignore the footnote and come away with the impression that acrylonitrile induces 'astrocytomas'? (2) As an alternative, how many readers will ignore a footnote to the title of Table 2-7, for example, indicating that

"[a] Brain tumors in acrylonitrile-treated rats are now considered to be malignant microglioma tumors (Kolenda-Roberts et al 2013)". (3) The option preferred by this reviewer is to include in any relevant table or figure giving differential diagnosis for acrylonitrile-induced CNS tumors to be listed as 'Glial cell tumor' for each relevant bioassay (or summary of bioassays) and then add a footnote indicating the particular differential diagnosis applied to these CNS tumors in each distinct cancer bioassay. This third approach is the one recommended for appropriate pages under Suggested Edits below. ATSDR scientists reviewing the above comments should determine which option to undertake; however, for reasons elaborated upon below, no reader of this toxicological profile should reach the mistaken conclusion that acrylonitrile induces 'astrocytomas' in the rat.

RESPONSE: *A discussion of the findings of the Kolenda-Roberts et al. (2013) study was added to Section 2.19:*

> Kolenda-Roberts et al. (2013) conducted an investigation to further characterize acrylonitrile-induced brain tumors observed in rat studies. Immunohistochemical characterization was conducted on 39 spontaneously occurring brain tumors in rats (5 oligodendrogliomas, 14 astrocytomas, 8 gliomas/mixed gliomas, and 1 severe case of gliosis which later considered to be an oligodendrogliomas) obtained from the National Toxicology Program (NTP) and 9 astrocytomas from a 2-year acrylonitrile drinking water study (no additional information on the source was provided). Based on immunohistochemical analysis, 16 tumors were characterized as oligodendrogliomas (previously diagnosed as 5 oligodendrogliomas, 6 glioma/mixed glioma, 4 astrocytomas, and 1 gliosis), 9 were characterized as malignant microglial tumors (previously diagnosed as astrocytomas), 3 were characterized as mixed tumors (previously diagnosed as 2 gliomas and 1 astrocytoma), and 11 were diagnosed as granular cell tumor. Based on immunohistochemical analysis, all nine astrocytomas from acrylonitrile-exposed rats were identified as malignant microglial tumors. This finding is supported by the results in the Bigner et al. (1986) acrylonitrile study that reported that the observed brain lesions were similar from spontaneously occurring tumors, which have been generally classified as astrocytomas; however, there was no evidence that the tumors were astrocytic in lineage or relatedness, and the tumors were negative for glial fibrillary acidic protein which is an astrocyte marker. These finding suggest that the tumors referred to as astrocytomas in the acrylonitrile studies were likely malignant microglial tumors or possibly oligodendrogliomas. For this toxicological profile, ATSDR has opted to refer to these tumors as glial cell tumors.

Additionally, Table 2-6 (formerly Table 2-7) was revised, and the tumors were referred to as glial cell tumors with a footnote indicating the tumor name used by the study investigators.

COMMENT 4: The "*Cancer Effects*" section under the "SUMMARY OF HEALTH EFFECTS" includes no mention of the cancer outcomes in chronic gavage study of acrylonitrile led by Burhan Ghanayem for the National Toxicology Program. The following has been suggested (under Specific Comments and Suggested Edits) for inclusion at the end of the first paragraph in the "*Cancer Effects*" section:

"In a single chronic study in mice (Ghanayem et al 2002; NTP 2001), acrylonitrile-treated animals had increased incidences of Harderian gland adenomas and carcinomas and forestomach papillomas/ carcinomas. Neoplasms of the ovary and lung in female mice may have been related to administration of acrylonitrile."

This reviewer would prefer that both the NTP (2001) and Ghanayem et al (2002) reports be cited when results of the cancer bioassay of acrylonitrile are noted in the toxicological profile. It is far easier for an interested party to search for specific details in the 10-page report by Ghanayem et al (2002) compared to

the nearly 200-page NTP Technical Report, plus Burhan's paper has color photomicrographs of neoplasms.

RESPONSE: *The text in Section 1.2 was revised to include the forestomach tumors observed in the NTP (2001) study. Note that this is not intended to be an exhaustive list of all tumor types observed in the animal studies; rather, the list includes tumor types observed in more than one study.*

> In contrast, a number of animal studies have consistently found increases in the incidence of several cancer types including glial cell tumors in the brain and spinal cord of rats (the study investigators categorized these tumors as astrocytomas; see Section 2.19 for additional details), Zymbal gland carcinomas in rats, and forestomach tumors in rats and mice.

Regarding the comment on the Ghanayem et al. (2002) paper, ATSDR opted to only reference this study as NTP (2001) since the Ghanayem et al. (2002) paper does not include the acute or intermediate mouse studies. A statement was added in the LSE table indicating that the results of the chronic NTP (2001) study were also published by Ghanayem et al. (2002).

COMMENT 5: Genotoxic effects and biomarkers of exposure and effect – The information and discussion in Section 2.20 GENOTOXICITY, and relevant studies listed in Tables 2-8 and 2-9 are not complete and, in particular, are missing mention of two recent reports showing the *in vivo* induction of gene mutations in acrylonitrile-exposed rats and mice. Instead of this reviewer providing a list of several pertinent references missing from these two tables it is advisable for the profile to cite a comprehensive review by Albertini et al (2023, in press) that examined "all currently available reports of acrylonitrile's genotoxicity" and studies of biomarkers of effect in humans with the goal of characterizing the potential carcinogenic mechanisms of action of the compound. The tables in the review by Albertini et al (2023) are exhaustive and includes, along with a given endpoint and results for each/multiple relevant references, the methods applied and insightful comments on each finding.

In the paragraph preceding Table 2-8, the text can be changed to mention review by Albertini et al (2023) as well as offer a brief summary of the most salient findings from Tables 2-8 and 2-9 at the end of the paragraph. For example, the end of the paragraph can be changed to read:

"Increases in gene mutations were observed in studies in Drosophila and in rats and mice exposed *in vivo* to acrylonitrile (Walker et al 2020a, 2020b). In contrast to the positive studies of gene mutations, most studies assessing chromosome level mutations arising in somatic cells *in vivo* in mice or rats administered acrylonitrile by a variety of routes have yielded negative results (reviewed in Albertini et al 2023). Thus, there is a disparity between the lack of clastogenic effects of acrylonitrile *in vivo* and the more numerous positive *in vitro* studies listed in Table 2-8."

Two recent reports showing that acrylonitrile caused mutations in exposed rats and mice need to be added to Table 2-9 since these publications are the first to demonstrate the induction of mutations by this chemical in vivo in mammals and these findings have significantly impacted observations and conclusions in two recent reviews concerning the mechanisms of action of acrylonitrile as a rodent carcinogen (Albertini et al 2023; Kobets et al 2022) (suggested edits to Table 2-9 are indicated below). Furthermore, the novel findings in these in vivo gene mutations studies of acrylonitrile should be summarized in a brief paragraph at the end of section 2.20. For this purpose, the following paragraph is included for consideration, review, and editing by ATSDR scientists:

"Dose-related increases in the frequencies of *Hprt* mutations in T lymphocytes were found in rats, conventional mice, and *lacZ* transgenic mice exposed to acrylonitrile (Walker et al 2020a, 2020b). The spectra of mutations in acrylonitrile-treated rats overlapped significantly with those previously reported

for 1,3-butadiene-exposed rats, suggesting a shared mutagenic mechanism. In CYP2E1-null mice, devoid of cytochrome P450 2E1 that yields the epoxide intermediate cyanoethylene oxide, *Hprt* mutant frequencies were significantly increased only at a high dose of 60 mg/kg acrylonitrile that is lethal to wild-type mice. Exposures of wild-type and CYP2E1-null mice to acrylonitrile produced no elevations in micronucleated erythrocytes, but induced significant dose-related increases DNA damage, detected by the alkaline (pH > 13) Comet assay, in one target tissue (forestomach) and one nontarget tissue (liver) of wild-type mice only. These combined studies indicated that metabolism of acrylonitrile to cyanoethylene oxide is central DNA damage and mutation induction *in vivo*, but acrylonitrile itself may also contribute to its mutagenicity/carcinogenicity via mechanisms involving direct and/or indirect DNA reactivity."

At the end of section 3.3.1 Biomarkers of Exposure, consider mentioning the study by Osterman-Golkar et al (1994 8001224):

"In rats exposed sub-chronically (105 days) to acrylonitrile, the relationship between levels of N-(2-cyanoethylene)valine and water concentration was linear to concentrations of 10 ppm but increased sublinearly at higher concentrations."

In section 3.3.2 Biomarkers of Effect, there is a general description of a variety of effects following acrylonitrile exposure in humans and animals (**Albertini et al 2023**); however, there is no consideration of any of a handful of studies performed in humans. Rather than adding a succinct discussion of studies of biomarkers of effect in humans, this reviewer suggests adding the review by Albertini et al (2023) at the end of the first sentence (as shown in bold below). Dick Albertini has done a great job reviewing endpoints, methods, results (with comments) for each of 9-10 referenced studies of biomarkers of effects of acrylonitrile in humans.

"A variety of effects have been demonstrated following acrylonitrile exposure in humans and animals (**Albertini et al 2023**)".

RESPONSE: *The Albertini et al. (2023) paper was added to the profile in Section 2.20 and a statement was made regarding the lack of clastogenic alterations in* in vivo *studies:*

> The genotoxicity of acrylonitrile has been extensively studied in *in vitro* (Table 2-7) and *in vivo* (Table 2-8) studies and reviewed by Albertini et al. (2023). Mixed results have been found in studies of bacterial and mammalian system *in vitro* assays when tested with or without metabolic activation. Increases in gene mutations were observed in *in vivo* studies in rats, mice, and *Drosophila*. In contrast, most studies assessing chromosome level mutations arising in somatic cells *in vivo* in mice or rats administered acrylonitrile by a variety of routes have yielded negative results.

The increase in gene mutations in rats and mice observed in the Walker et al. (2020a, 2020b) studies were added to Section 2.20 and Table 2-8 (formerly Table 2-9):

> Increases in gene mutations were observed in *in vivo* studies in rats, mice, and *Drosophila.*

The Osterman-Golkar et al. (1994) study on hemoglobin adduct formation was added to Section 3.3.1:

> In a study in rats exposed to various doses of acrylonitrile (3–300 ppm) in drinking water for 105 days, a dose-related increase in N-(2-cyanoethyl)valine levels were found (Osterman-Golkar et al. 1994). At doses of 0.74 mg acrylonitrile/kg (10 ppm in drinking water) and lower, there was a linear relationship between dose and hemoglobin adduct levels. A sublinear relationship, indicative of saturation, was observed at higher doses.

Regarding the Reviewer's comment on Section 3.3.2, ATSDR notes that this section is not intended to be a review of the health effects of acrylonitrile. Rather it is a discussion of effects that could be used to identify and/or quantify exposure to acrylonitrile.

COMMENT 6: Mechanisms of acrylonitrile as a rodent carcinogen – While the Introduction of the toxicological profiles indicates that "mechanisms of action are discussed along with the health effects data" when available, there is no consideration of the potential modes of action of acrylonitrile as rodent carcinogen. Mechanistic considerations for the carcinogenicity of acrylonitrile as an animal carcinogen are complex and outside the scope of a toxicological profile because of the need to review abundant and often equivocal findings. However, at the end of the section 2.20 GENOTOXICITY, consider adding a short statement directing readers to two recent reviews that consider a number of potential key effects that may underly the mode of action of acrylonitrile as a rodent carcinogen. For example, consider adding either the first sentence only versus the complete short paragraph:

"Two groups (Albertini et al 2023; Kobets et al 2022) have recently reviewed the genotoxicity profile and other databases to identify key events that potentially play a role in oncogenesis in rodents, including in part direct-DNA reactivity, oxidative stress and oxidative DNA damage, and/or epigenetic changes induced by acrylonitrile and its metabolites. Differences in opinion by these two reviews about general processes (also known to occur in humans) reflects the ongoing and unsettled debate over the key characteristics of acrylonitrile as a rodent carcinogen and their relevance to humans. However, the current data do not allow unequivocal determination of acrylonitrile's mode of action(s) as an animal carcinogen (Albertini et al 2023; Haber and Patterson 2005; Kobets et al 2022)."

The following unpublished summary is provided to illustrate the complexity of deriving an unequivocal mode(s) of action of acrylonitrile as a rodent carcinogen; these mechanistic considerations are not meant for inclusion in the toxicological profile and copies of publications cited immediately below are not included with the Summary Report:

It is notable that both ethylene oxide and 1,3-butadiene are considered to be direct genotoxic compounds, acrylamide is considered to induce both direct genotoxicity and oxidative stress as accepted modes of action, and acrylonitrile has been recognized by Rice and Wilbourn (2000) as an important case where the mechanism of carcinogenic action remains to be established. Since the publication of the pivotal report by Kolenda-Roberts et al. (2013), the ECHA background report (2018) and the current manuscript do not provide brief descriptions of the locations, relative cell numbers, functional differences, etc. between astrocytes and microglia in mice versus rats and how the change in the classification of acrylonitrile-induced rat brain tumors from astrocytomas to malignant gliomas will ultimately impact the interpretation of varied reports focused on whole brain or astrocytes. Caito et al. (2014, 2017) have highlighted some of the differences between astrocytes and microglia cells, but did not mention some important features of microglia and the incidence of microgliomas in humans. While glial cells (astrocytes, microglia, and oligodendrocytes) are derived from multiple progenitor pools and diverge into hundreds of different, mostly post-mitotic phenotypes, microglia are a unique mono-lineage, derived from a macrophage precursor, accounting for ~12% of the total cellular population in the mammalian brain that monitors tissue for debris and pathogens and maintains homeostasis in the parenchyma via phagocytic activity (Soulet and Rivest 2008 PMID-18487084; Soulet and Rivest 2008 PMID-18579087; Tamashiro et al. 2012 PMID-22929966; Sierra et al 2013 PMID-23386811; McCarthy 2017 PMID-28728016). While mature astrocytes do not typically proliferate, microglia cells can readily undergo proliferation in the face of a challenge or when depleted in the mature adult, either chemically or genetically, and undergo re-colonization via bone marrow macrophage stem cells that populate the CNS and differentiate into functional parenchymal or perivascular microglial cells (Soulet and Rivest 2008 PMID-18579087; McCarthy 2017 PMID-28728016). Microglia exhibit regional heterogeneity in number, morphology, activational state, and gene expression (McCarthy 2017 PMID-28728016). Only the smallest astrocyte protrusions are motile and alter their shape based upon neuronal activity, while microglial cells can be amoeboid, migrate, and change functional status depending upon the location in the CNS (Soulet and Rivest 2008 PMID-18579087; McCarthy 2017 PMID-28728016). Among these differences between

astrocytes and microglia, the possibility of increases in proliferation of microglial cells in response to acrylonitrile-induced direct DNA damage from cyanoethylene oxide, oxidative stress, and indirect oxidative DNA damage, and/or cytotoxicity could be important elements leading to the fixation of initiation events in the rat brain.

Table 1. Reports on potential effects of oxidative stress or non-genotoxic mechanisms in brain of acrylonitrile-exposed rats or in astrocytes exposed in vitro to acrylonitrile (CAN), and relevance to microglial cells

Published Report	***Experimental model (and postulate for studies)***	***Major findings***	***Relevance to microglia***
Jiang et al. 1998	Brain and liver from ACN-exposed rats (postulate - ACN induces astrocytomas)	Data showed selective induction of oxidative stress and oxidative damage in rat brain but not liver	Data do not establish whether oxidative stress was induced by ACN specifically in microglial cells
Whysner et al. 1998b	Brain from ACN-exposed rats (postulate - ACN induces gliomas of elusive classification)	Data showed increased levels of 8-oxodeoxyguanosine (8-oxodG) in DNA isolated from brains of ACN-exposed rats	Data do not establish whether 8-oxodG was induced by ACN specifically in DNA of microglial cells
Kamendulis et al. 1999a	DITNC1 astrocyte type 1 phenotype cells and primary rat hepatocytes (postulate - ACN induces astrocytomas)	Data showed selective induction of oxidative stress and oxidative damage in rat astrocytes but not hepatocytes	Data do not provide evidence of oxidative stress or damage in microglia cells or their potential role in ACN-induced microgliomas
Kamendulis et al. 1999b PMID-10580550	DITNC1 rat astrocyte transformed cell line (postulate - target cell type for ACN) and 1?rat hepatocytes (non-target cells)	Data showed that ACN caused a selective inhibition of GJIC in astrocytes but not in rat hepatocytes; reversed by treatment with antioxidants	Data do not provide evidence of ACN-induced inhibition of GJIC (an epigenetic phenomenon) in microglial cells
Jacob and Ahmed 2003b	Primary human astrocytes (postulate - ACN induces gliomas of elusive classification)	Data showed that ACN caused dose-related depletion of GSH and increases in GSSG, ROS, and oxidative DNA damage	Data do not establish whether ACN induces these molecular changes specifically in microglial cells
Esmat et al. 2007 PMID-17606339	Primary glial cells isolated from rat pups (postulate - ACN induces unspecified 'gliomas')	Data showed that 95% of isolated glial cells were astrocytes, and in vitro ACN induced lipid peroxidation and oxidative stress and depleted GSH; NAC pre-treatment afforded some protection	Data do not establish whether ACN induces these molecular changes specifically in microglial cells
Pu et al. 2009	Brain and WBCs of ACN-exposed rats (postulate - ACN induces astrocytomas)	Data showed no evidence of direct DNA damage vs dose-related increases of oxidative DNA damage (fpg comet and 8′hydroxyl-2-deoxyguanosine) in brain and WBCs that was mitigated by dietary NAC	Data do not provide evidence of ACN-induced oxidative DNA damage in microglial cells

Wang et al. 2015 PMID-26332274	DITNC1 rat astrocytes (postulate - ACN induces astrocytomas)	Data showed that ACN induces 8-OHdG or oxidative damage in mtDNA & sustains mitochondrial bioenergetic/biogenic changes	Data do not provide evidence of ACN-induced oxidative DNA damage or changes in mitochondria of microglial cells

Kamendulis et al. (1999a) noted that the brain contains a mixed population of cell types and that in vivo studies "demonstrating the selective induction of oxidative stress by acrylonitrile in the (whole) rat brain (of exposed animals) could not establish whether oxidative stress was produced specifically in the astrocyte", the presumed target of acrylonitrile-induced neuro-oncogenesis in the rat in the majority of reports available for consideration. Once the cell type differences between astrocytes and microglia have been described in future literature related to acrylonitrile, then it will be increasingly difficult to make the case that multiple studies evaluating effects (i.e., the potential role of oxidative stress, GSH depletion, and oxidative damage) in whole brain of acrylonitrile-exposed rats or astrocytes/astrocyte cell lines exposed in vitro to acrylonitile mirror or reproduce molecular events in varied populations of microglial cells that differ somewhat in function depending upon their location in the CNS. Such attempts to use experimental data from astrocytes to predict molecular events in microglia is equivalent to using information gained from lung epithelial cells to forecast responses to chemically-induced insults in macrophages residing in lung or other solid tissues. Transformed macrophages lead to histiocytic sarcomas in lung/other solid tissues (Marlowe et al. 2018 PMID-30246460; Emile et al. 2016 PMID-26966089) as a counterpart to malignant microgliomas in the CNS. These cell-specific differences in brain as well as potential tissue- and species-specific differences are sure to be a major topic of debate among experts in the next IARC Working Group who will update the review of longstanding and recently published data for acrylonitrile.

RESPONSE: *A discussion of the possible mechanisms of acrylonitrile carcinogenicity, as reviewed by Kobets et al. (2022) and Albertini et al. (2023) was added to Section 2.19:*

> The mechanism of acrylonitrile carcinogenicity in rats and mice has not been fully elucidated. Kobets et al. (2022) suggested that multiple mechanisms are likely involved, but the mechanisms do not likely involve direct DNA damage. Likely mechanisms for brain and forestomach tumors are direct and indirect (due to oxidative damage) cytotoxicity and compensatory cell proliferation. Kobets et al. (2022) suggested that glutathione depletion in the brain and forestomach (and various other tissues) is a critical initiating event. Glutathione depletion results in increases in the metabolism of acrylonitrile to 2-cyanoethylene oxide and cyanide. These metabolites, as well as acrylonitrile, could initiate pro-inflammatory signaling and sustained cell and tissue injury which could lead to compensatory cell proliferation, cell transformation, and neoplastic development (Kobets et al. 2022). Albertini et al. (2023) also suggested that multiple mechanisms are involved in acrylonitrile's mutagenicity. The investigators suggested that acrylonitrile's mutagenic mechanism of action likely involves indirect mutagenicity caused by oxidative DNA damage.

COMMENT 7: Relevance of rodent cancers to humans – This reviewer is uncertain as to where in the toxicological profile to consider the 'human biological plausibility' for induction of microgliomas in acrylonitrile-exposed people. In the Charge Questions for reviewers, the most relevant query (Question 2) occurs under questions for Chapter 1 (Relevance to Public Health). In the opinion of this reviewer, however, the question of "Are the (carcinogenic) effects only observed in animals likely to be of concern to humans?" should indeed be addressed in brief in Chapter 2 on page 61, just prior to noting the categorization of acrylonitrile as a human carcinogen by regulatory agencies and IARC (see the second paragraph below).

The 'human biological plausibility' for induction of microgliomas in acrylonitrile-exposed humans appears to be remote. While microglia frequently exhibit cross-talk with high-grade gliomas and intimately interact, invade, and co-evolve with malignant tumor cells of glial cell origin (Roesch et al. 2018 PMID 29389898), there appear to be only two cases of microgliomas occurring in humans that have been listed in PubMed. The first case of a microglioma in a 50-year-old woman was confirmed by immunohistochemistry (Hulette 1996 PMID 8685234), and a second case of a microglioma in a child was more recently reported by Matthews et al. (2016 PMID 27191913). It appears that it is now considered a factual error that glioblastomas can rarely arise from microglial cells based upon immunohistochemistry panels that can distinguish malignant glial cells in the neoplasm from infiltrating microglia (Roesch et al. 2018 PMID 29389898).

Consider adding the following paragraph in some edited form to the toxicological profile:
"The major types of cancers induced by acrylonitrile in animals are not likely to be of concern to humans beyond the fact that the chemical is a multi-site carcinogen in rats and mice exposed chronically to high concentrations of the agent. However, the evidence for the "human biological plausibility" for acrylonitrile-induced cancers is diminished by the nature of the chemically-induced rodent tumors including those of the Zymbal gland (rat), the Harderian gland (mouse), forestomach (rat and mouse), and the brain and spinal cord as malignant microgliomas. The Harderian gland is either absent or vestigial in primates (Payne 1994 PMID 7559104). The Zymbal gland is an auditory sebaceous gland of the external ear in the rat, while the ears of primates have an apocrine ceruminous gland. Likewise, humans lack a forestomach, or a region consisting of cornified, stratified squamous epithelium proximal to the glandular stomach in rodents. Last, immunohistochemical studies revealed that acrylonitrile-induced brain and spinal cord tumors in the rat are microglial/histiocytic in origin (Kolenda-Roberts et al 2013) and a significant portion of rare spontaneous brain tumors in control rats are microgliomas, suggesting a tendency for microglial cells to undergo malignant transformation in this species. In contrast, microgliomas are exceedingly rare in humans and are an unlikely target for acrylonitrile-induced carcinogenesis in people (Hulette 1996 PMID 8685234; Matthews et al. 2016 PMID 27191913)."

RESPONSE: *ATSDR toxicological profiles are developed in accordance with ATSDR's "*Guidance for the Preparation of Toxicological Profiles*." In the absence of a weight-of-evidence evaluation of the epidemiological carcinogenicity data and an extensive evaluation of the carcinogenicity mechanisms of action, it is beyond the scope of the toxicological profile to assess whether the results of the rodent carcinogenicity studies are relevant to humans. ATSDR notes that the Department of Health and Human Services (HHS) considers acrylonitrile to be reasonably anticipated to be a human carcinogen, and EPA and IARC categorized acrylonitrile as probably or possibly carcinogenic to humans, respectively. Additionally, the Kobets et al. (2022) recent review of cancer mechanisms of action noted that further dose-effect and mechanistic investigations are required to evaluate the relevance of the findings in rodent studies to humans.*

COMMENT 8: The Roman numerals in the table of Contents page preceding Chapter 1 need to be corrected: the Foreword should be 'iii' and the Contents should be 'vii'. Roman numeral 'ix' should be added to the List of Figures page and 'x' should be added to the List of Tables page.

RESPONSE: *The Contents page has been corrected in accordance with the Reviewer's comment.*

COMMENT 9: Page iv, line 3: Consider changing the text to read "…health-related authorities…" because 'health-related' comes before the word it modifies (in this case "authorities").

RESPONSE: *The suggested revision was made to the Foreword:*

CERCLA section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health-related authorities" of the statute.

COMMENT 10: For Questions 1-3 in the Charge to the Reviewer, this reviewer agrees with "those effects known to occur in humans as reported in the text" (Question 1) are accurately summarized and that the "exposure conditions have been adequately described" (Question 3) for a brief overview of the Relevance to Public Health.

RESPONSE: *No response needed.*

COMMENT 11: Regarding Question 2, while the available human studies "do not support an increased risk of cancer and acrylonitrile occupations exposure" (page 4, line 12), regulatory agencies have categorized acrylonitrile as being a possible (IARC) to probable (NTP) carcinogen in humans based in large part upon its carcinogenicity in rodents, especially the reported increased incidence of brain and spinal cord tumors classified as astrocytomas based upon tumor morphology in four chronic studies in the rat (Quast et al 1980; Quast 2002; Johanssen and Levinskas 2002a, 2002b). As noted above in the Summary Report, it may be that the current IARC classification of Group 2B is the more accurate categorization because the reclassification of rat brain tumors as malignant microgliomas make the chemical induction of these neoplasms in rats "less likely to be of concern to humans".

The issue of "human biological plausibility" for acrylonitrile-induced cancer was addressed above and a recommendation was made for including a related paragraph under section 2.19 instead of in Chapter 1.

RESPONSE: *See the Response to Comment 7.*

COMMENT 12: Regarding Questions 4-6, this reviewer agrees with each MRL derivation and has confidence in the MRL database assessment.

RESPONSE: *No response needed.*

COMMENT 13: Page 1, line 20: Make a change here to define the 'forestomach' as the 'nonglandular stomach' upon its first use since the term 'forestomach' is used 20 times before it is currently first mentioned as 'the nonglandular stomach' on page 50, line 28. Thus, the suggested change in reading should be "…inhalation exposure, nonglandular stomach (i.e., forestomach) damage following oral exposure. Then, it is a good idea to leave both terms on page 50, line 28 to less-informed readers.

RESPONSE: *The suggested revision was made to Section 1.2:*

… the most sensitive effects appear to be nasal lesions following inhalation exposure, nonglandular stomach (i.e., forestomach) damage following oral exposure, neurological effects, and cancer.

COMMENT 14: Page 2, Figure 1-1: Consider changing 'Glial cell astrocytomas' to 'Glial cell tumors' (or more accurately, microglial cell tumors). Consider adding a 'Concentration' level of 60-80 ppm since 'Chronic' exposures at this level of acrylonitrile induced 'Zymbal gland tumors in rats'.

RESPONSE: *The suggested revision to Figure 1-1 was made.*

COMMENT 15: Page 3, Figure 1-2: Under a 'Dose' of 31-40 mg/kg/day, consider changing 'Astrocytomas' to 'Glial cell tumors' (or more accurately, microglial cell tumors). Under the 'Dose' level of 1.1-10 mg/kg/day, consider changing 'Gliosis and perivascular cuffing in the brain' to read 'Gliosis and perivascular cuffing in the brain of rats'. Under the 'Dose' level of 1.1-10 mg/kg/day, consider adding 'Harderian gland tumors in mice' since 'Chronic' exposure to ≥ 2.5 mg/kg/day induced this cancer type in this species.

RESPONSE: *The suggested revision to Figure 2-1 was made.*

COMMENT 16: Page 4, line 7: Consider changing 'glial cell astrocytomas' to 'glial cell tumors' and add the citation for Kolenda-Roberts et al (2013) in addition to the Quast citations.

RESPONSE: *The suggested revision to Section 1.2 was made:*

Other neurological effects include decreased sensory nerve conduction velocity (Gagnaire et al. 1998) and glial cell tumors and perivascular cuffing in the brain (Quast 2002; Quast et al. 1980a).

COMMENT 17: Page 4, line 13: Change to read "In contrast, a number of chronic studies of acrylonitrile in rats have…".

RESPONSE: *The suggested revision was not made to Section 1.2, because NTP (2001) reported forestomach papilloma or carcinoma in mice.*

COMMENT 18: Page 4, line 14: Consider changing "including astrocytomas" to read "glial cell tumors" in the brain and spinal cord…".

RESPONSE: *The suggested revision to Section 1.2 was made:*

In contrast, a number of animal studies have consistently found increases in the incidence of several cancer types including glial cell tumors in the brain and spinal cord of rats (the study investigators categorized these tumors as astrocytomas; see Section 2.19 for additional details), Zymbal gland carcinomas in rats, and forestomach tumors in rats and mice.

COMMENT 19: Page 4, line 17: Add the following sentence for a more complete summary of 'Cancer Effects'. "In single chronic study in mice (Ghanayem et al 2002; NTP 2001), acrylonitrile-treated animals had increased incidences of Harderian gland adenomas and carcinomas and forestomach papillomas/carcinomas. Neoplasms of the ovary and lung in female mice may have been related to administration of acrylonitrile."

RESPONSE: *Section 1.2 is intended to provide a brief overview of the sensitive health effects. The suggested revision was not made because this level of detail would exceed that of the other sections in Section 1.2. ATSDR toxicological profiles are developed in accordance with ATSDR's "*Guidance for the Preparation of Toxicological Profiles."

COMMENT 20: Page 4, line 18: Consider adding a version of the following paragraph, as discussed above: "While there has been significant agreement in the morphologic appearance of microscopic brain

tumors from differing cancer bioassays in rats, several differential morphologic diagnoses (based upon standard histological findings) have been applied to brain and spinal cord tumors in acrylonitrile-treated rats, including microgliomas (Fed Reg 1978), astrocytomas (Quast et al 1980a, 1980b; Johannsen and Levinskas 2002a, 2002b), oligodendrogliomas (Maltoni et al 1988), and brain tumors-difficult to classify (Bigner et al 1986). Designating these brain tumors as astrocytomas also was based upon the observation that acrylonitrile-induced glial cell tumors closely resembled spontaneous brain tumors that occur at a low incidence in rats and are most often classified as 'astrocytomas' (Bigner et al 1986; Maltoni et al 1988; Quast 2002; Johannsen and Levinskas 2002b; Ward and Rice 1992 PMID 6953796). To further characterize the histogenesis, cellular phenotype, and appropriate classification of spontaneously occurring and acrylonitrile-induced glial cell tumors in the rat, Kolenda-Roberts et al (2013) used a panel of immunohistochemistry stains to distinguish between astrocytes, microglia, and oligodendrocytes. These immunohistochemical studies found no evidence of astrocytomas among the sets of spontaneous and acrylonitrile-induced brain tumors; on the contrary, oligodendrogliomas followed by microgliomas were more common as spontaneous tumors, while acrylonitrile-induced gliomas were microglial/histiocytic in origin."

RESPONSE: *ATSDR toxicological profiles are developed in accordance with ATSDR's "*Guidance for the Preparation of Toxicological Profiles*." The suggested addition exceeds the intended level of detail of Section 1.2. A statement was added to Section 1.2 that the study investigators categorized the tumors as astrocytomas and referring the reader to Section 2.19 for more information:*

> In contrast, a number of animal studies have consistently found increases in the incidence of several cancer types including glial cell tumors in the brain and spinal cord of rats (the study investigators categorized these tumors as astrocytomas; see Section 2.19 for additional details), Zymbal gland carcinomas in rats, and forestomach tumors in rats and mice.

COMMENT 21: Regarding Chapter 2 Charge Questions, For Questions 1 and 11, a number of additional publications related to the cancer effects and genotoxicity of acrylonitrile supporting related discussions above have been included in the Summary Report.

RESPONSE: *As noted in responses to other comments, additional publications were added to Sections 2.19 and 2.20.*

COMMENT 22: For the most part, this reviewer could not make suggestions for improving the considerations of issues of concern in Questions 2-9. Minor suggestions related to use of abbreviations associated with Question 9 were included below in association with page 8.

RESPONSE: *No response needed.*

COMMENT 23: For Question 10, the absence of possible mechanisms of action of acrylonitrile as a carcinogen were addressed and discussed above, with suggestions for inclusion of a short paragraph.

RESPONSE: *A discussion of possible mechanisms of acrylonitrile carcinogenicity was added to Section 2.19:*

> The mechanism of acrylonitrile carcinogenicity in rats and mice has not been fully elucidated. Kobets et al. (2022) suggested that multiple mechanisms are likely involved, but the mechanisms do not likely involve direct DNA damage. Likely mechanisms for brain and forestomach tumors are direct and indirect (due to oxidative damage) cytotoxicity and compensatory cell proliferation. Kobets et al. (2022) suggested that glutathione depletion in the brain and forestomach (and various

other tissues) is a critical initiating event. Glutathione depletion results in increases in the metabolism of acrylonitrile to 2-cyanoethylene oxide and cyanide. These metabolites, as well as acrylonitrile, could initiate pro-inflammatory signaling and sustained cell and tissue injury which could lead to compensatory cell proliferation, cell transformation, and neoplastic development (Kobets et al. 2022).

COMMENT 24: Page 8, line 34 versus page 9, line 5; Figures 2-2 and 2-3: It is recommended that the abbreviation for the classification of "serious LOAEL" be used preferably over "serious SLOAEL" to avoid the redundancy of the "S" in SLOAEL preceded by "serious" in the text and table. Columns in Tables 2-1 and 2-2 distinguish between Less serious "LOAEL" and "Serious LOAEL" while the keys to data in Figures 2-2 and 2-3 use "SLOAEL". Help the reader by using only "Serious LOAEL" in the text and by adding it to the key to the aforementioned figures in lieu of "SLOAEL"; thus, matching a parallel heading in Tables 2-1 and 2-2. Then, in the footnotes to Tables 2.1 and 2.2, the abbreviation of "SLOAEL" can be deleted. Likewise, under the column of "Effects", "Serious LOAEL" can be used in lieu of "SLOAEL" for studies for studies 9 and 12 (Dudley and Neal 1942),

RESPONSE: *The text in the Section 2.1 boilerplate was corrected:*

LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects (serious LOAELs) are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death).

*ATSDR toxicological profiles are developed in accordance with ATSDR's "*Guidance for the Preparation of Toxicological Profiles." *SLOAEL is a standard acronym in the toxicological profile, and the suggested revision was not made.*

COMMENT 25: Figure 2.1, page 11: If "members of the public" are intended for inclusion in the "principal audiences for the toxicological profiles" as stated in the Foreword (page iii, lines 34-35), then help the less-informed reader understand the pie graphs compared to the bar graphs by using different color codes. The color codes for the bar graphs are clear. However, determining that the color codes in the pie charts requires a little extra thought that can be avoided by using a different color scheme (e.g., different shades of gray to black) so that the reader does not think mistakenly, for instance, that 7% of Dermal exposure only occurs in animals and 46% of Oral exposure only occurs in humans (and which species are exposed by inhalation) in the upper pie chart.

RESPONSE: *ATSDR thanks the Reviewer for the suggestion, and will consider it in future updates of the guidance document for toxicological profiles (ATSDR's "*Guidance for the Preparation of Toxicological Profiles*").*

COMMENT 26: Tab1e 2.1, page 16, Study 19 (Quast et al 1980a): Consider changing 'glial cell astrocytomas' to 'glial cell tumors'.

RESPONSE: *The suggested revision was made to Table 2-1.*

COMMENT 27: Table 2.2, page 27, Study 6 (Friedman and Beliles 2002): Consider changing from 'astrocytomas' to 'glial cell tumors'

RESPONSE: *The suggested revision was made to Table 2-2.*

COMMENT 28: Table 2.2, page 31, Study 16 (Bigner et al 1986): Consider changing from 'brain tumor' to 'glial cell tumors'.

RESPONSE: *The suggested revision was made to Table 2-2.*

COMMENT 29: Table 2.2, page 33, Study 18 (Johannsen and Levinskas 2002b): Consider changing from 'brain and spinal astrocytomas' to 'brain and spinal glial cell tumors'.

RESPONSE: *The suggested revision was made to Table 2-2.*

COMMENT 30: Table 2.2, page 34, Study 19 (Johannsen and Levinskas 2002b): Consider changing from 'astrocytomas' to 'glial cell tumors'.

RESPONSE: *The suggested revision was made to Table 2-2.*

COMMENT 31: Table 2.2, page 35, Study 20 (Johannsen and Levinskas 2002a): Consider changing from 'brain, spinal cord' to 'brain and spinal cord glial cell tumors'.

RESPONSE: *The suggested revision was made to Table 2-2.*

COMMENT 32: Tab1e 2.2, page 36, Study 22 (Quast et al 2002): Consider changing 'brain glial cell astrocytomas' to 'glial cell tumors'.

RESPONSE: *The suggested revision was made to Table 2-2.*

COMMENT 33: Table 2.2, page 37, Study 23 (NTP 2001): Consider adding Ghanayem et al (2002 12075111) along with NTP 2001 as references, and then capitalize 'Harderian' as the gland that is named after Johann Jacob Harder, who first described the gland in 1694.

RESPONSE: *Table 2-2 was revised to include a note that the results of the chronic mouse NTP (2001) study was also published by Ghanayem et al. 2002 and to capitalize Harderian.*

COMMENT 34: Page 48, line 4: Change the sentence to read "No studies were located regarding body weights in humans following exposure to acrylonitrile". It is necessary to delete "or animals" from the original sentence given that the subsequent paragraph describes effects of acrylonitrile on body weights of laboratory animals.

RESPONSE: *The suggested revision was made to Section 2.3:*

No studies were located regarding body weight effects in humans following exposure to acrylonitrile.

COMMENT 35: Page 49, lines 22 and 23: Rearrange the sentence to read "…for 1 or 2 years in rats (Johannsen…) or 40 mg/kg for 14 weeks…in mice (NTP 2001).

RESPONSE: *The suggested revision was made to Section 2.4:*

> Histopathological evaluation of lung tissues showed no lung injury at doses up to 25 mg/kg/day for 1 or 2 years in rats (Johannsen and Levinskas 2002a, 2002b; NTP 2001; Quast 2002) or 40 mg/kg for 14 weeks or 20 mg/kg for 2 years in mice (NTP 2001).

COMMENT 36: Page 50, line 28: A request was made to indicate that the 'forestomach' is the 'nonglandular stomach' the first time 'forestomach' is used in the text.

RESPONSE: *The suggested revision was made to Section 1.2; see Response to Comment 13.*

COMMENT 37: Page 51, lines 1-3: Consider changing to read "… and mice (NTP 2001), respectively, following intermediate-duration exposure…and mice (Ghanayem et al 2002; NTP 2001), respectively, following chronic-duration exposure."

RESPONSE: *The results of the intermediate-duration NTP (2001) study was not reported in the Ghanayem et al. (2002) paper.*

COMMENT 38: Page 51, line 27: For completeness add 'hemoglobin content' to this sentence to read "… in decreases in hematocrit, hemoglobin content, mean cell volume…". After this sentence, add the following: "In addition, decreased total white blood counts and lymphocyte counts were observed in female mice exposed to 40 mg/kg for 14 weeks (NTP 2001)."

RESPONSE: *The suggested revision to the discussion of the Farooqui and Ahmed (1983) study in Section 2.7 was made:*

> A single gavage dose of 80 mg/kg resulted in decreases in hematocrit, mean cell hemoglobin concentration, mean cell volume, and platelet count in rats (Farooqui and Ahmed 1983).

A discussion of the alterations in leukocyte cell parameters was also added to Section 2.7:

> In addition to the alterations in red cell parameters, decreased lymphocyte counts were observed in male and female mice at administered 20 and 40 mg/kg, respectively, for 14 weeks and decreased total leukocyte counts were observed in females at 40 mg/kg (NTP 2001).

COMMENT 39: Page 53, line 2: To highlight the contrast in findings, change this sentence to read "…slightly lower liver weight (Gut et al 1984); whereas, increases in liver weight…"

RESPONSE: *The suggested revision was made to Section 2.9:*

> Alterations in liver weight have also been reported in some studies. Inhalation exposure of rats for 5 days to 129 ppm acrylonitrile resulted in slightly lower liver weight (Gut et al. 1984), whereas increases in liver weight were reported in rats following acute-duration oral exposure to 65 mg/kg/day (Murray et al. 1978) . . .

COMMENT 40: Page 53, line 34: Consider changing this sentence to read "This phenomenon is presumably…"

RESPONSE: *The suggested revision was made to Section 2.11:*

This phenomenon is presumably a direct irritant effect of acrylonitrile on the skin. In contrast, no signs of skin irritation were observed in humans following a 2-day patch test with 0.1% acrylonitrile (Kanerva et al. 1999).

COMMENT 41: Page 55, lines 15 and 16: It appears that the citation of Vogel and Kirkendall (1984) should be moved up after the preceding sentence, as in "a man accidentally sprayed with acrylonitrile (Vogel and Kirkendall, 1984). Dizziness, redness…".

RESPONSE: *The referenced sentence in Section 2.15 was revised:*

Signs of cyanide poisoning were exhibited by a man accidentally sprayed with acrylonitrile; dizziness, redness, nausea, vomiting, and hallucinations were reported (Vogel and Kirkendall 1984).

COMMENT 42: Page 56, line 13: Consider changing 'glial cell astrocytomas' to 'glial cell tumors'.

RESPONSE: *The suggested revision was made in Section 2.15:*

Chronic exposure resulted in glial cell tumors and perivascular cuffing in the brain of rats exposed to 80 ppm acrylonitrile via gavage 6 hours/day, 5 days/week for 2 years (Quast et al. 1980a)

COMMENT 43: Page 56, lines 15-16: Consider deleting the sentence reading "The gliosis appeared to be a pre-malignant lesion related to the formation of brain tumors" based upon the following: Bigner et al (1986) observed that 'gliosis' has been reported in brain sections of rats exposed to various neuro-oncogenic chemicals in long-term studies; however, gliosis is not regarded [by most experts in the field of experimental neuro-oncogenesis (see references in Bigner et al, 1986)] as a transitional stage in the development of gliomas.

RESPONSE: *The suggested revision was made to Section 2.15.*

COMMENT 44: Page 61, line 9: Consider changing 'glial cell astrocytomas' to 'glial cell tumors' … following oral exposure in rats.

RESPONSE: *The suggested revision was made to Section 2.19:*

Multiple studies have reported glial cell tumors in the brain and spinal cord, carcinomas in the Zymbal gland, and mammary gland following inhalation or oral exposure and forestomach papillomas/carcinomas following oral exposure.

COMMENT 45: Page 61, line 12: Change the sentence to read "NTP (2001) and Rice and Wilbourn (2000) noted …for other compounds such as 1,3-butadiene, vinyl chloride, benzene, glycidol, and ethylene oxide…".

RESPONSE: *ATSDR disagrees with the Reviewer that the Rice and Wilbourn (2000) study supports the referenced statement. No change was made based on this comment.*

COMMENT 46: Page 61, line 16: Consider adding the following paragraph is some edited form to the toxicological profile:

"The major types of cancers induced by acrylonitrile in animals are not likely to be of concern to humans beyond the fact that the chemical is a multi-site carcinogen in rats and mice exposed chronically to high concentrations of the agent. The evidence for the "human biological plausibility" for acrylonitrile-induced cancers is diminished by the nature of the chemically-induced rodent tumors including those of the Zymbal gland (rat), the Harderian gland (mouse), forestomach (rat and mouse), and the brain and spinal cord as malignant microgliomas. The Harderian gland is either absent or vestigial in primates (Payne 1994 PMID 7559104). The Zymbal gland is an auditory sebaceous gland of the external ear in the rat, while the ears of primates have an apocrine ceruminous gland. Likewise, humans lack a forestomach, or a region consisting of cornified, stratified squamous epithelium proximal to the glandular stomach in rodents. Last, immunohistochemical studies revealed that acrylonitrile-induced brain and spinal cord tumors in the rat are microglial/histiocytic in origin (Kolenda-Roberts et al 2013) and a significant portion of rare spontaneous brain tumors in control rats are microgliomas, suggesting a tendency for microglial cells to undergo malignant transformation in this species. In contrast, microgliomas are exceedingly rare in humans and are an unlikely target for acrylonitrile-induced carcinogenesis in people (Hulette 1996 PMID 8685234; Matthews et al. 2016 PMID 27191913)."

RESPONSE: *As noted in the Response to Comment 7, the available data are inadequate for ATSDR to evaluate the relevance of the animal carcinogenicity findings to humans.*

COMMENT 47: Table 2-7, page 65: Consider changing the putative diagnosis for brain tumors for each study referenced to 'Brain glial cell tumors' or 'Brain and spinal glial cell tumors' and then add a footnote "[a] Originally diagnosed as astrocytomas; now classified as malignant gliomas (Kolenda Roberts et al 2013) for each reference except Bigner et al (1996). For Bigner et al (1996) add a footnote "[b] Originally diagnosed as primary brain tumors; now classified as malignant gliomas (Kolenda Roberts et al 2013). Change original footnote "[a]" to footnote "[c]".

RESPONSE: *The suggested revisions were made to Table 2-7.*

COMMENT 48: Page 66, line 7: Consider extending this paragraph by adding the following: "Increases in gene mutations were observed in studies in Drosophila and in rats and mice exposed *in vivo* to acrylonitrile (Walker et al 2020a, 2020b). In contrast to the positive studies of gene mutations, most studies assessing chromosome level mutations arising in somatic cells *in vivo* in mice or rats administered acrylonitrile by a variety of routes have yielded negative results (reviewed in Albertini et al 2023). Thus, there is a disparity between the lack of clastogenic effects of acrylonitrile *in vivo* and the more numerous positive *in vitro* studies listed in Table 2-8."

RESPONSE: *The results of the Walker et al. (2020a, 2020b) studies were added to the text in Section 2.20:*

Increases in gene mutations were observed in *in vivo* studies in rats, mice, and *Drosophila*.

The Albertini et al. (2023) paper was added to Section 2.20:

The genotoxicity of acrylonitrile has been extensively studied in *in vitro* (Table 2-7) and *in vivo* (Table 2-8) studies and reviewed by Albertini et al. (2023). Mixed results have been found in studies of bacterial and mammalian system *in vitro* assays when tested with or without metabolic activation. Increases in gene mutations were observed in *in vivo* studies in rats, mice, and *Drosophila*. In contrast, most studies assessing chromosome level mutations arising in somatic cells *in vivo* in mice or rats administered acrylonitrile by a variety of routes have yielded negative results.

COMMENT 49: Table 2-9, page 69: Immediately above the heading "Non-mammalian systems" add two final entries under "Mammalian Species" as follows:

Species (exposure route)	Endpoint	Results	Reference
Rat t-lymphocytes (oral)	Gene mutations	+	Walker et al 2020a
Wild-type mouse T-lymphocytes (oral)	Gene mutations	+	Walker et al 2020b
CYP2E1-null mouse T-lymphocytes (oral)	Gene mutations	(+)	Walker et al 2020b

RESPONSE: *The Walker et al. (2020a, 2020b) studies were added to Table 2-8 (formerly Table 2-9).*

COMMENT 50: Page 70, after line 2: As discussed above consider adding some form of the following the two paragraphs as the final entries under 2.20 GENOTOXICITY:

"Dose-related increases in the frequencies of *Hprt* mutations in T lymphocytes were found in rats, conventional mice, and *lacZ* transgenic mice exposed to acrylonitrile (Walker et al 2020a, 2020b). The spectra of mutations in acrylonitrile-treated rats overlapped significantly with those previously reported for 1,3-butadiene-exposed rats, suggesting a shared mutagenic mechanism. In CYP2E1-null mice, devoid of cytochrome P450 2E1 that yields the epoxide intermediate cyanoethylene oxide, *Hprt* mutant frequencies were significantly increased only at a high dose of 60 mg/kg acrylonitrile that is lethal to wild-type mice. Exposures of wild-type and CYP2E1-null mice to acrylonitrile produced no elevations in micronucleated erythrocytes, but induced significant dose-related increases in DNA damage, detected by the alkaline (pH > 13) Comet assay, in one target tissue (forestomach) and one nontarget tissue (liver) of wild-type mice only. These combined studies indicated that metabolism of acrylonitrile to cyanoethylene oxide is central to DNA damage and mutation induction *in vivo*, but acrylonitrile itself may also contribute to its mutagenicity/carcinogenicity via mechanisms involving direct and/or indirect DNA reactivity."

"Two groups (Albertini et al 2023; Kobets et al 2022) have recently reviewed the genotoxicity profile and other databases to identify key events that potentially play a role in oncogenesis in rodents, including in part direct-DNA reactivity, oxidative stress and oxidative DNA damage, and/or epigenetic changes induced by acrylonitrile and its metabolites. Differences in opinion by these two reviews about general processes (also known to occur in humans) reflects the ongoing and unsettled debate over the key characteristics of acrylonitrile as rodent carcinogen and their relevance to humans. However, the current data do not allow unequivocal determination of acrylonitrile's mode of action(s) as an animal carcinogen (Albertini et al 2023; Haber and Patterson 2005; Kobets et al 2022)."

RESPONSE: *The suggested text insert was not added to the profile since it exceeds the level of detail of the rest of Section 2.20. This is in accordance with ATSDR's "Guidance for the Preparation of Toxicological Profiles." The results of the Walker et al. (2020a, 2020b) studies were added to Table 2-8 (formerly Table 2-9).*

COMMENT 51: This reviewer thought that the section related to toxicokinetics was well-written and organized, and has no concerns about the issues in Questions 1-3 under *Toxicokinetics*. The same is true for the questions under sections entitled *Children and Other Populations that are Unusually Susceptible* or *Interactions with Other Chemicals*. However, this reviewer had comments and suggested additions to address questions in the section entitled *Biomarkers or Exposure and Effect*.

RESPONSE: *No response needed; see Comments 52 and 53 for the comments on the Biomarkers of Exposure and Effect sections.*

COMMENT 52: Page 83, line 3: Consider adding the following sentence to the end of this paragraph: "In rats exposed sub-chronically (105 days) to acrylonitrile, the relationship between levels of N-(2-cyanoethylene)valine and water concentration was linear to concentrations of 10 ppm but increased sublinearly at higher concentrations (Osterman-Golkar et al 1986)."

RESPONSE: *The results of the Osterman-Golkar et al. (1994) study was added to Section 3.3.1:*

> In a study in rats exposed to various doses of acrylonitrile (3–300 ppm) in drinking water for 105 days, a dose-related increase in N-(2-cyanoethyl)valine levels were found (Osterman-Golkar et al. 1994). At doses of 0.74 mg acrylonitrile/kg (10 ppm in drinking water) and lower, there was a linear relationship between dose and hemoglobin adduct levels. A sublinear relationship, indicative of saturation, was observed at higher doses.

COMMENT 53: Page 83, line 7: Consider adding a highly pertinent reference to the first sentence of this section (3.3.2 Biomarkers of Effect):
"A variety of effects have been demonstrated following acrylonitrile exposure in humans and animals (**Albertini et al 2023**)".

RESPONSE: *The Albertini et al. (2023) citation was not added to Section 3.3.2; this review of the genotoxicity only briefly discusses the non-genotoxic health effects of acrylonitrile.*

COMMENT 54: Regarding Chapters 4-7, the Reviewer commented: This reviewer had no constructive criticisms to offer for these chapters, except to fill data gaps by bringing attention to publications that were overlooked or missed in generation of the database. Where missing, copies of relevant reports have been included with this Summary Report.

RESPONSE: *No response needed.*

COMMENT 55: Page A-16, line 17: Consider changing "glial cell astrocytomas" to "glial cell tumors".

RESPONSE: *The suggested revision was made to the MRL worksheet for the chronic-duration inhalation MRL in Appendix A:*

> Quast et al. (1980a) reported death and glial cell tumors at the lowest concentration tested (20 ppm) and decreased body weight, nasal mucosal irritation, and focal gliosis at 80 ppm.

COMMENT 56: Page A-32, line 14: Consider changing "brain glial cell astrocytoma" and "spinal cord glial cell astrocytoma" to read "brain glial cell tumor" and "spinal cord glial cell tumor", respectively.

RESPONSE: *The suggested revision was made to the MRL worksheet for the chronic-duration oral MRL in Appendix A:*

At 8.0/10.7 mg/kg/day, significant increases in the incidence of brain glial cell tumors (females only), spinal cord glial cell tumors (not examined in males), Zymbal's gland carcinoma, and forestomach squamous cell papilloma/papilloma (females only).

Annotated Comments on the Toxicological Profile

The Reviewer provided annotated comments on the toxicological profile which were the same as Comments 8–56.

Additional Comments Submitted by the Reviewer

After the initial submission, the Reviewer submitted an additional comment.

COMMENT 57: In my review of the draft Toxicology Profile for Acrylonitrile that I completed in January, I made numerous but significant references to an upcoming review concerning the potential mode of action of acrylonitrile as a carcinogen. Yesterday, this review by Richard Alberitini et al appeared as an open assess PDF at the on-line site for the journal Critical Reviews in Toxciology. The journal name is quite appropriate in this case because this nearly 50-page article should be given close attention by the scientists who wrote the draft Toxicology Profile for Acrylonitrile. As I pointed out in my review, the profile wisely does not attempt in any detail to address the basic mechanisms underlying the actions of acrylonitrile as a rodent carcinogen. However, it is important for the profile to reference this review by Albertini et al where appropriate (as suggested for specific places in the profile in my review) and to refer readers to two reviews with differing points of view on mechanisms of acrylonitrile's carcinogenicity expressed by Albertini et al versus Kobets et al (Acrylonitrile induction of rodent neoplasia: Potential mechanism of action and relevance to humans; 2022).

RESPONSE: *The Albertini et al. (2023) paper was added to the profile; see the Responses to Comments 5 and 6.*