

**Disposition of Peer Review Comments For
Two Unpublished Studies or Toxicological Profile For Acrylonitrile**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Peer reviewers for two unpublished studies added to the post public comment draft of the Toxicological Profile for Acrylonitrile were:

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Comments provided by Peer Reviewer #1

Reviewer Comments on Unpublished Studies

Kiplinger 2005

Kiplinger GR. 2005. Acute inhalation toxicity study of acrylonitrile in albino rats. Innovene. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8(e). WIL-54200. OTS0601033. 8EHQ-0805-16067. <https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0601033.xhtml>. October 3, 2024.

COMMENT 1: This report details an acute inhalation toxicity study of acrylonitrile in male and female rats using a nose-only exposure method. The study was conducted in 2005 at WIL Research Laboratories, a well-established Contract Research Organization (CRO) recognized for its extensive experience with inhalation studies in rodents. It is stated that the study was conducted “in general compliance with [OECD Test Guideline 403],” although the specific version of TG 403 followed is not mentioned. Based on the study's design, it likely adhered to the original 1981 version of TG 403, predating revisions introduced in 2009 and 2024. These later updates included significant procedural changes, such as recommending nose-only exposure, reducing animal use via sighting studies, and adopting a step-wise study design.

This study utilized the now-preferred nose-only exposure method (the 1981 guideline included whole-body exposures), but it did not incorporate a sighting study or step-wise dose escalation – both features of OECD TG403 since 2009. While these omissions do not detract from the overall quality of the study, they should be acknowledged. Other aspects, including animal selection, group size, exposure duration, procedures, concentration range, and number of dose groups, were appropriate for determining the LC50 as intended. The exposure apparatus was state-of-the-art at the time of the study, and the staff were undoubtedly well-versed in its operation.

The study's reporting aligns with GLP compliance, offering comprehensive data tables and detailed documentation of exposure characterization. However, there is no mention of approval by an appropriate animal care and use committee.

In summary, the report provides sufficient information, and the derived LC50 value appears to have been calculated in accordance with the test guideline procedures in effect at the time of the study.

RESPONSE: *ATSDR thanks the Reviewer for their comments. The Reviewer agreed with study conclusions; therefore, no response is needed.*

Wang et al. 1995

Wang Z, Zhilan L, Wei X. 1995. Study on acrylonitrile to teratogenesis of rats sperm. AN Group, Inc. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8(e). OTS0559911. 89000000313. 8EHQ-0900-14711. <https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0559911.xhtml>. October 3, 2024.

COMMENT 2: The document is an English translation of a report originally published by Wang et al. (1995) in China. The study investigates the time- and dose-dependent effects of acrylonitrile exposure (60, 90, or 120 mg/m³) via inhalation for up to 28 days (2 hours/day, 6 days/week). The 28-day exposure group included both negative and positive controls, while the 7- and 14-day exposure groups lacked time-matched controls. The methods section provides limited detail, making it difficult to fully understand the

study design and treatment protocols. Notably, the species used in the study is unclear. Although both rats and mice are mentioned, the average listed animal weight (~24.5 g) and reference to Kunming mice—a common outbred strain used in Chinese research—suggest that mice were likely used. There is no information indicating whether the study adhered to animal welfare standards or received ethical oversight. The rationale for selecting specific acrylonitrile concentrations is also not provided.

The study aimed to include 12 animals per group; however, 7 animals did not survive until the end. Mortalities included 2 animals in the positive control (cyclophosphamide) group, 2 in the 60 mg/m³ group (28-day exposure), 2 in the 120 mg/m³ group (7-day exposure), and 1 in the 120 mg/m³ group (14-day exposure). Although the authors reported that the animals exhibited "no obvious intoxication," no cause of death was offered. Additionally, body weight data during exposure periods are absent.

The methodology for sperm counts and abnormality assessments is poorly described. The study states that "500 sperm with good dispersion" were examined, presumably per mouse, as the data tables indicate 5,000–6,000 sperm were analyzed per group. This number is relatively low compared to typical studies of chromosomal abnormalities in rodent spermatozoa, which often examine significantly larger samples. Statistical analyses are inadequately explained, with no description of tests performed or standard deviations included in the data tables.

Despite these reporting limitations and the uncertainty surrounding the accuracy of the English translation, the study does have utility. Its design includes both time- and concentration-response evaluations, which is a positive feature. The data tables provide sufficient information to conclude that acrylonitrile exposures at 60 mg/m³ or higher, for 28 days, significantly increase sperm abnormalities. However, the specific types of abnormalities are difficult to determine based on the available information. The inclusion of both positive and negative controls for the 28-day exposure group lends credibility to these findings.

Overall, this study can be used to provide supporting evidence of the effects of acrylonitrile exposure on sperm abnormalities. However, due to its methodological limitations and incomplete reporting, it is unsuitable for dose-response analysis.

RESPONSE: *ATSDR thanks the Reviewer for their comments. The Reviewer agreed with study conclusions; therefore, no response is needed.*

Comments provided by Peer Reviewer #2

Reviewer Comments on Unpublished Studies

Kiplinger 2005

Kiplinger GR. 2005. Acute inhalation toxicity study of acrylonitrile in albino rats. Innovene. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8(e). WIL-54200. OTS0601033. 8EHQ-0805-16067. <https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0601033.xhtml>. October 3, 2024.

COMMENT 1: A review of the quality and relevance of the findings in this unpublished study depends heavily upon an understanding of the current guidelines and protocols established in the U.S. and other developed countries for performing an acute inhalation toxicity (single-dose) study to determine acute toxic manifestations, gross behavior, and the median lethal concentration (LC50) of an environmental exposure resulting in 50% mortality in the tested groups of experimental animals, which are preferably young adult rats for inhalation tests. In brief, the classic LC50 study is designed for exposure of rodents for a recommended 4 hours (or up to 24 hours) to test an environmental chemical or pharmaceutical agent in increasing concentration levels, **with a control group and at least four groups receiving one concentration per group**. Each control and treatment group has at least five animals of each sex, including nulliparous and non-pregnant females. Animals are observed for a relatively standardized set of clinical signs or death during the exposure period. After the exposure, each test animal should be the sole occupant of a cage and be observed for 1-2 hours and thereafter at regular intervals for clinical signs or death over a subsequent observation period of 14 days. Surviving animals are weighed before initiation of exposure, at the end of the exposure period (or 24 hours later or on Day 1 post exposure), at intervals during the observation period, and before scheduled necropsy for observing and recording gross pathological changes. The LC50 data represent the exposure level yielding 50% mortality but the value may not reflect the acute toxic properties of a chemical/xenobiotic and may not correlate well with known mechanisms of action of a toxic agent.

COMMENT 2: A brief evaluation using the following questions as prompts:

- Did the study use an adequate number of animals and practice good animal care?
- Did the study account for competing causes of death?
- Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

The “Acute Inhalation Toxicology Study of Acrylonitrile in Albino Rats” used a standard protocol that was conducted in compliance with FDA guidelines, GLP regulations/standards, the OECD guidelines, the standard operating procedures of the contract laboratory performing the study, and within the options proposed by the sponsor for the study to determine the LC50 in SD rats, but the final protocol had several shortcomings.

First, while each treatment group had an adequate number of male and female rats (5 per sex for each acrylonitrile exposure level) to establish a defined LC50, the contract laboratory opted to include a fifth exposure level in lieu of incorporating a sham-exposed control group (see page 163 of 170). The lack of sham-exposed control groups of male and female rats undoubtedly reduced the overall quality and quantity of possible assessments of the acute toxic manifestations and gross behavior of animals exposed to differing levels of acrylonitrile. 2

Second, there is a mismatch between the age and body weight information for young adult male and female SD rats given in the study report (page 17 and Tables 3). A narrower range in body weights in young adult rats than the ~8-12 week-old range noted in the study report (page 17) is preferable for establishing a uniform distribution of similar sized animals for each control and treatment group in an LC50 study. Based upon growth charts from Charles River Labs as the supplier of the SD rats, and the experience of this reviewer, it appears that in this study the age range for male rats was indeed narrower at 8-10 weeks of age. However, the range in weights of female SD rats at 242 g to 264 g suggests that the females were likely older at 11-13 weeks of age, slightly complicating the assessment of age on the toxic responses to acrylonitrile exposure in females versus males. Nonetheless, all male and female rats in these age groups had reached sexual maturity and their growth rates were beginning to slow.

Third, while the report provides evidence of the use of excellent methods for characterizing the acrylonitrile aerosol exposure levels, it would have been helpful if the report had included a basis for the range in and odd numbers for the selected exposure concentrations from 539 to 1181 ppm acrylonitrile. Preliminary range-finding experiments are often initiated to minimize the extent of no lethality or 100% lethality, thus reducing the number of groups in the total study and improving the precision of the LC50 determination. Ultimately, the study did include a sufficient number of dose groups but used a sham-exposed control group in lieu of the highest exposure level and still determined well defined LC50 values for acrylonitrile in both male and female rats using the selected range of exposure levels between 539 and 1006 ppm acrylonitrile.

Fourth, the report lacks a list of clinical observations during the 4-hour acrylonitrile exposures and the lists of possible clinical signs immediately following exposure and during the 14-day post-exposure period appear to be incomplete based upon the experience of this reviewer as a veterinary pathologist. The key personnel who appear to have made clinical observations in this study report are either a trained laboratory animal technician (LAT, 2-year associate degree) and/or PhD scientists/toxicologists but with no veterinarian involved in the study (pages 160-162). This reviewer has experience training laboratory technicians and other scientists in performing clinical observations during acute, subacute, and chronic animal toxicity and/or carcinogenicity studies, and there is a high possibility that the list of clinical signs that were considered during the observation periods were incomplete. Additional possible clinical signs include in part increased motor activity, arching and rolling, clonic convulsions, salivation, lacrimation, ptosis, muscle spasms, opisthotonus, writhing, hyperesthesia, blanching, and cyanosis. It is uncertain if all or some of these clinical signs were considered but not seen, but some have been observed by this reviewer during subacute exposures of rats to acrylonitrile in drinking water. Thus, it is possible that some acute toxic manifestations and gross behavior of the rats to acrylonitrile were overlooked.

Last, it is critical to note that in the absence of a sham-exposed control group of male and female rats, it is very possible for a trained laboratory technician (LAT) and other trained personnel to not recognize or overlook subtle gross pathological changes at necropsy of animals in an acute toxicity study. While a veterinary pathologist is often involved in the gross and histopathological analysis of tissues from rats (as well as other animal species) in subacute (e.g., 4- or 13-week) and chronic (2-year or life-time) toxicity studies, this is typically not the case with acute toxicity studies to define the 50% lethal dose of a chemical/drug. As one example, since tissues are not weighed during necropsy after an acute toxicity study, it is quite difficult to judge possible swelling or shrinkage of tissues without a sham-exposed control group. Based upon previous experience of this reviewer in finding gross toxicological lesions in rats and mice following subacute exposure to lower concentrations of acrylonitrile versus control groups,

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it is highly likely that subtle gross lesions could have been found in one or more acrylonitrile exposure groups if a control group had been available for comparisons.

While the study did not consider any alternative cause of death other than the acute toxic effects of inhaled acrylonitrile, the ‘Mortality’ finding reported under section 5.2 (page 20) are consistent with the acute exposures being responsible for the dose-related deaths observed during the 4 hour nose-only exposures or withing 2 days post-exposure.

COMMENT 3: For each of the unpublished studies, prepare a brief evaluation using the following questions as prompts:

- If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.
- Do you agree with the conclusions of the author? If not, please explain.

While the “Acute Inhalation Toxicology Study of Acrylonitrile in Albino Rats” was not adequately designed to allow robust comparisons between a sham-exposed control group and the potential acute toxic manifestations and gross behavior of rats exposed nose-only to differing acrylonitrile concentrations, the study design using five treatment levels of acrylonitrile with no control group does not negate the utility of the study. Based upon the well-executed portion of the study characterizing the exposure atmospheres of acrylonitrile, this reviewer agree with the conclusion that the LC50 was 946 ppm (866-1032 ppm, 95% confidence limits) when male and female SD rats were exposed to a nose-only vapor of acrylonitrile for a single, 4-hour exposure period.

RESPONSE: *ATSDR thanks the Reviewer for their comments. The Reviewer agreed with study conclusions; therefore, no response is needed.*

Wang et al. 1995

Wang Z, Zhilan L, Wei X. 1995. Study on acrylonitrile to teratogenesis of rats sperm. AN Group, Inc. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8(e). OTS0559911. 89000000313. 8EHQ-0900-14711.

<https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0559911.xhtml>. October 3, 2024.

COMMENT 4: An immediate understanding of the quality and meaning of the findings in this unpublished study is hindered by a poor translation of a Chinese vernacular into formal English that often fails to use language typically describing the Materials and methods, Results, and Discussion/Summary associated with an inhalation study of a toxic chemical. Several salient examples are given below where a re-interpretation of the English improves the understanding and evaluation of the study.

First, the study title and text appear to misinform the reader as to the species used in this evaluation of the effects of acrylonitrile inhalation on teratogenicity in sperm of rodents. As a veterinary pathologist, this reviewer has no knowledge of a rat strain exhibiting an average weight of 24.47 ± 3.46 g at 7 weeks-of-age purportedly used in this study. Likewise, there appears to no history of previous use of male rats of “Kunming type” in research studies. On the other hand, this reviewer is familiar with published reproductive toxicity of environmental chemicals using male Kunming mice. For example, male Kunming mice weighing $23.2 \pm$ g at 6 weeks-of-age were used in a report of the reproductive toxicity study of the effects of cantharidin in mouse testis (Xiao et al. Cantharidin-induced toxic injury, oxidative stress, and autophagy attenuated by Astragalus polysaccharides in mouse testis. *Reprod. Toxicol.* 2024 Jan;123:108520. doi: 10.1016/j.reprotox.2023.108520. PMID: 38056682. (See attached PDF) The ~1 g smaller body weight in male Kunming mice in the Xiao et al. study compared to the male “Kunming type” rodents used in the Wang et al. study provides strong presumptive evidence that 7-week-old male Kunming mice were exposed by inhalation to test the teratogenesis of acrylonitrile in ‘mouse’ sperm. The following review is based upon the premise that there was an unfortunate mistranslation and the following

review is based upon animal weights providing proof that male mice were exposed to acrylonitrile. However, it is concerning that ‘educated guesses’ have been made about issues critical to this work, where the species of animal is just one example. Other findings would not be affected by this process. As indicated below, the Wang et al. study evaluated “the significance of the testicular effects on reproductive capability in mice ... and would be very valuable” (as noted on page 115 in *Toxicological Profile of Acrylonitrile*) for inclusion in a brief review (on page 56, Section 2.16) and summary (page 115, Section 6.2) of Reproductive Toxicity in the profile. Since there was an expectation that the Wang et al. reproductive toxicology study of acrylonitrile was performed in rats, and the *Toxicological Profile of Acrylonitrile* notes on page 115 that “No effects on male reproductive organs have been reported in rats”, this reviewer suggests that the profile be revised [pages 56 (Section 2.16) and 115 (Section 6.2)] to include brief summaries of two published reports of significant toxicological effects in sperm and testis in male Sprague-Dawley rats treated by gavage with 46 mg acrylonitrile/kg for 4 weeks (6 days/week) or 50 mg acrylonitrile/kg for 12 weeks (6 days/week). These reports provide significant evidence of acrylonitrile-induced sperm and testis injury in rats along with some persuasive evidence of a mechanistic basis for the reproductive toxicity in male rats exposed subchronically to relatively high concentrations of acrylonitrile. 2

These studies are:

1. Dang et al. Protective effects of apigenin against acrylonitrile-induced subchronic sperm injury in rats. *Food Chem. Toxicol.* 2017 Nov;109(Pt 1):517-525. doi: 10.1016/j.fct.2017.09.025. PMID: 28963002. (See attached PDF)
2. Shi et al. Protection of apigenin against acrylonitrile-induced sperm and testis injury in rats: involvement of activation of ASK1-JNK/p38 signaling pathway. *Toxicol. Res. (Camb)*. 2021 Mar 11;10(2):159-168. doi: 10.1093/toxres/tfab017. PMID: 33884167. (See attached PDF)

Second, the translation of the Wang et al. study described the acrylonitrile treatment groups as receiving different amounts of acrylonitrile “contamination” instead of the usual description of groups of mice receiving inhalation exposures to acrylonitrile. Notably, the text did not indicate whether the inhaled acrylonitrile vapors occurred via nose-only inhalation or whole-body exposures. However, this oversight does not negatively impact interpretation of data based upon different inhalation exposure levels of 60, 90, or 120 mg/m³ (or ~28, 40, or 50 ppm) for 28 days (2 hours/day, 6 days/week) or to the high-level exposure for 7, 14, or 28 days (2 hours/day, 6 days/week).

Third, the use of 50 mg/kg cyclophosphamide as a positive control is misleading when the route of exposure was described as injections into the ‘stomach’ once a day for two consecutive days.

Cyclophosphamide was surely injected intraperitoneally as a standard practice.

Fourth, the use of the “Cecity method” for animal observations apparently meant that observations of animals during the treatment period was carried out in a blinded fashion in terms of the treatment group.

Last, the use of the terms “sperm aberration rates” and “sperm distortion” were inferred as meaning “sperm abnormality rates” and “sperm deformity”, respectively.

RESPONSE: The Dang et al. (2017) and Shi et al. (2021) reproductive toxicity studies have been added to Section 2.16 of the toxicological profile:

Decreases in sperm motility and concentration and increases in sperm morphological alterations were observed in rats administered 20 mg/kg/day, 6 days/week for 12 weeks (Dang et al. 2017) or 46 mg/kg/day, 6 days/week for 28 days (Shi et al. 2021).

Section 6.2 has also been revised to indicate that studies examining sperm parameters in rats have been conducted:

Studies in male rats and mice have shown that exposure to acrylonitrile results in increases in sperm aberrations, decreases in sperm motility and concentrations, and increases in sperm head and tail morphological alterations.

COMMENT 5: A brief evaluation using the following questions as prompts:

- Did the study use an adequate number of animals and practice good animal care?
- Did the study account for competing causes of death?

The brief descriptions in the ‘Material and method’ of the animal management, assignment to treatment groups, application of the experimental design, and observation and sacrifice of mice suggest that the study was conducted according to planned and adequate protocol. The highly significant sperm abnormality rate for each of the three acrylonitrile exposure levels in groups of mice exposed for 28 days compared to control mice (Table 1) suggests that the selected starting sample size of 12/group and 10-12/group at the end of exposure was more than sufficient to detect a toxic effect in testis. This conclusion is supported by (i) the P -values <0.005 for each acrylonitrile exposure level even though there were relatively small differences in the average rates of abnormality between the 60, 90, and 120 mg/m³ exposure groups and (ii) the observation of a highly positive correlation between increasing acrylonitrile exposure levels and higher rates of sperm abnormality. The authors reported a significant correlation coefficient of 0.9712 while a reanalysis by this reviewer using Excel yielded a correlation coefficient of 0.9857 for observed sperm abnormality rates and a correlation coefficient of 0.9333 for the induced rates above background (i.e., the observed abnormality rate minus the control mouse rate for each acrylonitrile exposure level). The study did not provide an accounting for the reduction in group size from 12 to 10 mice/group in the cyclophosphamide positive control group and the 60 mg acrylonitrile/m³ inhalation exposure group (Table 1). Likewise, the study did account for the reduction in animal numbers by 1 or 2 mice in 3 of 5 treatment groups in the time course study of mice exposed to sham exposure control air, cyclophosphamide, or 120 mg/m³ acrylonitrile for 7, 14, or 28 days (6 days/week) (Table 3). An additional shortcoming of this time-course study is that (based upon the total number of groups used in the overall study, i.e., $8 \times 12 = 96$ mice) there were no sham-exposed control mice for comparison to mice exposed to 120 mg/m³ acrylonitrile for 7 or 14 days (matched controls were only necropsied along with mice exposed for 28 days).

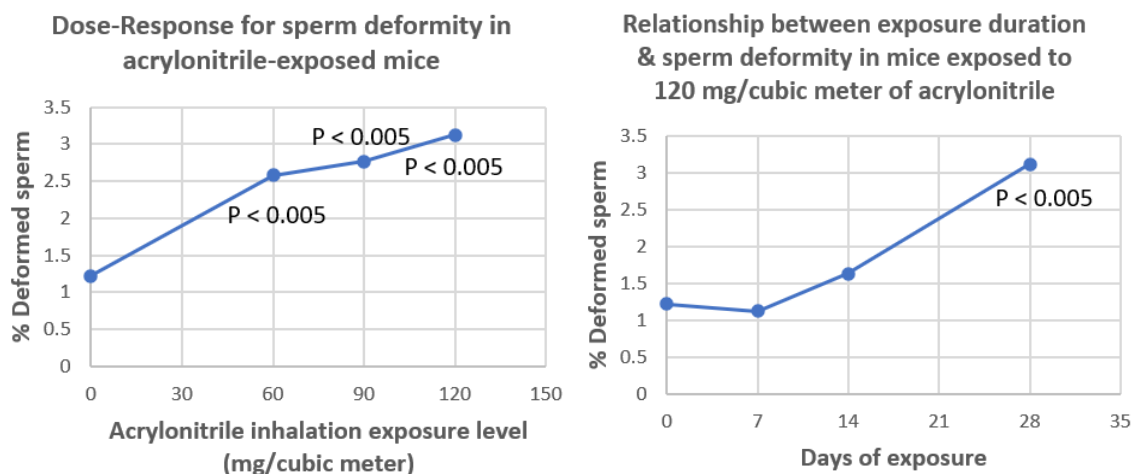
COMMENT 6: A brief evaluation using the following questions as prompts:

- Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?
- If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

The study had a sufficient number of dose groups by using three inhalation exposure levels compared to sham-exposed controls; however, the range in the exposure levels at roughly 28, 42, and 55 ppm acrylonitrile (i) were not sufficiently spread apart, the magnitude of the high dose was too low, (ii) the inhalation exposure duration at 2 hours/day was too brief, and (iii) the study duration for examining the toxic effects of differing exposure levels after 28 days was too short. The study design would have generated superior dose-response data (Table 1), time course data for the high-dose exposure level (Table 3), and comparisons of the proportions of specific abnormalities in sperm morphology (Table 2) had the investigators mimicked selected parameters in several published studies (listed above) using an ‘intermediate’ exposure duration (e.g., 13 weeks or greater), at a wider range of acrylonitrile concentrations previously used to evaluate (in part) reproductive toxicity of inhaled acrylonitrile (e.g., 0,

20, 40, and 80 ppm or 0, 25, 50, and 100 ppm) in rats for a longer exposure period per day (e.g., 6 or 8 hours).

Despite the limitations in the study design, the applied protocol provided useful evidence that induction of sperm abnormalities/alterations by inhaled acrylonitrile is dependent upon the dose delivered, as determined by both duration of exposure and ambient concentration. A plot of the dose-response relationship between exposure to 0, 60, 90, or 120 mg/m³ for 28 days (2 hours/day, 6 days/week) and the percentage of sperm deformity clearly illustrates the impact of the ambient exposure level upon the dose of acrylonitrile delivered to mice. Likewise, a plot of the relationship between duration of acrylonitrile exposure and the percentage of sperm deformity plainly shows the influence of exposure duration upon the dose of acrylonitrile delivered to mice. These following graphs were made by this reviewer.



In contrast, the duration of exposure to the selected levels of ambient acrylonitrile in mice exposed for 28 days (2 hours/day, 6 days/week) was not sufficient to permit detection of treatment related changes in sperm morphology (Table 3). In summary, the weaknesses in the study design do not negate the utility of significant findings (i) in the dose-response experiment showing a significant correlation between 28 days of exposure and sperm abnormalities over a narrow range of acrylonitrile concentrations and (ii) in the time-course experiment illustrating that exposures greater than 14 and up to 28 days at 120 mg/m³ acrylonitrile induces in sperm abnormalities.

COMMENT 7: For each of the unpublished studies, prepare a brief evaluation using the following questions as prompts:

- Do you agree with the conclusions of the author? If not, please explain.

The authors' conclusions in the Discussion and Summary are generally supportable with few exceptions. An important conclusion is that the overall findings show that induction of sperm abnormalities by inhalation exposure of mice to acrylonitrile is indeed dependent upon both "time" (or duration) and "dosage" (as a substitute for concentration/level) when applying different 'doses' (concentration x time). While the study protocol had weaknesses, the research did show that "in a short period of time inhalation of low" concentrations/levels "can cause sperm aberration rates to increase".

The chief area of disagreement with the authors concerns the conclusions in the paragraph following Table 3 in the Results. The data in Table 3 and the second graph above clearly show that there was a

significant relationship between the duration of acrylonitrile exposure at 120 mg/m³, with a significant effect observed after 28 days of exposure but not after 7 or 14 days of exposure. Therefore, the last sentence in this paragraph (i.e., “Therefore, this does not show any significant difference.”) is either a misinterpretation of the data or a mis-translation of the Chinese to English.

RESPONSE: *ATSDR thanks the Reviewer for their comments. The Reviewer agreed with study conclusions; therefore, no response is needed.*

Comments Provided by Peer Reviewer #3

Reviewer Comments on Unpublished Studies

Kiplinger 2005

Kiplinger GR. 2005. Acute inhalation toxicity study of acrylonitrile in albino rats. Innovene. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8(e). WIL-54200. OTS0601033. 8EHQ-0805-16067. <https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0601033.xhtml>. October 3, 2024.

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT 1: The study included both sexes in its determinations, but the sample sizes of n=5 seem quite minimal for the endpoints that were measured in the study, since changes would always be 20% or greater within a sex. This small sample size may be the reason why data were not analyzed separately by sex, but observation of the raw numbers suggests a potential earlier vulnerability of females; since females weighed less than males, this could mean that they were effectively exposed to a higher concentration than males, but it is not possible to determine if this is the case. Animal care is well described as are in general the other methods that were used in the study.

QUESTION: Did the study account for competing causes of death?

COMMENT 2: Mortality was one of the endpoints in the study, but no specific causes of death were investigated. There were clinical observations made including statements of gas-filled stomach and of dark red discoloration of the lungs, but no other macroscopic findings. Hence the study cannot contribute any information as to the actual basis of mortality that was seen. As per above, it would be useful to know whether mortality ever occurred with a 0 ppm exposure just from the procedure itself.

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT 3: The study did include 5 different exposure concentrations of acrylonitrile, but there does not appear to have been a 0 ppm control group included in the study. Thus all comparisons occurred in animals that had exposure, and this is also the data from which an LC₅₀ value was calculated. In addition, this makes the clinical observation data collected immediately following exposures more difficult to interpret.

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT 4: While the study design here is clearly limited, both in terms of sample sizes and the absence of a 0 ppm control, its data on clinical observations at day 14 as well as the overall mortality data is still useful given that two concentrations (539 and 775 ppm) show no mortality and mortality levels do increase with subsequent increases in exposures such that the LC₅₀ value is likely accurate within the range of these exposure concentrations. Furthermore, at the highest concentration, there was effectively

90% mortality indicating that concentrations were sampled from a no effect level to effectively a maximal mortality level.

With respect to the clinical observations, it isn't clear how these were actually defined or measured or whether individuals who were doing the clinical observations were actually blinded to treatment conditions. In addition, the sample sizes for these were quite small. Thus, the clinical observation data at the best seems suggestive.

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT 5: The one conclusion presented is an LC₅₀ value for combined males and females for a single 4 hr exposure to acrylonitrile. This is consistent with the mortality data presented. However, as noted above, some of the data shown seem to suggest a greater vulnerability of females, including some of the immediate clinical observations as well as mortality data, but unfortunately sample sizes were so small that sex was not included as a variable in any statistical analyses. Thus, this leaves a residual question of whether this LC₅₀ value is accurate for both sexes and whether the LC₅₀ value, and the inherent toxicity of acrylonitrile is greater in females. For that reason, I think the limitation of the conclusion reached should have been qualified as to these limitations.

RESPONSE: *ATSDR thanks the Reviewer for their comments. The Reviewer agreed with study conclusions; therefore, no response is needed.*

Wang et al. 1995

Wang Z, Zhilan L, Wei X. 1995. Study on acrylonitrile to teratogenesis of rats sperm. AN Group, Inc. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8(e). OTS0559911. 89000000313. 8EHQ-0900-14711. <https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0559911.xhtml>. October 3, 2024.

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT 6: While the study used an adequate number of animals for each exposure group, unfortunately they did not include both sexes in the study, so results for females are not known in this case due to the fact that it was focused on changes in sperm characteristics.

It is not possible to ascertain whether there was good animal care, as, there is virtually no information as to animal care included in the study. One comment of particular concern in this regard was the statement that there were 6 days of inhalation exposure and the text states: "During those 6 days, no food or water was fed to the animals." Presumably they meant that no food or water was provided during the two hour exposure period during that day, but it is not clear, and no body weight data are provided to know whether body weights were maintained during this period or were influenced by the exposure.

QUESTION: Did the study account for competing causes of death?

COMMENT 7: Mortality was not examined in this study, as its focus was on sperm aberrations. Consequently, animals were sacrificed following the inhalation exposure. What is unclear is the timing of the collection of the sperm relative to the end of the exposure period. If there was mortality it is not possible to ascertain as the initial group sample sizes are not provided. However, Table 1 includes a

column of No. of alive mice that suggests (but it is not stated) that there may have been two deaths in the positive control group and two in the 60 mg/m³ group.

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT 8: A strength of the study is the inclusion of 3 different inhalation concentration groups as well as a positive control group that were exposed for 28 days. In addition, for the highest exposure concentration group, additional groups were included at the highest concentration that had differences in the total duration of exposure of either 7, 14 or 28 days. One problem is that the data for the normal control data and the positive control groups in Table 1 (all groups after 28 days of exposure) were the same as that presented in Table 3 that includes different durations of the highest exposure concentration, suggesting that the normal control data and the positive control data results in Table 3 were not concurrent with either the 7 day or 14 day exposures.

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT 9: One concern about the study design relates to the lack of concurrent controls for the high concentration exposure at both 7 and 14 days in terms of number of disordered sperm. It could be the case that the handling and exposure at 7 and 14 days might be even higher in normal controls at those time points due to the early effects of handling and likely associated stress that would eventually dissipate with continued handling and exposure. In addition, the discussion of Table 3 in the Results is inconsistent with what is presented in Table 3 and the text states that this does not show any significant difference but it is not clear as to what this statement refers, as Table 3 itself indicates a significant difference from control in the 28 day inhalation group.

Another concern however is that no real information is provided as to the methods of the statistical analyses that were carried out, particularly for Table 1.

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT 10: As to the conclusions, it does seem, even with the limitations that the inhalation exposure to acrylonitrile does increase sperm aberrations. However, at what concentrations this occurs is actually not clear to me as the methods of statistical analyses are not provided. It is also not clear in terms of the presentation as to how the numbers of sperm aberrations are distributed within each of the groups. Are these just numerous such aberrations in a couple of the animals within each group, or a more broad distribution across subjects?

RESPONSE: *ATSDR thanks the Reviewer for their comments. The Reviewer agreed with study conclusions; therefore, no response is needed.*