

**Disposition of Peer Review Comments For
Two Unpublished Studies For Vinyl Acetate Toxicological Profile**

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Peer reviewers for the two unpublished studies for the Vinyl Acetate Toxicological Profile were:

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Comments provided by Reviewer #1

ATSDR Charge Questions and Responses and Reviewer Comments

Study 1:

JRBC (Japan Bioassay Research Center). 1995. Report on carcinogenicity studies on vinyl acetate orally administered (mixed in drinking water) to rats and mice. 138 pgs.

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

COMMENT 1: The study used 50 male and 50 female animals – rats and mice - in each group, with one control and three treatment groups (exposed to 400, 2000, and 10000 ppm vinyl acetate in drinking waer). Therefore, a total of 400 rates and 400 mice were used in the study. This seems to be appropriate. The amination care was acceptable.

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

COMMENT 2: Animal in-life conditions were observed; body weight, water consumption, and food consumption were measured. No significant difference in survival rates between treatment groups and the control group was noted in neither rats nor mice. However, for the rats, the highest-exposed group (10000-ppm group) had consistent lower water consumption compared to the control group (83% and 75% as much water consumed as the control group for the male and female rats, respectively) and had a decrease in body weight gain (8% in males and 10% in females) compared to the control group. Not sure if these contributed to the lower survival rates in the highest-dose treatment group, which were 78% and 74% for the males and the females, respectively, whereas it was 88% and 82% in the control group for male and female rats.

The drop of the water consumption and body weight gain in male mice was even more substantial, as describe in the original report: “At the end of the study, body weight in males and females of the 10000ppm groups were significantly less (30% and 18%, respectively) than controls” and “Water consumption was significantly less than control group in males and females of the 10000ppm group, especially in the latter period of this experiment” (pp. 109 of the document). It is unclear if these are accounted for in the study.

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

COMMENT 3: The study had one control and three treatment groups with the target vinyl acetate concentrations in drinking water of 400, 2000, and 10000 ppm. These concentrations were determined based on preliminary studies that were conducted for 2- and 13-weeks, which seemed relatively short for data to be used to determine concentrations in a cancer study. The doses were five times different between groups from low to medium to high. It might not be sufficient for a carcinogenicity study. This is probably reflected in the results, as most of increased tumor rates only occurred in the highest dose group.

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

COMMENT 4: Although the study design and data reporting could be improved, the results from this study still contribute to our understanding of the carcinogenicity of vinyl acetate.

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

COMMENT 5: The study provided some evidence on the types of tumors as summarized in the Conclusion of the original document (pp. 111 – 112 of the document). However, I am not sure if I would say there was clear evidence about all types of tumors listed in the summary. For example, for squamous cell carcinoma of esophagus in female rats, there was on one case that occurred in the 10000-ppm dose group (Table 5, pp. 76 of the document), with no statistical analysis results provided (Table 7, pp. 77). Another example is the squamous cell carcinoma of esophagus in male mice, the number of cases is hard to read in Table 14 (pp. 89), which seemed to be 2 cases in the 10000-ppm group. Again, statistical analysis was not provided for this. Furthermore, most of the tumors only occurred in the highest dose group and thus there is lack of evidence on the dose-response relationship.

RESPONSE: *ATSDR thanks the Reviewer for their comments. ATSDR reviewed the original data presented in the industry report by JRBC (1995). However, after review, ATSDR ultimately cited only the peer reviewed version by Umeda et al. (2004) in the profile.*

Study 2:

Krieger et al. or Dow 2020. Vinyl acetate: Evaluation of epithelial cell proliferation in nasal airways of Crl:CD(SD) rats repeatedly exposed to vinyl acetate vapors. 335 pgs.

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

COMMENT 6: The study used 84 -105 male rats, with 21 in each group, which seemed to be appropriate. However, only 8 were used for the endpoints reported in the study under review, which is not a large sample size but acceptable. The animal care was acceptable.

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

COMMENT 7: No animal death was reported in the study.

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

COMMENT 8: The study had one control group and 4 treatment groups, which are exposed to 0 (control), 50, 200, 600, or 1000 ppm vinyl acetate vapors. The exposure levels were selected based on a previous study (four-week) that evaluated the effects of inhaled vinyl acetate on rat nasal epithelium. The magnitude of dose levels seemed to be appropriate.

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

COMMENT 9: No issues noted.

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

COMMENT 10: I agree with the conclusions.

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

Comments provided by Reviewer #2

Reviewer General Comments

Study 1

This study, by the Japan Bioassay Research Center (JBRC), was entitled “Report on Carcinogenicity Studies on Vinyl Acetate Orally Administered (Mixed in Drinking Water) to Rats and Mice”. It is dated 1995, so the research project was probably conducted around the time of related work published by Maltoni et al. (1997), Belpoggi et al. (2002) and Minardi et al. (2002). The JBRC study differed from the other three studies in that it involved oral administration of vinyl acetate (VA) in drinking water to a single generation of mice and rats. The other 3 studies involved drinking water exposure of both the FO and F1 generations of mice and rats. The strains of rodents also differed from one study to another.

The overall findings of the cancer hazards of high level oral VA exposure by the Japanese and Italian research groups were quite similar (i.e., chronic, repeated ingestion of high concentrations of VA caused portal entry dysplasia and neoplasia in a variety of sites in the oral cavity and GI tract of several strains of mice and rats. It is noteworthy that cancers were observed (in some female rats) at a lower exposure level (i.e., 400 ppm) by JBRC than was observed in the Italian studies.

The JBRC research design appeared to be quite adequate, in terms of use of a sufficient number of animals, animal care, number of dose groups, range of doses, and reporting of results. The data clearly show that the GI tracts of both mice and rats are susceptible to induction of hyperplasia, dysplasia and carcinogenesis when subjected chronically to high levels of VA.

RESPONSE: *ATSDR thanks the Reviewer for their comments. ATSDR reviewed the original data presented in the industry report by JRBC (1995). However, after review, ATSDR ultimately cited only the peer reviewed version by Umeda et al. (2004) in the profile.*

Study 2

This study, by Dow Chemical Company (Sponsor: The Vinyl Acetate Council) was entitled “Vinyl Acetate: Evaluation of Epithelial Cell Proliferation in Nasal Airways of Crl:CD(SD)Rats Repeatedly Exposed to Vinyl Acetate Vapors,” is a logical follow-up and expansion of a prior/key chronic toxicity and oncogenicity investigation of inhaled VA by Bogdanffy et al. (1994a). Dow (2020) assessed effects of the same vapor concentrations of VA (i.e., 50, 200 & 600 ppm), as well as one higher level (i.e., 1,000 ppm). It is important to recognize that : (1) the nature and magnitude of adverse effects of VA on the olfactory and respiratory epithelia were quite similar at 50, 200 and 500 ppm in both studies, despite the dissimilar durations of the exposure regimens (i.e., up to 104 weeks for Bogdanffy et al. (1994a) versus a maximum of 13 weeks for Dow (2020)). It was interesting to see that 50 ppm and 200 ppm were the NOAEL and LOAEL, respectively, in each study. The magnitude of adverse effects on the nasal transitional epithelium (NTE) did not appear to become much more pronounced with longer duration of exposure to 200 or 600 ppm in either study, although inhalation of 1,000 ppm for only 1 or 5 days resulted in necrosis and degeneration of the NTE (Dow, 2020).

It is noteworthy that Dow (2020) examined effects of VA on a relatively wide variety of NTE parameters. Measures of cellular proliferation, epithelial thickness, olfactory marker protein and AB/PAS positive substance were monitored. Time- and VA-concentration-dependent site-specific effects were manifest in the olfactory epithelium and the NTE. A 1-day exposure to just 200 ppm induced nasal epithelial injury and evidence of modest cellular proliferation. The extent and degree of cellular proliferation increased in a VA concentration- and duration-dependent manner. Necrotic and atrophied olfactory epithelial cells were exfoliated and replaced, resulting in a metaplastic respiratory epithelium. There was an increase in nuclear Ki-67 (an index of proliferation) in the 200, 600 and 1,000 ppm exposure groups.

The olfactory and respiratory sites examined by Dow (2020) coincided with the locations of nasal carcinogenesis observed in the previous investigation by Bogdanffy (1994a). It has been previously proposed that VA nasal tumors are dependent upon chemically-induced cytotoxicity and persistent cell proliferation, which are necessary for fixation of mutations. The findings in the Dow (2020) study are consistent with this hypothesis. These were shown to be high-dose phenomena, suggesting that VA doses too low to elicit such effects are not a cancer risk. Unfortunately, numerous technical and logistical problems precluded completion of studies of VA-induced DNA adducts and genomic analyses of the nasal epithelium by Dow (2020). Acetaldehyde, an obligate metabolic product of VA, is known to produce protein and hemoglobin adducts *in vitro*. The extent of their formation and relevance to genotoxicity *in vivo* with relatively low VA exposures remains unclear.

Specific Questions to be Addressed

1. An adequate number of animals per dose group was utilized, as were an adequate number of doses. It is assumed that good animal care was practiced, as this was a GLP study conducted by an experienced toxicology laboratory. The number of dosage groups and actual doses coincided with those in a previous investigation of VA by Bogdanffy et al. (1994a). Animal fatalities were not a parameter of relevance. I agree with the authors' conclusions about the nature and sequence of adverse effects of VA on the rodents' nasal transitional epithelium. I also concur, as noted previously, that exposures to relatively low VA vapor levels are likely to be without appreciable cancer risk.

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

Comments provided by Reviewer #3

ATSDR Charge Questions and Responses and Reviewer Comments

Krieger et al. or Dow 2020. Vinyl acetate: Evaluation of epithelial cell proliferation in nasal airways of Crl:CD(SD) rats repeatedly exposed to vinyl acetate vapors. 335 pgs.

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

COMMENT 11: The study did not use the number of animals that would be expected of a standard 28- or 90-day study. The overall study design (animal numbers in each group) has considerable limitations. The study was conducted under GLP and there are no issues with the study conduct or execution.

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

COMMENT 12: No concerns.

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

COMMENT 13: The dose groups and time points were well selected. The issue is the number of animals examined and the fact that several original goals (in terms of endpoints and biomarkers) were not met.

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

COMMENT 14: Please see my written comments for details.

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

COMMENT 15: I do not agree with several conclusions. Please see my written comments for details.

Additional Comments

The Report “Vinyl Acetate: Evaluation of Epithelial Cell Proliferation in Nasal Airways of Crl:CD(SD) Rats Repeatedly Exposed to Vinyl Acetate Vapors” details the results and analyses of data from a GLP-compliant study of inhalation of Vinyl Acetate Monomer. The study used male Crl:CD(SD) rats were exposed for 5 days a week (6 hrs/day) to concentrations of 0, 50, 200, 600, and 1000 ppm of vinyl acetate vapors by a whole-body exposure method. Time points examined were 1, 4 and 13 weeks (5, 20 and 65 days of actual exposures, respectively). Because the study aimed to examine multiple endpoints, two different time points were selected for sacrifice after the last exposure in each time-point group. For studies of microscopic evaluation of nasal sections and site-specific evaluation of nasal

epithelial cell proliferation (Ki-67-positive nuclear area), epithelial thickness, OMP expression, and AB/PAS-positive intraepithelial mucosubstances (olfactory epithelium lining the dorsal meatus (DM) only), animals were sacrificed 18 hrs after last exposure was stopped (up to 8 animals/group were selected for these analyses). For studies of DNA adducts, animals were sacrificed 2 hrs after the last dose (up to 9 animals/group). For additional future molecular analyses (e.g. gene expression), animals were sacrificed 2 hrs after the last dose (up to 4 animals/group).

The information in the report is organized in several sub-sections, in a logical and clear manner typical of most animal study reports. Experimental details are sufficient and well-presented. Data summaries are presented as Text Figures/Tables; additional detailed study data is included as voluminous Appendix Material.

Comments on the Overall Report:

- The study was not conducted according to standard 28- or 90-day NTP study designs with respect to the animal numbers and inclusion of both sexes. The report provides no information on why only male rats were examined and whether there are any sex-specific differences in toxicokinetics/toxicodynamics of vinyl acetate.
- The animal numbers selected for each study' arm has not been well rationalized. Why 8 animals were selected for histopathology and other analyses is not clear, likewise samples from only 6 of 8 animals was processed which further decreases the power of this study.
- The remaining samples from 13 rats per study group were not analyzed – neither DNA adduct data nor gene expression analyses were performed for a number of reasons. The lack of these data makes it very difficult to interpret the other findings in the context of the potential relationship between cell proliferation and mutagenesis.
- The study authors were unable to collect interpretable data on BrDU incorporation into DNA, an endpoint which is considered a preferable marker of cell proliferation, for technical reasons. While Ki-67 staining is an acceptable biomarker, it is known to be highly variable among animals in the same group, notion that is supported by the data in this study. High variability in control and treated animals, combined with a fairly small group size, make these data difficult to interpret in terms of either dose- or time-course.
- Overall, the study can be considered as severely underpowered (no formal power analysis was conducted by the authors to justify group size) and it is regretful that the original study goals to collect a comprehensive dataset on both proliferative and genotoxic events, along with a comprehensive mechanistic data from gene expression, went largely unrealized. As such, while the results of this study may be used for some dose-response analyses and are confirmatory of the previous observations; however, this dataset may have limited utility because of the methodological considerations stated above.
- The Report suffers from a number of regretful typos. Statistical significance asterisks and Greek symbols in many Figures did not convey in the PDF reviewed herein. While one can surmise what is being meant, the quality of the document preparation is lacking.

Comments on Statistical Analysis:

- The description of the Methods (p. 33-34) is adequate and detailed; however, question remains whether the use of Grubbs test for outlier detection was appropriate because it can detect only one

outlier. Alternative methods, such as ROUT could have been used as they can detect any number of outliers in a dataset.

- What is more concerning is that Figures and Tables in the Text did not provide sufficient details on the exact type of a statistical test that was used in each analysis. This may result in confusion when “text figures/tables” are examined at their face value. Because the raw data was provided in Appendix materials, independent analyses can be performed on most salient datasets. Regardless, a formal BMD modeling should be conducted if these data are to be used for quantitative dose-response assessment.

Comments on Results and Discussion:

- Based on the data presented herein, this reviewer is satisfied with the overall description of the findings in the Results. While the statistical analyses may need to be documented better (see above), the findings are rather clear showing significant effects (despite wide variability in some phenotypes within each dose/time group) at the highest doses tested, most of the effects were detected in the olfactory epithelium.
- The Report includes representative histopathological images that provide important context for the findings.
- The authors use different terminology throughout the results. It appears that where the effects were undeniable, they would rely on the “significant” descriptor to characterize the data. However, in some parts of the Report, the authors would characterize effects as “relatively minor” even though they were statistically significant. For example (as noted above), Ki-67 staining is prone to high inter-animal variability – something that was clearly present in the results (Text Figure 7). Still, the Report should have used consistent (e.g., unbiased) language when describing effects or lack thereof.

Comments on Summary and Conclusions:

- The authors correctly state that most of the pathological and microscopic effects observed in this study occurred in animals exposed to 600 and 1000 ppm of vinyl acetate and were time-dependent.
- The authors have not provided a similarly detailed overview of the effects that were significant at concentrations of 200 and even 50 ppm; the latter being a clear dose-response effect on olfactory epithelial thickness at 5 day (1 week) exposure time point.
- The authors suggest that “the metaplastic respiratory epithelium, that developed with continuous long-term exposure to high concentrations of vinyl acetate, had a high cellular turnover rate as evident by the
- increased nuclear Ki-67 labelling of epithelial cells in the [dorsal meatus].” This suggestion appears to be without solid evidence, however, because Dorsal Meatus proliferation rates (derived from Ki-67 staining) are increased at all time points examined herein and only one biomarker of metaplasia (AB/PAS-positive staining) is presented as evidence for the epithelial transition. The latter effects appears to be non-monotonous with respect to time effects and it is difficult to determine what fraction of the T2 region may be replaced by metaplastic respiratory epithelium.

- The authors also state that “It has been hypothesized that vinyl acetate-induced nasal tumors that developed at nasal airway locations lined by olfactory epithelium [...] are dependent on chemical-induced cytotoxicity and persistent cell proliferation that is necessary to fix mutations in the affected tissue. The results of our study are in line with this hypothesis for those tumors that arose from intranasal sites normally lined by olfactory epithelium.” This hypothesis remains to be proven, in the opinion of this reviewer, because the study provides only cell proliferation data and did not reach its goals of demonstrating DNA adducts or gene expression changes.

RESPONSE: *ATSDR thanks the Reviewer for their comments. The Reviewer agrees with major study conclusions regarding the results of the microscopic evaluations, which are the basis for the NOAEL/LOAEL determinations in the Toxicological Profile for Vinyl Acetate. However, as noted in the additional comments, the Reviewer notes the difficulty in interpreting the cell proliferation findings reported based on nuclear Ki-67 labeling. Consistent with this, ATSDR did not base NOAEL/LOAEL determinations based on cell proliferation data due to: (1) lack of clear dose- and duration-response and (2) failure of evaluation of the standard BrdU evaluation of proliferation (by study authors) due to “overloading of BrdU.” Therefore, NOAEL/LOAEL determinations for Krieger et al. (2020) in the profile are based on histopathological lesions, with brief reporting of cell proliferation findings in Chapter 2.*