External Peer Review

U. S. Environmental Protection Agency Propionaldehyde: Combined Repeated-Exposure and Reproductive/Developmental Toxicity Study in CD Rats Final Report

Final Compilation of Reviewer Comments And Responses to Charge Questions

Prepared for
Integrated Risk Information System (IRIS) Program
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency

Prepared by
ORISE IRIS Technical Assistance Team
Oak Ridge Institute for Science and Education
Oak Ridge Associated Universities

August 2007

This document was prepared for the EPA by ORISE under interagency agreement No. DW-89939822-01-0 between EPA and the U.S. Department of Energy. ORISE is managed by Oak Ridge Associated Universities under a contract with DOE.

EPA PROPIONALDEHYDE REVIEW

TABLE OF CONTENTS

EXTERNAL PEER REVIEWERS

Rogene F. Henderson, Ph.D.
Lovelace Respiratory Research Institute

Stephen B. Hooser, DVM, Ph.D.
Purdue University

Jerold Last, Ph.D.
University of California, Davis

Marion Miller
UC Davis

The ORISE IRIS Technical Assistance Team has neither altered nor edited these comments for grammatical or other errors.

PEER REVIEW PROJECT MANAGERS

John E. Whalan, Project Manager
U.S. Environmental Protection Agency

Leslie Shapard, Peer Review Manager
Oak Ridge Institute for Science and Education

CHARGE TO EXTERNAL PEER REVIEWERS

Subject: Peer review of report entitled, "Propionaldehyde: Combined Repeated-Exposure and Reproductive/Developmental Toxicity Study in CD® Rats", generated by Union Carbide Chemicals and Plastics Company in 1993 at their Bushy Run Research Center facilities

The U.S. Environmental Protection Agency's (EPA) National Center for Environmental Assessment (NCEA) is currently developing a human health assessment of the chemical propionaldehyde (CASRN 123-38-6). The report from the Union Carbide Chemicals and Plastics Company entitled, "Propionaldehyde: Combined Repeated-Exposure and Reproductive/Developmental Toxicity Study in CD® Rats" (1993), is proposed as principal support for the development of one or more toxicity values for propionaldehyde, but this report has not been subjected to a formal peer review process. Such a peer review process is important to establishing the appropriateness, validity, and robustness of the study design, conduct, and interpretation of findings of the reported investigation.

As indicated by the report title, the study described is that of a combined repeated-exposure and reproductive/developmental toxicity study in CD® rats. Young adult rats (15/sex/group) were exposed to propionaldehyde for 6 hours/day, 7 days/week via whole-body inhalation, during a 2 week pre-mating period and a 14-day mating phase. The males continued to be exposed until sacrifice in week 7, for a total of 52 exposures. The mated females were exposed daily through day 20 of gestation. The females were then allowed to deliver their litters naturally and raise their offspring until day 4 of lactation.

CHARGE QUESTIONS

Question 1 - Based on your knowledge of toxicological protocols, please comment on the experimental design of this investigation. Do you see any significant issues with the test system or test article employed, inhalation exposure equipment and monitoring of atmosphere, endpoints recorded, terminal procedures, statistical analyses, and quality assurance?

Question 2 - Are there physiological/toxicological endpoints that should have been assessed that were not part of the investigation?

Question 3 - Please comment on the strength, credibility, and relevance of the toxicological results. Were the individual animal data correctly summarized and interpreted?

Question 4 - Are the reported conclusions supported by the data? Are there any observations that were excluded from the conclusions that should have been included? More specifically, were there any observations that were excluded that are contradictory to the reported conclusions?

Question 5 - In your opinion, was this investigation properly planned, conducted, and reported? Are there any procedures, observations or analyses that would have added to the quality of this investigation?

QUESTION 1 - Based on your knowledge of toxicological protocols, please comment on the experimental design of this investigation. Do you see any significant issues with the test system or test article employed, inhalation exposure equipment and monitoring of atmosphere, endpoints recorded, terminal procedures, statistical analyses, and quality assurance?

Response from Rogene F. Henderson

This was a well conducted study using GLP and with an appropriate protocol, adequate quality control and good record keeping. The inhalation exposures were done well with adequate monitoring and good control and characterization of the exposure atmosphere. Animal husbandry was well done. The exposure article was well characterized as 99% pure, both before and after the study. Statistical analyses were appropriate and well described. The histopathological assessment of the adults in the study were thorough and well described.

A preliminary range-finding study was conducted before selection of exposure concentrations for the main study. The preliminary study found that the NOAEL for maternal toxicity was 500 ppm propionaldehyde (PA) and for developmental toxicity was 1500 ppm. The report of this study in Appendix 9 indicates it was also a well conducted study using GLP. (I would like to note that on page 254, the table giving the experimental design for the preliminary study lists the concentrations of PA used in the final study, not the preliminary study. But this is an editorial error and of no consequence for the quality of the study.) The results of the preliminary study were used to choose the concentrations to be used in the main study and the choices were appropriate.

The protocol used follows, in general, the protocols for a one generation fertility test published by the EPA (1985) and the OECD (1983). One deviation from these protocols was the use of 15 animals per group rather than 20. I do not think that deviation affected the quality of the results.

Another difference is the duration of the dosing of males and females prior to mating. The standard protocols suggest administration of the test material for 10 weeks prior to mating for both sexes (EPA) or 10 weeks for males and 2 weeks for females (OECD). In the current study both sexes were exposed by inhalation for 2 weeks prior to mating. Without any toxicokinetic data, it would be difficult to calculate the difference between the dose that would be received in a 10 week feeding period versus a 2-week inhalation exposure. Based on the range of exposure concentrations, which included a high level that caused some toxicity in the parental animals, I think the pre-mating exposure schedules were adequate to test the agent.

Response from Stephen B. Hooser

1. Based on your knowledge of toxicological protocols, please comment on the experimental design of this investigation. Do you see any significant issues with:

a. the test system or test article employed,

These appear to be adequate, although in retrospect, euthanasia of the F1 pups at a longer time point, perhaps lactational day 14 or so, with periodic measurements during this time period, may have given a better indication of the trend of body weight gains of the F1 generation (see the answers to question 4 for explanation).

b. inhalation exposure equipment and monitoring of atmosphere,

These appear to me to be adequate.

c. endpoints recorded,

i. Identification of individual F1 pups to allow monitoring of body weights and crown-rump length on an individual basis, and a complete necropsy with gross and microscopic pathological evaluation might have been useful to make an assessment of developmental effects (see question 4 for explanation). Otherwise, the endpoints appear adequate.

ii. Were vaginal smears performed on the F0 females during the mating period? The section, "Mating, Gestation, and Lactation", pg 10, makes reference to checking for vaginal sperm, but the method of evaluating this is not given.

d. terminal procedures,

These appear to be appropriate.

e. statistical analyses,

This is not my area of expertise, but consultation with a statistician might be warranted to assess the value of additional statistical analyses of the body weights of the F1 pups, especially at Day 4 of lactation. In addition, "Data Analysis", pg 13, indicates that the unit of comparison for the F1 offspring is the litter. If appropriate, statistical analysis of the F1 individuals at Day 4 of lactation might be useful.

f. quality assurance?

This appears to be adequate.

Response from Jerold Last

The inhalation exposures were appropriately designed and carried out. Rats were acclimatized for 14 days prior to exposure before being randomly assigned to exposure groups, and each exposure group started at comparable weights. The test material was taken from 2 barrels of production grade propionaldehyde, and was therefore representative of the product in commerce (>98% purity). Detailed analytical information by several methods was obtained for the starting material, and the analytical results are documented in the report. Impurities were

identified and quantified; none was present at high enough levels to affect the toxicological studies. The inhalation chambers were appropriately designed and constructed, with adequate testing for homogeneity of the chamber atmosphere with respect to maintenance of constant temperature and relative humidity during exposure, and homogeneity of distribution of the test material in the chamber during the exposures. I could not find any mention of whether cages were inserted into the chambers in the same relative position for each exposure; optimal experimental design would have included rotation of cages with each exposure to correct for any lack of homogeneity of vapor distribution during exposure, so that each individual animal received the same total dose during the study (with a molecular weight of 58, propionaldehyde is denser than air, so relative concentrations could have been higher in the lower areas of the chambers, especially in areas that were not perfectly mixed by chamber airflow). Concentration of propionaldehyde in each chamber (at the sampling points) was maintained at target concentrations within acceptable limits, and cross-contamination into the control chamber did not seem to occur (control values for propionaldehyde were below the limit of detection for the assay used to measure concentration in the chamber atmosphere). Temperature and relative humidity were at appropriate levels at all times sampled. At 1 chamber volume change per 4 minutes, flow rate for incoming air was slightly slower than optimal (we generally use a volume change every 2 minutes), but within acceptable limits based on the measured values within the chambers. The selected analytical method for chamber propionaldehyde concentration determination, gas chromatography, was appropriate, and the analytical standard curve was linear over the selected concentration range. The experimental design with respect to inhalation exposure to the test substance was adequate for the toxicological testing protocol.

The terminal procedures used for sacrifice were typical for this type of study, quality assurance seems to have been within standard guidelines, and statistical analysis used standard methods for evaluating significance of the findings. However, there were individual animals whose data differed from the mean values by more than 2 SD, for example body weights in animal number 28188 in the 150 ppm group, 28190 in the 750 ppm group, and 28179 in the 1500 ppm group. While GLPs require inclusion of all animals in the reported results, scientific convention would have discarded these individuals as statistical outliers, thereby reducing the large coefficient of variation in the group and perhaps making the apparent decreases in body weight for the various groups statistically significant. Similar considerations pertain with regard to the food consumption data for the same individual animals. It might have been appropriate to calculate the data both with and without inclusion of the statistical outliers, and determine whether one or both calculations caused the data to be significantly different than that obtained with the control rats.

Response from Marion Miller

The test material and the inhalation system utilized were well documented as was the monitoring of the atmosphere in order to ensure that exposure was at the required dose levels. In terms of the analytical work, there were a couple of very minor issues. One was the availability of standards for the breakdown products and impurities indicated as present in the propionaldehyde test substance. However, since the levels of contaminants were very low the reviewer does not consider this a significant issue. A second consideration in the analytical section is the use of a standard (calibration) curve with only 4 data points. Again this is of

minor importance as the line shows excellent fit and there is a high level of confidence in the levels reported.

Toxicological endpoints were body weights, food consumption, a limited number of reproductive indices including litter size and pup weight and gross evaluation of pup physical abnormalities, gross and microscopic pathology as well a blood hematology and chemistry. The procedures utilized to collect this data were well documented. Wire cages may not be currently acceptable to this type of study due to additional animal stress. Terminal procedures, statistical analyses and QA were all appropriate for the study.

QUESTION 2 - Are there physiological/toxicological endpoints that should have been assessed that were not part of the investigation?

Response from Rogene F. Henderson

The word "should" in this question is a little problematic. The study included the endpoints that are a part of standard protocols. Of course, one would always like to have more information on internal dosimetry, to allow extrapolations between methods of administration and between species. But that type of data is not part of a standard protocol for one generation fertility studies. I also note that only the males were assessed for clinical pathology endpoints and no explanation was given for that. It may be that it was not convenient to fast the females during lactation but I wonder if additional females might have been added to the study to get that information.

Response from Stephen B. Hooser

Identification of individual F1 pups to allow monitoring of body weights and crown-rump length on an individual basis, and a complete necropsy with gross and microscopic pathological evaluation might have been useful to make an assessment of developmental effects in the F1 animals (see the answers to question 4 for further explanation).

Response from Jerold Last

The key endpoints measured were body weight gain, food consumption, clinical chemistry, mating performance, fertility, litter size, pup viability, pup body weights, and histological and pathological endpoints. The main exposure-related observations noted were lower body weights in the pups at 4 days with dose of propionaldehyde (both genders, significant with female pups) and decreased weight of the thymus in adult males. There seemed to be immune system effects (thymus and lymph nodes) in both genders exposed to higher levels of the propionaldehyde. Effects on the nose were noted at all doses in both genders, some of these effects were severe in a few of the animals at the higher concentrations (squamous metaplasia).

Additional endpoints that should have been assessed to determine the significance of these observations might have included allowing the pups to mature beyond weaning (to maturity - 6 weeks or so) to see if the body weight changes persisted, and complete necropsies on the F1 generation (were there effects on thymus and lymph nodes in these animals?). Effects on the immune system could very well adversely affect development in many ways, and persistent changes in lymph nodes or thymus as a consequence of maternal exposure would be cause for concern. Examination of neonatal histology and/or pathology on the pups would also seem to be obligatory to be able to make any kind of statement with regard to putative teratogenicity of the propionaldehyde.

For example, Cavieres et al. examined pups that had been allowed to mature to 6 weeks, as suggested above (as described below): "The 8 remaining pups were kept with the mother until weaning at 3 weeks (postnatal day 21) and then were allowed to grow until week 6 for

additional immune, endocrine, and behavioral assays." (Maria Fernanda Cavieres, James Jaeger, Warren Porter. Developmental toxicity of a commercial herbicide mixture in mice: I. Effects on embryo implantation and litter size. Environmental Health Perspectives, 2002 110:1081-1085).

In Appendix 2, page 3, we are told that the pups were killed on postnatal day 4 and "discarded without pathological evaluation". It would seem that pathological evaluation of the pups, even if at 4 days, would have been a better experimental design, with more information being obtained at a relatively low incremental cost. While the omission of necropsies and pathological evaluation of the pups postnatally may have adhered to the letter of the minimal requirements for a reproductive and developmental study (at least in 1993), one would like to have seen a more proactive approach to the evaluation of possible adverse health effects in the data to be used for a major risk assessment by a regulatory agency like EPA.

Response from Marion Miller

As indicated in the summary this appears to be a well conducted study. However, it is not a study that comprehensively addresses reproductive and developmental toxicity. For example the males were exposed for 2 weeks prior to mating. If there had been an effect on the integrity of spermatogenesis in the testis it would not have been readily detected within this short time frame. However, there is data suggesting no effect on the male reproductive system since both testes and epididymides were weighed and assessed histologically after 7 weeks exposure and no changes were detected. No sperm analyses were included in the study design which would have further documented the integrity of spermatogenesis after propionaldehyde. It was documented that ovaries, vagina and pituitary were collected and fixed but no histological evaluation was performed on these tissues again indicating the lack of a comprehensive approach to evaluating potential effects on reproduction. A more important deficiency is in the developmental toxicity component of the research. While *in utero* exposure is dealt with in the study design there was limited assessment of pup health and this was indicated only by pup weight and the presence of broadly defined physical abnormalities. In addition to a more in depth evaluation of the pups, a more comprehensive study would have included exposure of the F_1 generation to allow detection of effects that occur throughout the entire period of development including peripubertal and adult phases. A multigenerational study would have also included the F_2 generation.

QUESTION 3 - Please comment on the strength, credibility, and relevance of the toxicological results. Were the individual animal data correctly summarized and interpreted?

Response from Rogene F. Henderson

The animal data were correctly summarized and interpreted. The most significant finding of the study was the consistency of the nasal lesions. There was no level of exposure for which this lesion was not found. Because PA is a water soluble, reactive vapor, one would expect heavy deposition in the nose. At the highest exposure concentration (1500 ppm) the lesions changed from vacuolization of the olfactory epithelium (seen at the low and mid level concentrations) to atrophy and even some squamous cell metaplasia (males). This was a relatively short term exposure (52 days) and only 15 animals were studied in each group. The nasal findings suggest that longer term studies with more animals might result in observations of some nasal neoplasms. The current study is not adequate to assess the carcinogenicity of PA, nor was the study designed to do so.

Response from Stephen B. Hooser

The toxicological results in this investigation appear to be strong, credible, and relevant. Please see the answers to question 4 below for discussion of the data summary and interpretation.

Response from Jerold Last

Yes, the individual animal data were correctly summarized and were interpreted consistently with standard toxicology testing. The strength of the data was diminished by the experimental omissions noted above (but, we should note that the study was not performed with the intent of its being used to generate data for a major risk assessment), and perhaps by the study group size. We are not told how the exposure group size was set to N = 15 per chamber, nor is there any evidence of appropriate power calculations to ensure that the study protocol would have detected an effect on a given percentage of the population had it occurred. Given that a standard N = 50 per group is used for NTP protocols, the use of N = 15 seems low for this type of study, especially if the toxic effect of interest occurs infrequently. This statistical concern may diminish the overall credibility of the study results, especially its negative findings. The relevance of the positive findings in the toxicological results speaks for themselves if this study is really underpowered.

Response from Marion Miller

The results are well and clearly presented both in summary and for the individual animal data. Occasional data gaps were justified and/or reported, e.g., due to food spillage, a nonpregnant animal, a second mating.

QUESTION 4 - Are the reported conclusions supported by the data? Are there any observations that were excluded from the conclusions that should have been included? More specifically, were there any observations that were excluded that are contradictory to the reported conclusions?

Response from Rogene F. Henderson

I found the conclusions to be supported by the data and I did not see any excluded observations that might contradict those conclusions. Because the study was designed to test the effects on fertility, I think the authors did not pay much attention to the nasal lesions. However, the nasal lesions were certainly included in the conclusions and summary statements.

Response from Stephen B. Hooser

a. For the most part, the conclusions are supported by the data, however…

b. Are there any observations that were excluded from the conclusions that should have been included? Yes, as follows:

    i. The last summary paragraph of the Summary (pg. 6) states, "A slight decrease in body weight gain in the 1500 ppm offspring was the only finding of significance in the neonates". Technically, this appears to be correct, however, examination of Table 18, pg. 36, "Summary of pup body weight and weight change (grams) per litter", shows that while the only statistically significant finding was a decrease in pup body weight change (lactational day 0 to 4) at 1500 ppm (for entire litter and for female pups), there is a very noticeable dose-related trend for decreased body weights of F1 pups at lactational day 4 which also manifests itself as a dose-related decrease in body weight changes, lactational day 0 to 4. While not significant by the statistical methods used, there appears to be a trend that as the dose of propionaldehyde increased, the F1 pup body weights and changes in body weights (lactational day 0 to 4) decreased. Whether this represents an adverse effect on the F0 females (such as decreased milk production or some other effect) or whether it represents an *in utero* effect on the F1 pups is unclear.

    ii. In the Summary, pg. 6, it states, "The decline in the severity of the nasal lesions in females relative to males is likely attributable to the 6-day (approximately) period between the cessation of exposure after gd 20 and the sacrifice on lactation day 4 for the females". Is 6 days (approximately) long enough for the resolution of squamous metaplasia?

    iii. In Table 4, pg. 22, Summary of Body Weight Gain (grams), F0 Adult Males, Week 5 to 6: What is the explanation for the very large Standard Deviations in all treatment groups?

iv. In the Summary, pg. 5 and Table 10, pg. 28, Summary of Gestational Body Weight and Weight Change (grams), F0 Adult Females: The overall Summary, pg. 5 states, "During gestation, the body weights of the high concentration females were less than controls on Days 0, 7, and 14". Table 10 indicates that these lower body weights in the 1500 ppm females were statistically, significantly lower than the controls although this statistical significance was not stated in the Summary. In addition, at day 21, the body weights of the 1500 ppm females were lower than controls, but not at the $p<0.05$ level. It would be interesting to see what the p value was for this time point. Please see the related answers, v. and vi, below.

v. Table 11, pg. 29, Summary of Gestational Food Consumption (grams/animal/day), F0 Adult Females: The food consumption for the 1500 ppm females is significantly lower than for the controls. This may help explain the lower body weights for the females in this group.

vi. Table 12, pg. 30, Summary of Lactational Body Weight and Weight Change (grams), F0 Adult Females: The Day 0 Lactational Body Weights for the mid and high dose (750 and 1500 ppm) groups are significantly lower than controls. These weights are lower, but not significantly lower, than controls at Lactational Day 4.

Do these (Nos. vi, v., and vi.) imply that the mid- and high-doses of propionaldehyde adversely affect the pregnant females and that they start to recover their body weight once the have delivered and propionaldehyde exposure has stopped?

vii. Table 10, pg. 28 and Table 12, pg. 30:

Table 10 shows an "N" value of 15 for the 150 ppm females and an "N" value of 14 for the 750 ppm females at gestation Day 21.

Table 12 shows an "N" value of 14 for the 150 ppm females and an "N" value of 15 for the 750 ppm females on lactational Day 0.

What is the reason for this discrepancy in the number of animals in each of these groups, and if there is an error, does it affect the statistical analysis?

viii. Table 14, pg. 32, Summary of Gestational Length and Reproductive Parameters, F0 Adult Females: Preimplantation Loss and Postimplantation Loss need to be defined in the section on Reproductive Indices on page 11.

xi. Table 18, pg. 36, Summary of Pup Body weight and Weight Change (grams) per Litter, F1 pups: See 4.b.i. above for discussion.

x. Table 19, pg. 37, Summary of Organ Weights (grams), F0 Adult Males: The Thymic Region Weights in the 1500 ppm F0 Adult Males are significantly lower than those of the controls (no treatment-related lesions noted on histological exam). This was not included in the overall Summary on pg. 5 with the statement of the increase of kidney weights (as a percent of the body weight) in the males from the 1500 ppm group.

Response from Jerold Last

The report has three major sections: (1) A summary of the findings, (2) 9 appendices, and (3) 26 Tables. The 9 appendices present individual reports on chamber atmosphere, anatomical and clinical pathology findings (summary and individual), individual animal data "in-life", reproductive parameters, and detailed protocol as performed. The Tables summarize the data underlying the appendices---chamber atmosphere, clinical findings, body weight and food consumption data, data on litter size and pup size and sex ratios, pup survival, organ weights and other necropsy observations, and graded microscopic pathology observations. The individual animal data are presented in the appendices---the only data apparently excluded were from animals that were not part of the formal study (for example, rats used in the range finding studies). Only summary data are presented in the Tables. There is no evidence of selective exclusion of observations that might have been contradictory to the reported conclusions. The main reported conclusions from the study in the summary section were: (1) a striking lack of overt gross toxicity in the exposed male animals. Exposure-related findings included elevated erythrocyte counts, hematocrit, hemoglobin values and monocytes, as well as increased kidney weights. There was also a concentration-related injury noted in the olfactory epithelium in the anterior nasal cavity, which became progressively more severe with increasing concentrations of propionaldehyde in the exposure chambers. (2) Decreased food consumption and body weight gains in the female rats at the higher exposure concentrations and similar concentration-related injury noted in the olfactory epithelium in the anterior nasal cavity as was observed in the males, albeit less severe (no squamous metaplasia; however, this may have been due to experimental design rather than gender, as there was a 6-day recovery period for the females built into the protocol after exposure to allow for feeding pups for 4 days postnatally). (3) Decreased body weight in the pups from dams exposed to the highest concentration of propionaldehyde was the only reported affect in the F1 offspring as they were evaluated for overt effects at day 4 of life.

Response from Marion Miller

The conclusions were in general justified. However, it was indicated in the conclusion of the study report that only the nasal epithelium and not respiratory epithelium was affected by inhalation exposure. However, in the Anatomic Pathology report (appendix 2) "occasional metaplasia of the respiratory epithelium" was documented. Body weight changes were reported as minor and occurred in the females only. A consideration is the significantly lower body weight of the females at the 1500 ppm exposure level as they enter gestation. At both the 750 and 1500 ppm dose both food consumption and body weight gain were significantly decreased. However, body weight at the beginning of gestation was significantly less only at the 1500 ppm level. These data indicate potential body weight changes occurring at the lower dose level

that lost statistical significance due to inter-individual variation. A decreased pup body weight change between lactational day 0 and lactational day 4 was statistically significant at the 1500 ppm dose and reported in the conclusion. A similar trend was not reported for the 750 ppm dose level. Understanding the possible significance of this is limited by the absence of additional data from the F_1 generation.

QUESTION 5 - In your opinion, was this investigation properly planned, conducted, and reported? Are there any procedures, observations or analyses that would have added to the quality of this investigation?

Response from Rogene F. Henderson

In my opinion the study was well planned, conducted and reported. I can think of no addition procedures, other than those mentioned under charge question #2 above, that might have been conducted.

Response from Stephen B. Hooser

In my opinion, with the exception of the items noted above, this investigation was properly planned, conducted, and reported. The procedures, observations, or analyses that would have added to the quality of this investigation are also noted above.

Response from Jerold Last

As discussed above, I would have liked to see more complete studies on the F1 generation of pups--individual body weights and complete necropsies after growth to maturity (or at least through weaning) to determine the reversibility or persistence of the apparent changes reported in the thymus and lymph nodes, and to have examined the possibility of there being any teratogenic effects of the propionaldehyde. At the very least, complete necropsies on the pups, comparable to those performed on the adult rats, would have been a valuable addition to this study at a relatively low cost by comparison with the costs of exposure to the propionaldehyde. The parameters evaluated in this study--litter size and sex ratio, pup weights and viability--seem a very cursory approach to a complete toxicological evaluation of the F1 generation and clearly reflect at best a de minimis study of the possibility of effects of parental exposure on the offspring. In addition, some relatively simple power calculations (which could still be performed with the existing information) could have defined the extent of any toxic responses that would have had to occur for the analyses at the chosen group sizes to have found significant effects (and to have given either false negative or false positive outcomes). Such calculations of experimental power should be an obligatory component of any toxicological study of this type.

"The U.S. Environmental Protection Agency's (EPA) National Center for Environmental Assessment (NCEA) is currently developing a human health assessment of the chemical propionaldehyde (CASRN 123-38-6). The report from the Union Carbide Chemicals and Plastics Company entitled, "Propionaldehyde: Combined Repeated-Exposure and Reproductive/Developmental Toxicity Study in CD® Rats" (1993), is proposed as principal support for the development of one or more toxicity values for propionaldehyde." While we are not told which toxicity value(s) will be used from this report for the risk assessment, the most striking data on toxicity in the report are the findings of "an exposure-related effect on the olfactory epithelium in the anterior 2 sections of the nasal cavity". These effects included vacuolization in the low and intermediate dose groups, atrophy in the intermediate and high

dose groups, and squamous metaplasia in three of the males in the latter exposure groups. These results are not discussed at any length in the report, which was designed primarily to be a Reproductive and Developmental Toxicity Study, not a sub-chronic toxicity study as such, but the nasal epithelial effects represent a data set suitable for determination of a benchmark dose or LOAEL. If this is indeed the data set under consideration for the development of one or more toxicity values for propionaldehyde, then it is worth examining the quality of these data, as well as the Reproductive and Developmental Toxicity measurements. The summary of these results in Tables 25 and 26 (pages 43-44) suggests that these would indeed be useful data for such a risk assessment, and would appear to be relevant and credible data on which to base a risk assessment. While some of these changes in the nasal epithelium may be reversible, they are appropriately defined as "adverse health effects" for the purposes of a toxicology study. In addition, these toxicological results in the nasal epithelium are consistent with the other adverse effects observed, including lack of weight gain in the female rats (which could reflect decreased food consumption secondary to a systemic response to irritant aldehyde exposure) and the hematological changes and increased kidney weights in the males, which could have been caused by decreased water consumption in these animals (which could also reflect a systemic response to irritant aldehyde exposure). Thus, the adverse nasal effects observed are consistent with other changes observed, are the most sensitive indicator of exposure-related damage, and show a nice concentration-response behavior consistent with determination of a LOAEL by a linear dose response model.

Response from Marion Miller

This is a reasonable well conducted and reported study. However, it clearly is not a comprehensive study for assessing reproductive and developmental toxicity. As part of a group of studies it supplies useful information. However, the lack of a multigenerational component raises the possibility that reproductive and developmental effects would not have been detected using the reported study design.

Additional Comments

Response from Marion Miller

Summary:

The study is a combined repeated-exposure and reproductive/developmental toxicity study in CD® rats. Young adult rats (15/sex/group) were exposed to propionaldehyde for 6 hours/day, 7 days/week via whole-body inhalation, during a 2 week pre-mating period and a 14-day mating phase. The males continued to be exposed until sacrifice in week 7, for a total of 52 exposures. The mated females were exposed daily through day 20 of gestation. The females were then allowed to deliver their litters naturally and raise their offspring until day 4 of lactation.

The indicated goals of the study were to generate data on the toxicity of propionaldehyde in male and female rats as well as provide information on reproductive performance and developmental toxicity following inhalation exposure. The overall conclusion from the work

was that at the dose levels used (0, 150, 750, and 1500 ppm), propionaldehyde caused minimal toxicity in females at 750 and 1500 ppm but no effects were seen in males. The study also concluded that propionaldehyde had no effect on reproductive performance and had only a minor effect on pup weight at dose levels where there was evidence of maternal weight loss. The clearest toxicological finding was in the nasal epithelium where there was evidence for propionaldehyde associated vacuolization and atrophy.

This reviewer agrees with the findings of the study and found that in general it was well conducted. However, it clearly uses a limited number of reproductive endpoints and does not supply the level of information that would have been obtained in for example, a multigenerational reproduction assay.