

(Review only)

PROTOCOL TITLE
VINYL CHLORIDE: COMBINED INHALATION TWO-GENERATION
REPRODUCTION AND DEVELOPMENTAL TOXICITY STUDY IN CD RATS

RESPONSE TO THE ATSDR REVIEW

Prepared by
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The Dow Chemical Company
October 10, 1995

Reviewer: Dr. M. S. Christian

The reviewer requested CMA provide further justification and clarification on certain areas of the protocol dealing with study design and methods. Although we have chosen not to reiterate each specific comment here, all comments are addressed in the following section.

Route/Method of Exposure

Whole body exposures were selected over nose only exposures for the following reasons. 1) Vinyl chloride is highly volatile with a vapor pressure of 2,530 mm Hg at 20 C. Given the high volatility of vinyl chloride, the exposure concentrations to be used and the exposure conditions to be employed in this study, aerosol formation and deposition of vinyl chloride onto the fur or skin of the rats is expected to be very low and represent an insignificant source of both oral or dermal exposure. 2) Many of the reproductive and developmental endpoints to be evaluated in this study are highly sensitive to general stress. The stress associated with the confinement (immobilization) of nose only exposure is significant and represents an unnecessary confounder. Although the stress of nose only exposure can be controlled for, to a limited extent, through acclimating animals, the undesirable effects of this additional factor outweigh any potential benefits of reducing the limited amount of oral or dermal exposure due to whole body exposure. 3) The cost for a long term, large animal nose only exposure study is significantly greater than for a whole body exposure study due to extensive

animal handling and monitoring requirements. Since there is little scientific merit in conducting a nose only study, the additional cost associated with a nose only study would provide little or no value. 4) Whole body exposure is more representative of human exposure conditions.

The protocol states that animals will be exposed to the test material 5 days/week during the 10-week pre mating periods and 7 days/week during the breeding, gestation and lactation periods. The 5 day/week exposures were selected to simulate human exposures. Five days/week during a 10 week pre mating period is an adequate period of exposure to produce effects on male or female gonadal growth and function. The exposures were extended to 7 days/week following the pre mating period in view of the compressed time frame associated with the critical reproductive and developmental events of mating, gestation and lactation in the rodent, relative to humans. In rodents, embryonal/fetal (22 days) and neonatal (21 days prior to weaning) development are rapid, requiring daily exposure to assure the animals are exposed during all critical gestational and lactational events.

Areas in the protocol identifying information regarding exposure, housing and duration of exposure.

The following sentence in the protocol has been modified to provide greater clarification regarding animal housing procedures. The protocol modifications are in italics. Page 7, line 9. "All rats will be housed continuously in exposure chambers following the initial exposure to the test material, except during late gestation and throughout lactation periods when female rats will be housed outside of the exposure *chambers during non-exposure periods.*"

Exposure Levels Attained

Pharmacokinetics is beyond the scope and objectives of this study, and therefore, have not been incorporated into the design of this study. Pharmacokinetics are best obtained under a separate protocol and study design.

The rationale for housing females outside of the exposure chambers during late gestation and throughout lactation, during non exposure periods, is

logistical. Since the females will be held in the larger nesting boxes during late gestation and throughout lactation, following exposure, we chose to house the animals outside the actual exposure chambers to avoid crowding within the chambers. In addition, maintaining the nesting cages within the exposure chambers would necessitate exposing both the dam and pups during parturition and lactation. Exposing the dam during the process of parturition and the pups during lactation prior to weaning is unwarranted scientifically and not representative of human exposure. We are also not aware of any EPA Guideline inhalation studies designed for risk assessment that exposed the dam and pups during this period. It should also be noted that although the dam and pups will be housed outside the actual exposure chambers during late gestation and throughout lactation, following exposure, the rooms in which they will be housed will be identical unused exposure chambers. Since the choice of what room to house the animals in after exposure has no bearing on the study design, results, interpretation of the results or validity of the study, adding an extensive rationale to the protocol for this minor procedure is considered unnecessary.

Dams are withheld from the exposure chamber during the late gestation and early lactation period to allow the dam to deliver their litters and care for their pups during this critical period. Exposing the dams late in the gestation period (days 20-22) risks allowing the dams to deliver in the inhalation chambers and the loss of pups through the caging floor. The cages designed for inhalation exposure are made of wire mesh to allow unimpeded air flow and air circulation. The design of the inhalation cages are not compatible with maintaining the dams and their litters. Following parturition, the dams are allowed to remain with their litters for the first four days of lactation to clean and care for the young at a time in which the young are highly susceptible to neglect. It is well documented that removing the dams during the 6-hour exposure periods during lactation days 5 through 21 has an effect on pup growth. It is also well documented that infant weight and mortality are inversely correlated. Although we are not aware of any published data on whether or not increased pup mortality occurs if you remove the dam from the litter during the first four days of lactation, common sense tells us that we would exacerbate the well known growth retardation observed with removal of the dam during later periods of lactation and likely affect early mortality.

Certainly, pup mortality would be affected if the dams were allowed to deliver their litters in the chambers and have their pups fall through the wire mesh floor.

Comments regarding the ages of P2 animals at body weight and feed consumption measurement intervals. Mating of the P1 animals occurs over a two-week period. Past experience indicates that the majority of animals (70 to 100%) mate in the first week of mating. Therefore, the majority of the animals will be of similar age (within one week). For the pups from dams that mated during the second week of mating, it is clear that they will be one week younger than animals from the first week of mating. However, barring a highly significant treatment related effect on the timing of mating, the age spread of the animals should have no impact on the growth curves as the control group will have the same age distribution. In addition, by the time the P2 animals are mated at 13 to 14 weeks of age, the one week differences in age will not be a significant factor in growth or sexual maturation/development.

Day Weaning Occurs (page 8, culling and weaning)

The protocol, page 8, paragraph 1, will be revised as follows (revision made in italics). "Weaning of all litters will be done *on lactation day 21*." This correction should be sufficient to clarify the procedures. The repetitive definition of days 0 and 1 postpartum was not deemed necessary.

Culling (page 8, culling and weaning)

Culling of litters is allowed by US EPA guidelines. Due to the practices of the animal suppliers, the CD rat widely used for reproduction studies has been genetically selected for large litter sizes. As a result of this selective pressure, the litter size of the CD rat has increased significantly, beyond what is typical for this species. Culling of litters on lactation day 4 reduces the variation in pup growth associated with differences in litter size. The reduced interlitter variation can improve our ability to pick up true treatment-related differences in pup growth. The majority of pup mortality occurs early in lactation during days 0 through 4. Culling on lactation day 4 does not limit our ability to detect effects on viability during this sensitive period. We are also not aware of any data that documents that culling of litters on lactation day 4 confounds pup mortality in later periods of lactation. Regarding the

culling and definition of runts, the protocol clearly states that preferential culling of runts will not be performed. Extensive definitions of all terminology were not included in the protocol as this level of detail was considered unwarranted. However, the definition of runt will be included in the final report. Since the protocol clearly states that pups to be culled are selected using a computer generated randomization procedure, the reviewer's comment on needing a definition of runt to determine if preferential culling of runts will not occur makes no sense and is difficult to address beyond what has already been stated.

Feed Consumption (page 8)

Measuring feed consumption does not necessitate having feed containers in the chambers during the exposure period. In fact, the procedure for obtaining feed consumption during an inhalation study is to remove the feed containers during exposure and to return them following exposure. This procedure eliminates the potential for oral exposure due to absorption of the test material into the feed.

Body Weight (pages 8 and 9)

Historically, the body weight of dams from reproduction and developmental toxicity studies conducted in this laboratory, including inhalation studies, have been collected on gestation day 21. Therefore, the historical data from this laboratory include gestation day 21 data. Body weights in inhalation studies are generally collected in the morning prior to exposure and prior to the onset of delivery (gestation day 21). So as to assure collection of as much data as possible, morning measurements of gestation day 21 body weights will be recommended.

Litter Data (page 9)

On the day of parturition, dams and litters are observed for the number of pups delivered and the presence of dead pups. In order to reduce handling stress on the day of parturition, disturbances are kept to a minimum and data collection limited. The critical parameter observed on gestation day 0 is the live-dead count.

Reproduction - Pathology - Adult Rats (page 10)

The rats are to be euthanized after the last litter has been weaned. This necropsy schedule was chosen for logistical reasons and to allow for flexibility in the study design. Euthanizing the dam immediately following weaning of the litter would result in a two-week necropsy period (highly inefficient) and preclude breeding the dams for a second litter. A common necropsy date after the last litter has been weaned allows time to review all the litter data and make informed decisions as to the future direction of the study. Should the first litter produce equivocal results or results requiring follow-up investigations, additional litters (F1b or F2b) can be incorporated into the study protocol.

Reproduction - Organ Weights- Adults Rats (page 10)

The first 15 P1 and P2 adults were selected for organ weight measurements. Fifteen animals per group provide adequate sample size and power to detect biologically significant treatment related effects. Since the animals are randomly assigned to treatment group, and randomly placed within treatment group, the first 15 animals are, in effect, randomly selected. The selection of animals is not based on mating or delivery sequence.

Reproduction - Sperm Count, Motility and Morphology (page 11)

The rationale for selecting the first 15 males for sperm analyses is the same rationale provided above for organ weights. The source of sperm for motility analyses will be changed in the protocol to specifically state only caudal epididymis.

Statistical Evaluation (page 14)

The authors feel it is unnecessary to provide extensive definitions of terminology throughout the protocol, especially in the statistical analyses section. All terms required to interpret the results of the study will be defined in the final report.

Typographical Errors

All typographical errors identified by the reviewer have been corrected.

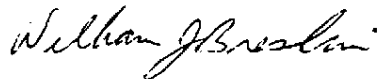
Reviewer: Dr. R. D. Hood

Reviewer comment no. 1 regarding the typographical error on the limit dose of 1 mg/kg body weight. The 1 mg/kg value has been corrected to read 1000 mg/kg/day.

Reviewer comment no. 2 regarding the calculated dose 1010 vs. 1012. Recalculation of the dose using the formula and data provided on page 6 of the protocol results in a value of 1012. The discrepancy between our numbers may be due to rounding differences.

Reviewer: B. F. Hales

No response, other than correcting typographical errors was required. Typographical errors were corrected.

A handwritten signature in cursive script, appearing to read "William J. Breslin".

William J. Breslin, Ph.D.

