

National Toxicology Program

**Peer Review of the Draft NTP Technical Reports on Prenatal
Developmental Toxicity Studies for: Tris(chloropropyl)
Phosphate, 4-Methylcyclohexanemethanol, Vinpocetine, and
Dimethylaminoethanol Bitartrate**

July 31, 2019

**National Institute of Environmental Health Sciences
Research Triangle Park, NC**

Peer Review Report

Table of Contents

1.	Attendees	3
2.	Introductions and Welcome.....	4
3.	Public Comments	4
4.	Background and Charge to the Panel	4
5.	Prenatal Developmental Toxicity Studies of Tris(chloropropyl) Phosphate.....	4
5.1.	Presentation and Clarifying Questions.....	4
5.2.	Peer Review Comments and Panel Discussion.....	5
5.2.1.	First Reviewer – Dr. Cheryl Broussard	5
5.2.2.	Second Reviewer – Dr. Alan Hoberman	5
5.3.	Vote on NTP Conclusion	6
6.	Prenatal Developmental Toxicity Studies of 4-Methylcyclohexanemethanol	7
6.1.	Presentation and Clarifying Questions.....	7
6.2.	Peer Review Comments and Panel Discussion.....	9
6.2.1.	First Reviewer – Dr. Mary Alice Smith.....	9
6.2.2.	Second Reviewer – Dr. Cheryl Broussard	9
6.2.3.	Third Reviewer – Dr. Linda Roberts	10
6.2.4.	Panel Discussion	10
6.3.	Vote on NTP Conclusion	11
7.	Prenatal Developmental Toxicity Studies of Vinpocetine	11
7.1.	Presentation and Clarifying Questions.....	11
7.2.	Peer Review Comments and Panel Discussion.....	13
7.2.1.	First Reviewer – Dr. Alan Hoberman	13
7.2.2.	Second Reviewer – Dr. Linda Roberts	13
7.2.3.	Third Reviewer – Dr. Kimberley Treinen	13
7.2.4.	Other Comments	13
7.3.	Vote on NTP Conclusion	13
8.	Prenatal Developmental Toxicity Studies of Dimethylaminoethanol Bitartrate.....	14
8.1.	Presentation and Clarifying Questions.....	14
8.2.	Peer Review Comments and Panel Discussion.....	14
8.2.1.	First Reviewer – Dr. Kimberley Treinen	14
8.2.2.	Second Reviewer – Dr. Mary Alice Smith	15
8.2.3.	Panel Discussion	15
8.3.	Vote on NTP Conclusion	15
9.	Closing Remarks on the Draft Reports.....	16
10.	Approval of the Peer Review Report by the Chair of the Peer Review Panel	16

1. Attendees

Peer Review Panel

Chair: George Daston, The Proctor and Gamble Company

Cheryl Broussard, Centers for Disease Control and Prevention

Alan Hoberman, Charles River Laboratories Preclinical Services

Linda Roberts, NapaTox Consulting LLC

Mary Alice Smith, University of Georgia

Kimberley Treinen, Sunovian Pharmaceuticals Inc.

National Toxicology Program Board of Scientific Counselors Liaison

Donald Stump, Charles River Laboratories International

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Brian Berridge

Barry McIntyre

Chad Blystone

Georgia Roberts

Bradley Collins

Kristen Ryan

Michelle Cora

Sheena Scruggs

Helen Cunny

Keith Shockley

Shawn Harris

Matthew Stout

Michelle Hooth

Vicki Sutherland

Angela King-Herbert

Nigel Walker

Elizabeth Maull, Designated Federal
Official

AtLee Watson

Mary Wolfe

Other Federal Agency Staff

Gonçalo Gamboa, FDA

Contract Support Staff

Susan Blaine, ICF

Steve McCaw, Image Associates

Dave Burch, ICF

Blake Riley, ICF

Lindsey Green, ICF

Samantha Snow, ICF

Ernie Hood, Bridport Services

2. Introductions and Welcome

The National Toxicology Program (NTP) convened a peer review panel for the *Draft NTP Technical Reports on Prenatal Developmental Toxicity Studies for: Tris(chloropropyl) Phosphate, 4-Methylcyclohexanemethanol, Vinpocetine, and Dimethylaminoethanol Bitartrate* on July 31, 2019, in Conference Room F193, Rall Building, National Institute for Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina (or via webcast).

- Dr. George Daston, panel chair, called the meeting to order at 8:30 a.m., welcomed everyone to the meeting, asked all attendees to introduce themselves, and reviewed the format for the peer review meeting for the panel and audience.
- Dr. Elizabeth Maull read the conflict of interest policy statement and briefed the attendees on meeting logistics.
- Dr. Donald Stump attended as the liaison to the NTP Board of Scientific Counselors.

3. Public Comments

Dr. Daston noted that no written public comments or requests for oral public comments on the draft technical reports had been received.

4. Background and Charge to the Panel

Dr. Chad Blystone gave a brief presentation on NTP draft technical reports, including information about the levels of evidence for developmental toxicity. He also described the Developmental and Reproductive Toxicity (DART) historical controls and the charge to the panel for the individual peer reviews:

- Review and evaluate the scientific and technical elements of each study and its presentation.
- Determine whether each study's experimental design, conduct, and findings support NTP's conclusions regarding the developmental toxicity of the substances tested.

5. Prenatal Developmental Toxicity Studies of Tris(chloropropyl) Phosphate

5.1. Presentation and Clarifying Questions

Dr. Kristen Ryan summarized the studies and conclusions reported in the *Draft NTP Technical Report on the Prenatal Developmental Toxicity Studies of Tris(chloropropyl) Phosphate (CASRN 13674-84-5) in Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats (Gavage Studies)*.

Tris(chloropropyl) phosphate (TCPP) is a flame retardant found in a variety of commercial and consumer products. It is ubiquitous but not bioaccumulative in the environment. Exposure can occur via dermal, oral, or inhalation routes. TCPP is a mixture constituted primarily of four isomers; the research focus is often on the primary isomer due to its abundance. The test article used for the NTP studies contained all four isomers. The goal of this study was to characterize the effects of TCPP exposure on pregnant rats and developing fetuses.

The dose range-finding study was conducted in 11 time-mated female rats using doses of 0, 300, 650, and 1,000 mg/kg/day, administered via gavage. Adverse signs at 1,000 mg/kg/day occurred throughout gestation. These results informed the use in the main study of doses of 0, 162.5, 325, and 650 mg/kg/day in 25 time-mated female rats per group. An additional 25 control dams were added to this study to supplement historical control data for maternal and fetal findings. The main study findings revealed:

- No maternal treatment-related effects on mortality or body weights during gestation.
 - Clinical observations were of low incidence and limited to the 650 mg/kg/day group
 - At 650 mg/kg/day, absolute and relative liver weights were increased approximately 26%
- No treatment-related effects on uterine or litter parameters, such as implantations, litter size, live fetuses per litter, or fetal weight
- Fetal skeletal malformations of limited toxicological relevance (e.g., lumbar rudimentary ribs) or those that occurred as single or sporadic incidence

Under the conditions of this prenatal study, NTP's draft conclusion was:

- *No evidence of developmental toxicity* of TCPP in Hsd:Sprague Dawley rats administered 162.5, 325, or 650 mg/kg/day in the absence of overt maternal toxicity.

There were no clarifying questions or comments about the presentation.

5.2. Peer Review Comments and Panel Discussion

5.2.1. First Reviewer – Dr. Cheryl Broussard

Dr. Broussard indicated that the study was clearly described, well conducted, and the conclusions followed logically from the presented findings. She agreed with NTP's draft conclusions. Dr. Broussard then recommended adding language explaining the rationale for limiting soft tissue examination to only 50% of the heads. She also requested that NTP clarify more specifically where the audit procedures and findings were located to aid in transparency. The comments regarding soft tissue allocations and audit procedures applied to all reports. Finally, Dr. Broussard questioned why blood was not collected from the dams for clinical pathology.

- Dr. Ryan noted that the allocation for fetal exams was based on the study guidelines, with every other fetus allocated for head examination. She agreed that NTP could consider adding more information on fetal exam allocations and the use and location of the audit procedures and findings, which are archived electronically, to the reports.
- Dr. Ryan stated that blood chemistry was not typically required in this type of study. Furthermore, these endpoints were not identified in the literature as a primary concern for TCPP exposure.

5.2.2. Second Reviewer – Dr. Alan Hoberman

Dr. Hoberman stated that the study was well conducted, and he did not disagree with the conclusion. However, he noted that the only individual data presented in the reports were fetal

data and that the public would benefit from having access to all individual animal data. This comment applied to all reports. The presence or absence of deviations should be included in the report. The historical control data lacked information on post-implantation loss percentages as well as other fetal information. The report failed to comment on an earlier study by Kawasaki (1982) that noted an increase in cervical ribs. Although Dr. Hoberman understood the use of two control groups, he noted that inclusion of group variations would have been informative. He also noted that this class of compound is known to produce enlarged livers, which may be considered an adaptive change rather than maternal toxicity. Because NTP referenced the changes in liver weights, the authors must have considered that the change in weight represented some sort of system perturbation. He recommended adding some discussion detailing why the enlarged liver was not considered as maternal toxicity.

In response to Dr. Hoberman's comments, Dr. Ryan indicated that:

- NTP would consider adding language to the report specifying the location of the individual animal data.
- Deviations are listed in the good laboratory practices report. NTP would consider adding a line to the main report such as “no other deviations were noted.”
- NTP is currently evaluating the historical control data and will be adding information (i.e., fetal and uterine parameters) to the database. Post-implantation loss observed in this study was limited to a single litter and was not considered an exposure-related finding.
- NTP evaluates cervical ribs as part of the fetal examinations. Although an increase in cervical ribs had been observed in the Kawasaki study, they were not seen in the NTP study, and, therefore, not populated in the historical control database. This information could be added.
- She reviewed the cross-reference data from dams to fetuses from the two control groups prior to the data being pooled and found that there were comparable findings in both control groups.
- NTP chose to report that no developmental toxicity was observed in the absence of overt maternal toxicity in this study and indicated that NTP would consider adding language to clarify the issues related to enlarged liver in the discussion.

5.3. Vote on NTP Conclusion

Dr. Daston called for a motion from the panel to approve the conclusion as written. Dr. Hoberman so moved and Dr. Kimberley Treinen seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusion as written.

6. Prenatal Developmental Toxicity Studies of 4-Methylcyclohexanemethanol

6.1. Presentation and Clarifying Questions

Dr. AtLee Watson summarized the studies and conclusions reported in the *Draft NTP Technical Report on the Prenatal Developmental Toxicity Studies of 4-Methylcyclohexanemethanol (CASRN 34885-03-5) in Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats (Gavage Studies)*.

4-Methylcyclohexanemethanol (MCHM) was the chemical involved in the 2014 Elk River Chemical Spill in West Virginia. An estimated 10,000 gallons of crude MCHM leaked into the river, contaminated the municipal water supply, and likely led to human exposure. This prenatal developmental toxicity study resulted from concern for women of child-bearing potential and developing embryos/fetuses, and provided an opportunity to evaluate the adequacy of the 1 part per million advisory level set forth by the Centers for Disease Control and Prevention (CDC) and the Agency for Toxic Substances and Disease Registry (ATSDR)¹ for MCHM in drinking water.

The dose range-finding study tested doses of 0, 150, 300, 600, and 900 mg/kg/day in groups of 10 time-mated female rats each and examined maternal and fetal endpoints. In this study, exposure to 600 and 900 mg/kg/day resulted in dose-related mortality and clinical observations of toxicity. These results informed the selection of doses of 0, 50, 100, 200, and 400 mg/kg/day for the main study in 25 time-mated female rats per group. Main study findings included:

- Reduced maternal serum total protein and globulin at doses ≥ 100 mg/kg/day
- Fetal findings at 400 mg/kg/day:
 - Decreased fetal body weights (15%) and gravid uterine weight (18%) compared with controls
 - Increased incidences of malformations of the axial skeleton
 - Misshapen adrenal glands (malformation)
- No exposure-related fetal findings at doses ≤ 200 mg/kg/day

Under the conditions of this prenatal study, NTP's draft conclusion was:

- ***Clear evidence of developmental toxicity*** of MCHM in Hsd:Sprague Dawley rats at 400 mg/kg/day in the absence of overt maternal toxicity based on findings of:
 - Reduced fetal weight
 - Malformations of the axial skeleton
 - Malformations of the adrenal glands

As a follow up to the presentation, panelists had the following clarifying questions and discussion:

¹ The CDC level was recommended the day of the spill (January 9th, 2014), according to "After Action Review of the January 9, 2014 Freedom Industries Chemical Spill" available at <http://dhsem.wv.gov/West%20Virginia%20Public%20Water%20Supply%20Study%20Commission/Documents/After%20Action%20Review.PDF>.

Topic – Malformation of the adrenal glands

- Dr. Linda Roberts asked for a description of the criteria for classifying the adrenal glands as misshapen.
- Dr. Hoberman asked if histopathology is routinely performed when necrotic masses are observed on adrenal glands. Although this finding appeared in three fetuses from different litters, the genealogy of the litters was unknown, which may play a role in the occurrence rate. Responding to a question posed by Dr. Roberts, Dr. Hoberman stated he could not recall ever seeing a misshapen adrenal with a necrotic mass. Dr. Sutherland agreed that it was an unusual finding.
 - Dr. Watson indicated that the misshapen adrenal designation was attributed to the presence of a necrotic mass on the adrenal glands.
 - Dr. Watson stated that while histopathology could inform whether the occurrence of a necrotic mass on the adrenal gland represents a permanent change or would alter postnatal or subsequent development, guideline prenatal developmental toxicity studies do not routinely call for it.

Topic – Clinical chemistry endpoints

- Dr. Daston inquired if the clinical chemistry findings on glucose, triglycerides, and blood urea nitrogen levels were also observed in other subchronic MCHM studies or if the changes in the clinical chemistry endpoints were specific to the pregnancy in the rat.
 - Dr. Watson noted that there was a decrease in some of the red blood cells in the repeat dose oral gavage study that was conducted by the Eastman Chemical Company. He indicated that Eastman Chemical Company did not observe the same glucose findings.

Topic – Potential MCHM review article

- Dr. Daston noted that NTP played a significant role in quickly developing information on MCHM and wondered if there will be a larger synthesis of information based on this and other recently conducted studies. He added that there would be interest in these types of summary reports from people who were exposed and who had made health decisions based on what the scientific community conveyed to them. The current report format may be difficult for the general public to understand given the dry and science-based conclusions they contain.
 - Dr. Watson indicated that NTP's website currently has summary findings, but the development of a report summarizing all MCHM-related NTP studies would be addressed in subsequent NTP discussions.
 - Dr. Blystone noted that prior communications to the stakeholders were less dry and more informal.

6.2. Peer Review Comments and Panel Discussion

6.2.1. First Reviewer – Dr. Mary Alice Smith

Dr. Smith indicated that the study was designed and conducted according to accepted DART guidelines. She stated that the findings in the study, including reduced fetal weight, adrenal malformations, and increased malformations of the axial skeleton, support the conclusion of clear evidence of developmental toxicity of MCHM in the fetuses from dams exposed to 400 mg/kg/day. Dr. Smith recommended adding historical normal pregnant rat clinical chemistry ranges (as reported for human studies) to the report, which would help interpret the exposure-related data. Adding to this comment, Dr. Daston asked if some of the qualitative statements on clinical chemistry endpoints found in the report might be expanded on to put this type of data in context. Dr. Smith recommended that the report clearly state that the dose-related changes are significantly different from the controls based on a dose-related trend or a pairwise comparison effect and to include this type of information in the conclusion statements. Finally, she requested inclusion of a 2018 human epidemiology study investigating the possible association of adverse birth defects with exposure to crude MCHM from the spill site.

- Dr. Michelle Cora, NTP Clinical Pathologist, responding to the clinical chemistry questions, noted that currently the NTP does not have historical control data for pregnant rats. She added that reporting values from the study's controls are preferred over those of historical controls due to the number of uncontrolled variables (i.e., animal diet, conditions of the study, type of machine the samples were run on) that influence historical control data. She added that the range for clinical chemistry data indicated in these reports is typically the standard error. Expansion beyond qualitative statements would not be feasible.
- Dr. Watson agreed that inclusion of dose-related response in the fetal body weight conclusion would improve the comprehension of the data but was concerned that it could overcomplicate the conclusion statement. He indicated that NTP would consider implementing this recommendation if it could be done in a concise manner.
- Dr. Watson indicated that he would incorporate the 2018 study, which found no adverse birth outcomes following the spill, in the report's discussion.

6.2.2. Second Reviewer – Dr. Cheryl Broussard

Dr. Broussard found the study design clearly described and well conducted, and that the conclusions followed logically from the presented findings. She agreed with the draft conclusion of clear evidence of developmental toxicity. She suggested adding the rationale for why approximately 50% of the heads were examined for soft tissue alterations, as well as being more transparent about where to find the audit procedures and findings. She wondered whether the Sentinel Animal Program described in some of the other reports was relevant here also.

- Dr. Watson replied that:
 - NTP would add the rationale to the methodology section of the report.
 - Given the short duration of these studies, a Sentinel Animal Program is not required. The dams received by the lab underwent a full evaluation by the staff

animal veterinarian before they were cleared to be included in the study. That information is included in the report.

6.2.3. Third Reviewer – Dr. Linda Roberts

Dr. Roberts indicated that the studies were conducted properly and agreed, with a single caveat, with the NTP conclusion. She was not as confident with a classification of “clear evidence” versus “some or equivocal” evidence based on the absence of statistical significance in misshapen adrenal glands in the historical controls. The strongest evidence for developmental toxicity was the reduction in fetal body weight. To clarify Dr. Roberts’ comments, Dr. Daston asked her to confirm that she thought there was clear evidence that MCHM causes developmental effects based on fetal weight and skeletal malformations, but not changes in adrenal malformation. Dr. Roberts confirmed that this was a correct interpretation of her thoughts.

Dr. Roberts also expressed appreciation that the fetal no-observed-effect level (NOEL) was included in the report and noted that the maternal NOEL of 50 mg/kg/day was based on the clinical chemistry endpoints. She stated that although there was statistical significance in these endpoints, she was less confident that there was biological significance.

- Dr. Watson agreed that additional information discriminating between structural malformations and alterations that might affect postnatal development would be useful to help understand the significance of the effect. It was difficult to confirm whether there was a pairwise significant difference in the highest dose group for this finding due to the very low incidences. Dr. Watson noted that NTP takes litter incidence into account. The fact that the findings occurred in three single fetuses from three separate litters support the conclusion that the adrenal malformation was a treatment-related effect.

6.2.4. Panel Discussion

Dr. Kimberley Treinen questioned the choice of reporting NOEL for maternal toxicity rather than the no-observed-adverse-effect level (NOAEL). She mentioned that the entire call was characterized as being “in the absence of overt maternal toxicity,” by which she assumes to be a NOAEL. Dr. Treinen also recommended adding a line to the summary table correcting for uterine weight. She noted that the study reported high non-pregnancy rates, along with a high rate of misshapen aortic valves. She would like to have seen a lower background rate, given concern about cardiovascular malformations in the controls. The relatively large increase in the axial skeletal malformations with limited variations in other endpoints was an unusual finding. Dr. Treinen recommended that further elaboration is needed in the report to describe the misshapen adrenal glands, perhaps by providing images, given that this is an unusual finding. Dr. Daston agreed that this issue needs more attention in the report.

- Dr. Watson indicated that NTP avoided distinguishing between adverse and non-adverse effects. Using the NOEL designation avoided some of the close calls that would have been generated by using NOAEL.
- Dr. Vicki Sutherland noted that:
 - NTP would consider adding language to the tables as recommended.

- At the time of the study, there was a concern about successful pregnancy rates, which has since improved with increased training, suggesting this was not a strain-related effect. NTP uses the same strain across all its studies.
- NTP will consider directing the lab to follow up with histopathology in the future if this finding is present. NTP will also ascertain if this finding is specific to this strain of rat.

Dr. Daston noted that the significant decrease in dam body weight with a significant increase in food consumption was a remarkable finding that, combined with the findings on blood glucose, suggests something interesting going on beyond general maternal toxicity—something that may yield an indication of a mechanism of action. The phenomenon deserved more treatment in the report.

- Dr. Watson said that data from a MCHM toxicogenomics study suggested that fatty acid metabolism may be involved as a mechanism of action. He indicated that NTP would add a discussion to the report.
- Dr. Cora remarked that although she thought the change in blood glucose levels was real, the rats would not be considered hypoglycemic, and the mild decrease is seen with some frequency. She said the triglycerides were affected by what the dams were eating and when they had last ingested food.

6.3. Vote on NTP Conclusion

Dr. Daston called for a motion to accept the conclusion as written, understanding that there would be information added to the report on the adrenal malformations. Dr. Roberts said she would prefer that the reference to adrenal gland malformations be removed from the conclusion. Dr. Smith moved to accept the conclusion as written and Dr. Broussard seconded. The panel passed the motion (4 yes, 1 no, 0 abstentions). Dr. Roberts voted no, citing her discomfort with including the adrenal malformations as the reason for her vote.

7. Prenatal Developmental Toxicity Studies of Vinpocetine

7.1. Presentation and Clarifying Questions

Dr. Sutherland summarized the studies and conclusions reported in the *Draft NTP Technical Report on the Prenatal Developmental Toxicity Studies of Vinpocetine (CASRN 42971-09-5) in Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and New Zealand White (Hra:NZW SPF) Rabbits (Gavage Studies)*.

Vinpocetine is marketed as a dietary supplement for cognitive enhancement. It is also a semi-synthetic/synthetic pharmaceutical agent for treatment of cerebrovascular and cognitive disorders. NTP chose to study vinpocetine due to concerns of consumer exposure through dietary supplement use, signals of developmental toxicity in the literature, and lack of adequate toxicity data.

The rat dose range-finding study used doses of 0, 20, 40, 80, 160, or 320 mg/kg/day via gavage, with 10 time-mated female rats per group. A dose-related decrease in maternal body weight correlated with fetal loss at the higher two doses in this study. These results informed the

selection of doses of 0, 5, 20, and 60 mg/kg/day for the main study in 25 time-mated female rats per group. Findings from the main study included:

- Dose-related increase in the incidence of vaginal discharge (20 and 60 mg/kg/day)
- Decreased maternal body weight
- Exposure-related increases in post-implantation loss (83% at 60 mg/kg/day)
- Fetal examination findings such as:
 - Increased incidences in the percent of fetuses with ventral septal defect (malformation)
 - Increased incidences of incomplete ossification of the thoracic centra (variation) and full thoracolumbar ribs (malformation)

The above findings provided sufficient concern to examine the effects of vinpocetine in a second species, the rabbit. The dosages chosen for the rabbit study were 0, 25, 75, 150, and 300 mg/kg/day, administered via gavage to eight time-mated female animals per group. The main rabbit study findings revealed:

- Decreased maternal body weight gains at 150 and 300 mg/kg/day
- Exposure-related effect on embryo-fetal survival at 300 mg/kg/day

Data from the rabbit study supported the findings observed in the rat dose range-finding study and rat prenatal developmental toxicity studies.

Under the conditions of the rat prenatal study, NTP's draft conclusion was:

- ***Clear evidence of developmental toxicity*** of vinpocetine in Hsd:Sprague Dawley rats in the absence of overt maternal toxicity based on findings of:
 - Increased post-implantation loss
 - Increased incidences of ventricular septum defects
 - Increased thoracolumbar ribs (full)
 - Increased incidences of incomplete ossification of the thoracic centrum

As a follow up to the presentation, participants had the following clarifying question and discussion:

Topic – No Effect Levels

- Dr. Roberts noted that the study did not include NOEL values and asked whether that was intentional.
 - Dr. Sutherland responded that NTP had internal discussion about the language; if the panel feels that NOELs should be included in all the reports, the team will consider modifying the text.

7.2. Peer Review Comments and Panel Discussion

7.2.1. First Reviewer – Dr. Alan Hoberman

Dr. Hoberman expressed appreciation to NTP for completing the study of this dietary supplement, approved performing the studies in both the rat and rabbit, and overall agreed with the conclusion. He recommended that individual animal data be made available for this report and all other studies and thought that including the onset and duration for clinical signs, such as vaginal discharge, could be informative. While recognizing that the studies were hazard assessments and not risk assessments, Dr. Hoberman also thought it would be beneficial to report how the animal doses in the study compared with human doses.

Dr. Sutherland responded that:

- The individual data are available online and indicated that NTP would consider how to make access more apparent in the reports.
- The vaginal discharge data did not directly correlate with embryonic loss.
- NTP considered risk assessment information outside the scope of this report.

7.2.2. Second Reviewer – Dr. Linda Roberts

Dr. Roberts commented that the study was well conducted and appreciated that a second species was included. She said that the body weight gain seen did not meet the criteria for overt maternal toxicity. She agreed with the clear evidence conclusion as written.

7.2.3. Third Reviewer – Dr. Kimberley Treinen

Dr. Treinen recommended that an additional line be added to the summary table with corrected numbers for maternal body weight. She noted that there was a comment made in the rabbit study that food consumption might have contributed to the body weight decrement, but it appeared that it was more attributable to the decrease in implants.

In response to Dr. Treinen's comments, Dr. Sutherland indicated that:

- NTP would consider adding corrected body weight in the text and tables if that would add clarity.
- The food consumption was not directly correlated to embryonic loss.

7.2.4. Other Comments

Dr. Gonçalo Gamboa, FDA, thanked NTP for keeping the FDA apprised as to the results. He noted that FDA released a statement cautioning women of child-bearing ages from consuming this chemical. He appreciated the good communication.

7.3. Vote on NTP Conclusion

Dr. Daston asked for a motion and second from the panel to approve the conclusion as written. Dr. Roberts so moved and Dr. Hoberman seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusion as written.

8. Prenatal Developmental Toxicity Studies of Dimethylaminoethanol Bitartrate

8.1. Presentation and Clarifying Questions

Dr. Sutherland summarized the studies and conclusions reported in the *Draft NTP Technical Report on the Prenatal Developmental Toxicity Studies of Dimethylaminoethanol Bitartrate (CASRN 5988-51-2) in Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats (Gavage Studies)*.

Dimethylaminoethanol bitartrate (DMAE) is a close structural analog of the essential nutrient choline. It is marketed as a dietary supplement to improve memory and general cognitive function. As there is both potential for widespread human exposure through its use in industrial and consumer products and limited evidence from the literature that it may be a teratogen and reproductive toxicant, NTP chose to study DMAE.

The dose range-finding study used doses of 0, 250, 500, and 1,000 mg/kg/day via gavage, with 10 time-mated female rats per group. No maternal or fetal toxicity were present at the doses used in the range-finding study. The same doses were employed in the main study, which used 25 time-mated female rats per group. Findings from the main study revealed:

- No treatment-related effects on mortality, body weights, or feed consumption
 - Effects were sporadic or without a dose response
- No effects on uterine or litter parameters such as implantations, litter size, live fetuses per litter, or fetal weight
- Fetal examination findings of:
 - Increased incidence of short thoracolumbar ribs (a variation) at the 1,000 mg/kg/day dose
 - Increased incidence in the number of supernumerary sites, or ossification sites, in the skull at the 1,000 mg/kg/day dose

Under the conditions of this prenatal study, NTP's draft conclusion was:

- ***Equivocal evidence of developmental toxicity*** of DMAE in Hsd:Sprague Dawley rats in the absence of overt maternal toxicity based on increased incidences of:
 - Short thoracolumbar ribs
 - Supernumerary sites in the skull

There were no clarifying questions or comments about the presentation.

8.2. Peer Review Comments and Panel Discussion

8.2.1. First Reviewer – Dr. Kimberley Treinen

Dr. Treinen commented that the study was well conducted and met the standard for this type of study. She wondered why the absent innominate artery in the high dose group was not considered a finding, even though it was statistically different from controls and was present across multiple litters. When combined with short innominate arteries, it potentially looked like a

dose-related effect. Dr. Hoberman commented that the absent innominate artery is a very common variation. However, he added that it and other similar variations do seem to indicate a perturbation in the system and should be investigated.

Dr. Treinen recommended breaking down the historical controls rather than lumping them together.

- Dr. Sutherland noted that the absent innominate artery is an extremely common finding and therefore was not included as a potential toxicity endpoint.

8.2.2. Second Reviewer – Dr. Mary Alice Smith

Dr. Smith thought that the maternal death in the 1,000 mg/kg/day dose group raised a question and recommended adding more historical control data in the report. She remarked that there was not a lot of evidence for dose-related outcomes in this study. In addition, Dr. Smith cautioned against concluding that there were no brain effects, and recommended qualifying the statement by indicating that there were no lesions noted in the brain because functional outcomes were not evaluated. Dr. Smith said that it should be made clear that there were no structural changes in the brain.

Dr. Sutherland responded that:

- More historical control data would be helpful.
- NTP only looked for structural changes in the brain. NTP will ensure that it is clear that there were no structural changes in the brain in the revised report.
- Individual data tables were available, but NTP needs to consider how to make them easier to access.
- The primary report focused on bringing forward positive findings; therefore, negative findings were not highlighted. She mentioned that this distinction would be clarified in the report.

8.2.3. Panel Discussion

Dr. Roberts indicated that the innominate artery finding should have received more attention in the report. Dr. Sutherland asked if she was suggesting more detail in the discussion or an addition to the conclusion. Dr. Roberts responded both.

8.3. Vote on NTP Conclusion

Dr. Daston proposed adding a third bullet to the draft NTP conclusion to read “increased incidence of absent innominate artery.” He called for a motion to add the bullet to the NTP conclusion. Dr. Treinen so moved and Dr. Smith seconded. Dr. Daston called for a vote on the conclusion, including the addition. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusion with the addition.

9. Closing Remarks on the Draft Reports

Dr. Daston welcomed additional panel comments on the overall organization of the reports. Dr. Hoberman suggested clarifying the definition of the term “natural death” used throughout the reports.

- Dr. Sutherland noted that they would revise the term to “found dead” in the reports.

Dr. Treinen recommended that litter data, as well as individual data, be added to the reports or compiled as a stand-alone report to assist with understanding the rate of resorptions and other important fetal findings. Dr. Hoberman added that it was standard to have that type of information in a toxicology report.

- Dr. Blystone remarked that NTP could explore adding some of the selected endpoints in an appendix.

Dr. Roberts appreciated having the pharmacokinetic information in the report along with its relevance to humans. She added that the value of including the NOEL eliminates the possibility of other researchers calculating their own NOEL based on the data in the report.

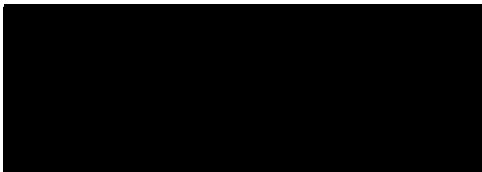
Closing the meeting, Dr. Maull thanked all the peer review panelists.

Dr. Daston added his thanks to NTP staff and the panel members for their efforts.

Dr. Daston adjourned the meeting at 11:22 a.m. EDT on July 31, 2019.

10. Approval of the Peer Review Report by the Chair of the Peer Review Panel

This peer review report has been read and approved by the chair of the July 31, 2019, Peer Review of the *Draft NTP Technical Reports on Prenatal Developmental Toxicity Studies for Tris(chloropropyl) Phosphate, 4-Methylcyclohexanemethanol, Vinpocetine, and Dimethylaminoethanol Bitartrate.*



George Daston, Ph.D.

Peer Review Panel Chair

Date: 10/18/19