National Toxicology Program

Peer Review of the Draft NTP Technical Reports on the Toxicology and Carcinogenesis Studies of Sodium Tungstate Dihydrate, Di-*n*-butyl Phthalate, and Di(2-ethylhexyl) Phthalate

April 2, 2021

National Institute of Environmental Health Sciences
Research Triangle Park, NC

Peer-Review Report

Table of Contents

1. Attendees[1]

Peer-Review Panel

Chair: Gabriele Ludewig, University of Iowa

Tracie E. Bunton, Eicarte, LLC

Michael R. Elwell, Apex Toxpath, LLC

Charles R. Mahrt, Retired, formerly with Flagship Biosciences

Daniel J. Spade, Brown University

John Pierce Wise, University of Louisville

National Toxicology Program Board of Scientific Counselors Liaison

Susan Felter, Procter & Gamble

National Institute of Environmental Health Sciences Staff

Mamta Behl

Chad Blystone

Mark Cesta

Sheba Churchill

Helen Cunny

Susan Elmore

Dori Germolec

Michelle Hooth

Madelyn (Mimi) Huang

Angela King-Herbert

Barry McIntyre

Georgia Roberts

Sheena Scruggs, Designated Federal Official

Kelly Shipkowski

Keith Shockley

Robert Sills

Stephanie Smith-Roe

Suramya Waidyanatha

Nigel Walker

Mary Wolfe

Other Federal Agency Staff

Shirisha Chittiboyina, National Institute for Occupational Safety and Health

Gonçalo Gamboa da Costa, U.S. Food and Drug Administration

[1]The meeting was held via webcast. Individuals who viewed the webcast are not listed except as noted.

Contract Support Staff

Amy Brix, EPL, Inc.

Canden Byrd, ICF

Lindsey Green, ICF

Shawn Harris, Social & Scientific Systems, a DHL Company

Elizabeth Maull, Kelly Government Services

Megan Rooney, ICF

Alessandria Schumacher, ICF

Cynthia Willson, Integrated Laboratory Systems, LLC

2. Introductions and Welcome

The National Toxicology Program (NTP) convened a peer-review panel for the *Draft NTP Technical Reports on the Toxicology and Carcinogenesis Studies of Sodium Tungstate Dihydrate, Di-n-butyl Phthalate, and Di(2-ethylhexyl) Phthalate* on April 2, 2021 via webcast.

- Dr. Gabriele Ludewig, panel chair, called the meeting to order at 10:00 a.m. and welcomed everyone to the meeting. She asked all attendees to introduce themselves, and reviewed the peer-review meeting format for the panel and audience.
- Dr. Mary Wolfe, Acting Deputy Director for Policy & Communication, welcomed all participants to the meeting.
- Dr. Sheena Scruggs read the conflict-of-interest policy statement and briefed the attendees on meeting logistics.
- Dr. Susan Felter attended as the liaison to the NTP Board of Scientific Counselors.
- Dr. Shirisha Chittiboyina attended as the liaison for the National Institute for Occupational Safety and Health.
- Dr. Gonçalo Gamboa da Costa attended as the liaison for the U.S. Food and Drug Administration.

3. Background and Charge to the Panel

Dr. Chad Blystone briefly presented the NTP draft technical report objectives, including a review of the levels of evidence for the potential carcinogenic activity and factors considered for tested chemicals. He also described how NTP collects historical control data[2] on neoplastic lesions and how these are utilized to provide context to report findings. Dr. Blystone provided the charge for the individual peer reviews:

- Review and evaluate the scientific and technical elements of the study and its presentation.
- Determine whether the study's experimental design, conduct, and findings support NTP's conclusions under the conditions of this study.

The peer-review meeting materials can be found on the NTP website.

4. Toxicology and Carcinogenesis Studies of Sodium Tungstate Dihydrate

4.1. Presentation and Clarifying Questions

Dr. Mamta Behl summarized the studies and conclusions reported in the *Draft NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Tungstate Dihydrate (CASRN 10213-10-2) in Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice (Drinking Water Studies)*.

[2] https://ntp.niehs.nih.gov/results/dbsearch/historical

Tungsten occurs naturally in the environment and can enter waterways through the weathering of rocks and soil. It was nominated for study due to concerns about potential widespread human exposure via contaminated drinking water. Sodium tungstate dihydrate was selected because it is a naturally occurring, water soluble form of tungsten. Drinking water was selected as the most likely route of exposure for the general population.

Dr. Behl first presented a summary of results from the perinatal and postweaning toxicity/carcinogenicity study in Hsd:Sprague Dawley® SD® rats. NTP exposed time-mated female rats to 0, 250, 500, or 1,000 mg/L sodium tungstate dihydrate in drinking water from gestational day (GD) 6 through postnatal day (PND) 21. NTP provided the F_1 generation rats with the same respective sodium tungstate dihydrate concentrations as their dam for 2 years (n=50/sex/concentration). In addition, F_1 generation rats were provided dosed drinking water or the vehicle control for 3, 6, 12, or 18 months for interim evaluations (n=40/sex/concentration).

Dr. Behl then presented a summary of results from the chronic toxicity/carcinogenicity study in B6C3F1/N mice. NTP exposed mice to 0, 500, 1,000, or 2,000 mg/L sodium tungstate dihydrate in drinking water for 2 years (n=50/sex/concentration). An additional 40 mice/sex/concentration were included for interim evaluations at 3, 6, 12, and 18 months.

Under the conditions of these 2-year studies, NTP's draft conclusions were:

- ***No evidence of carcinogenic activity*** in male Hsd:Sprague Dawley® SD® rats at 250, 500, and 1,000 mg/L.
- Exposure to sodium tungstate dihydrate in drinking water caused increased incidences of nonneoplastic lesions in the kidney of male rats.
- ***Equivocal evidence of carcinogenic activity*** in female Hsd:Sprague Dawley® SD® rats based on increased incidences of C-cell adenoma or carcinoma (combined) of the thyroid gland.
- Exposure to sodium tungstate dihydrate in drinking water caused increased incidences of nonneoplastic lesions in the kidney and uterus of female rats.
- ***Equivocal evidence of carcinogenic activity*** in male B6C3F1/N mice based on the occurrences of renal tubule adenoma or carcinoma (combined) in exposed animals.
- Exposure to sodium tungstate dihydrate in drinking water caused increased incidences of nonneoplastic lesions in the kidney, testes, and bone marrow of male mice.
- ***No evidence of carcinogenic activity*** in female B6C3F1/N mice at 500, 1,000, and 2,000 mg/L sodium tungstate dihydrate.
- Exposure to sodium tungstate dihydrate in drinking water caused increased incidences of nonneoplastic lesions in the kidney and spleen of female mice.

There were no clarifying questions or comments about the presentation.

4.2. Public Comments

Dr. Ludewig acknowledged the receipt of one written public comment from Dr. Ranulfo Lemus on behalf of the International Tungsten Industry Association. Dr. Ludewig noted that the panel did not receive requests for oral public comments on the draft technical report.

4.3. Peer-Review Comments and Panel Discussion

4.3.1. First Reviewer – Dr. Michael Elwell

- Dr. Elwell indicated that the dose selection was appropriate, the studies were well-conducted, the results were discussed clearly, and the rationale was clearly presented for neoplastic findings of equivocal evidence and several nonneoplastic effects. The important sodium tungstate-related findings were well-described and represented by quality pathology images in the report.
- Dr. Elwell noted some inconsistencies across report sections on the relationship of atypical hyperplasia in the uterus of female rats to sodium tungstate dihydrate exposure. Text on page 104 of the draft report indicates that the relationship is unknown; however, text in the abstract, summary, and conclusions states that atypical hyperplasia in the uterus is a nonneoplastic effect caused by sodium tungstate dihydrate.
    - Dr. Amy Brix stated that the sentence in the discussion about the unknown relationship to atypical hyperplasia was a typo and NTP may consider removing it. She stated that NTP believes that the effects were related to exposure and would consider make those edits.
- Dr. Elwell noted that on page 69 under "other tissues" for rats, several nonneoplastic findings were mentioned and considered to be of unknown biological significance. However, a significant decrease in fibroadenomas in the mammary gland was mentioned with no comment regarding biological significance or relationship to sodium tungstate dihydrate exposure. The fibroadenomas occurred in female rats with decreased body weights. He recommended that the report discuss the potential relevance of decreased body weight in relation to the tumors given that the effect of lower body weight on occurrence of this tumor has been noted in earlier NTP reports and in published studies.
    - Dr. Brix said that information about the mammary gland would be considered by NTP along with citing Dr. Haseman's article that compares body weight changes to certain tumor incidences. For the nonneoplastic findings in other tissues, NTP can make it clear that they do not consider them toxicologically significant, treatment-related, or biologically significant.
- Dr. Elwell questioned the rationale for including two of the effects listed in the abstract, specifically increased spleen hematopoiesis and bone marrow hyperplasia. For both findings, the increased incidences occurred in the low and mid-exposure groups with no significant effect in the high exposure group, and the average severity was similar across exposure groups. Given that the histopathology section states that the biological significance for the bone marrow and spleen is unknown, Dr. Elwell asked if there were other effects (e.g., inflammatory, hematologic, or hematopoietic) that supported listing

these as effects in the abstract? As a point of reference, Dr. Elwell noted that NTP concluded that the kidney pigment findings, which were significantly higher in the high exposure group, were of questionable toxicologic importance and therefore not brought into the abstract.

- Dr. Behl agreed with Dr. Elwell's comment on the spleen and explained that NTP noted an effect in bone marrow hyperplasia in males and hematopoietic cell proliferation in females at both low and mid doses. NTP is open to discussion about whether to bring these effects forward into the abstract. Dr. Behl asked if the panel recommended including spleen effects in the abstract.
  - Dr. Elwell commented that the other effects in the abstract were dose- or target organ-related; the spleen effects were weaker than what might be expected for a finding listed in the abstract.
  - Dr. Brix noted that for the spleen and bone marrow, the incidences were two to three times higher in the low and mid-dose groups, but agreed that it is a weak connection and that NTP may be open to removing them from the abstract based on the panel discussion.

4.3.2. Second Reviewer – Dr. John Pierce Wise

- Dr. Wise concurred with Dr. Elwell. The report was well-written, and the study was well-designed.
- Given the pressure to evaluate environmentally relevant concentrations, Dr. Wise recommended that NTP provide language on dose selection rationale to help readers unfamiliar with the NTP approach.
  - Dr. Behl noted that the comment on dose selection rationale was well-taken. She explained how NTP selected the concentrations and indicated that they have been criticized in the past for doses that failed to challenge the animals. The concentrations used in this study allowed NTP to state that tungstate at high levels does not result in overt toxicity. The effects observed in the kidney were common with this strain and species.
    - Dr. Wise clarified that it was not that NTP should use different doses. Rather, he recommended that NTP specify that their intention is to determine whether a substance causes cancer, not to define whether a substance causes cancer at the most environmentally relevant dose. It would help to add that context for a reader who does not understand that approach.
    - Dr. Behl said that NTP can add some clarifying language in the report.
- Dr. Wise recommended that NTP provide clarifying language to address the occurrence of pinworms in the rats for reader unfamiliar with rat use.
  - Dr. Behl explained that the rats were positive for pinworms for the duration of the study, and they did not receive medication for elimination. At study termination, the incidences of pinworms were similar between the exposed groups. Based on

histological sections, the pinworms were not associated with morphological changes in the large intestine and no inflammatory response was noted. NTP can clarify that point.

- Dr. Wise indicated it would be helpful to include that no medication was administered.

- Dr. Wise recommended that NTP add a second parameter in the table when presenting comet assay results, since it is standard to show two different measures such as tail length, olive moment, or tail moment.
  - Dr. Stephanie Smith-Roe noted that NTP uses percent tail DNA, which is the OECD guideline for comet assay. Some comet assays report more than one measure, with those measures usually based on tail length. As there is variability in electrophoretic conditions that can influence tail length, NTP has found that percent tail DNA is a more reliable measure.
  - Dr. Ludewig commented that she was unsure of how to interpret the significant comet effects even at the lowest concentration given the lack of pathology in the liver and other published positive comet assay results in the liver and other tissues.
    - Dr. Smith-Roe replied that the results suggest that sodium tungstate may be capable of damaging DNA, but the liver was not a target organ for neoplastic effects.

4.3.3. Third Reviewer – Dr. Charles Mahrt

- Dr. Mahrt commented that the studies were well-designed, well-conducted, and the report was clear. However, he suggested NTP clarify whether the progression in rats from uterine atypical hyperplasia to adenocarcinomas (noted in Table 31) also included an increase in uterine adenomas.
  - Dr. Brix agreed that it was unclear and indicated that NTP will consider adding language regarding the progression. From experience, adenomas are less common and NTP often sees a direct progression from atypical hyperplasia to adenocarcinomas.

4.3.4. Panel Discussion

Dr. Daniel Spade indicated he had no additional comments that were not already addressed by the other reviewers.

Dr. Ludewig noted that the comet assay should be brought forward into the abstract since DNA damage was observed in the liver, despite no associated pathology.

Dr. Tracie Bunton asked about the justification for the large number of interim sacrifices and questioned whether NTP could have obtained information on tungsten accumulation using fewer sacrifices.

- Dr. Behl explained that the study was started several years ago when there were no data in the literature on the accumulation of tungsten in tissues following repeat dosing. Because the kidney is a target, NTP included multiple time points to determine if accumulation continued over the course of the study or if saturation eventually occurred. In addition, a question of sex differences required that NTP use males and females.

Dr. Bunton agreed with Dr. Mahrt's comment about Table 31 and thought the discussion was sufficient. She asked why Table 31 did not include incidence and statistics.

- Dr. Brix clarified that the adenocarcinomas and adenomas were included in Table 31 to show that the incidences were not significantly increased and that there was no progression from atypical hyperplasia. There were no statistics included because they were all negative.

Dr. Bunton noted that for female mice, there was a significant increase in the incidence of hepatocellular adenomas and carcinomas in the 500 mg/L group and that they were included in the "other tissues" groups. She asked why that was lumped into the "other tissues" category rather than brought into the tumor category.

- Dr. Brix stated that hepatocellular tumors are a common background lesion in this strain of mice, so it is not uncommon to have a dose group with significant differences due to biological variation. However, there was no dose response or reason to consider these treatments related.

Dr. Bunton asked for additional language or reorganization to be added in the discussion to explain how NTP came to an equivocal conclusion for female rats. NTP stated that the conclusion was based on increased incidences of C-cell adenoma and carcinoma (combined) of the thyroid gland, but the statement is incomplete as written because that same rationale could apply for a carcinogenic conclusion.

- Dr. Brix said that NTP could look at the discussion and clarify.

Dr. Ludewig asked if NTP had any information about adipose tissue or lipid content in the liver, given that absolute liver weight decreased, there were positive comet assay results in the males, and the text mentions that sodium tungstate dihydrate is an antidiabetic agent.

- Dr. Nigel Walker said that NTP does not have these data.

4.4. Vote on NTP Conclusions

4.4.1. Male Hsd:Sprague Dawley® SD® rats

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Wise so moved, and Dr. Mahrt seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

4.4.2. Female Hsd:Sprague Dawley® SD® rats

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Wise so moved, and Dr. Elwell seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

4.4.3. Male B6C3F1/N mice

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Wise so moved, and Dr. Elwell seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

4.4.4. Female B6C3F1/N mice

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Wise so moved, and Dr. Mahrt seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

5. Toxicology and Carcinogenesis Studies of Di-*n*-butyl Phthalate

5.1. Presentation and Clarifying Questions

Dr. Madelyn (Mimi) Huang summarized the studies and conclusions reported in the *Draft NTP Technical Report on the Toxicology and Carcinogenesis Studies of Di*-n-*butyl Phthalate (CASRN 84-74-2) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice*.

Di-*n*-butyl phthalate (DBP) is commonly used as a plasticizer and is found in a variety of consumer products, such as vinyl fabrics and flooring, personal care products, pharmaceuticals, and food packaging. Human exposure primarily occurs through ingestion of food packaged in materials containing DBP; some inhalation and dermal exposure occurs as well, but to a lesser extent. In the gut, DBP is rapidly metabolized to mon-*n*-obutyl phthalate (MBP) and undergoes broad distribution throughout the body.

Dr. Huang presented a summary of results from the perinatal and postweaning toxicity and carcinogenicity study in Hsd:Sprague Dawley® SD® rats. Time-mated female rats were fed diets containing 0, 300, 1,000, 3,000, or 10,000 ppm DBP from gestation day (GD) 6 through postnatal day (PND) 21. NTP provided F_1 generation rats with the same respective DBP concentrations in feed as their dam for 2 years (generally two/sex/litter). In addition, select dams and their litters were removed on GD 18 and lactation day (LD) 4 to quantify the internal concentration of MBP.

Dr. Huang then presented a summary of results from the chronic toxicity and carcinogenicity study in B6C3F1/N mice. Mice were fed diets containing 0, 1,000, 3,000, or 10,000 ppm DBP for 2 years (n=50/sex/group).

Under the conditions of these 2-year studies, NTP's draft conclusions were:

- ***Equivocal evidence of carcinogenic activity*** in male Hsd:Sprague Dawley® SD® rats based on marginal increases in the incidence of pancreatic acinus adenomas.
- Exposure to DBP resulted in increased incidences of gross lesions of the male reproductive system and nonneoplastic lesions of the male reproductive system, liver, and pituitary gland pars distalis in male rats.

- ***No evidence of carcinogenic activity*** in female Hsd:Sprague Dawley® SD® rats at 300, 1,000, 3,000, or 10,000 ppm.
- Exposure to DBP resulted in increased incidences of nonneoplastic lesions of the liver in female rats.
- ***No evidence of carcinogenic activity*** in male B6C3F1/N mice at 1,000, 3,000, or 10,000 ppm.
- Exposure to DBP increased incidences of nonneoplastic lesions of the male reproductive system and liver in male mice.
- ***No evidence of carcinogenic activity*** in female B6C3F1/N mice at 1,000, 3,000, or 10,000 ppm.
- Exposure to DBP increased incidences of nonneoplastic lesions of the liver and kidney in female mice.

There were no clarifying questions or comments about the presentation.

5.2. Public Comments

Dr. Gabriele Ludewig acknowledged that there were no written public comments on the draft technical report. She also noted that the panel did not receive requests for oral public comments on the draft technical report.

5.3. Peer-Review Comments and Panel Discussion

5.3.1. First Reviewer – Dr. Tracie Bunton

- Dr. Bunton said the presentation of the rationale, methods, and results was clear and concise.
- She commented that she liked the introduction and found it helpful in getting up to speed on the properties, uses, and reduction in use of DBP. The rationale for the overall significance of the study is solid, especially given that perinatal exposure is a knowledge gap.
- She indicated that all the information about stability, homogeneity, dose selection, and number of animals per dose group is appropriate for the design of the study.
- Dr. Bunton suggested including the word “microscopic” or eliminate the word “gross” in the conclusion statement of the report as it currently specifies gross lesions in the male reproductive system but does not specify that the nonneoplastic lesions are not gross lesions.
    - Dr. Huang indicated that NTP is open to adding in the word “microscopic” to differentiate from gross lesions.
- Dr. Bunton noted that the findings to support the “equivocal” decision included an increased incidence in pancreatic acinar adenomas compared to controls, without a concurrent increase in hyperplasia, and a significant positive trend.

- She stated the lesion in the liver was compatible with effects of other phthalates, namely increased cytoplasmic alteration. This was an important point that was noted in the pathology review and in the discussion.
- She also agreed that the findings fit into the "equivocal" category.

5.3.2. Second Reviewer – Dr. Charles Mahrt

- Dr. Mahrt agreed with Dr. Bunton's comments and noted that the report was well-designed, conducted, and written.
- He appreciated the references to the literature to put the findings in perspective, especially the possibility of lesions related to peroxisome proliferation.

5.3.3. Third Reviewer – Dr. Daniel Spade

- Dr. Spade indicated that the study was well-conducted, and the report was clearly written.
- He noted that in the abstract, lines 39 to 40 indicate that there were fewer and less severe reproductive lesions in mice than in rats. He agreed with that conclusion, though suggested qualifying it by acknowledging the limits of cross-species comparison given that rats had perinatal exposure and mice did not.
    - Dr. Huang agreed that it was important to qualify the rat versus mouse comparison and said NTP could clarify those statements.
    - Dr. Nigel Walker explained that when NTP started adding the perinatal component to rat studies, people were trying to understand why the study design changed. With rat studies as perinatal and mouse studies as adults only, NTP has started to address that in the mid-2000's. In addition, it is difficult to do perinatal exposure on F_1 generation B6C3F1/N mice.
- Dr. Spade built on Dr. Bunton's comment about the equivocal conclusion for pancreatic acinar adenomas and asked if there was a significant trend test but not pairwise test.
    - Dr. Huang stated the affirmative.
        - Dr. Spade appreciated the response and agreed with the conclusion.
- He also asked why images were not included in some cases.
    - Dr. Huang clarified that NTP does not generally include images for common lesions or when the conclusion is equivocal.
        - Dr. Spade appreciated the response and agreed with the approach.

5.3.4. Panel Discussion

Dr. Michael Elwell noted that the last line of the discussion mentions that 2,4-dichlorophenoxyacetic acid and DBP did not produce hepatic lesions typical of peroxisome proliferators. DBP did result in hepatic lesions but did not produce hepatic neoplasms.

- Dr. Huang agreed that the last line should reference neoplasms instead of lesions, as Dr. Elwell suggested.

Regarding voting, Dr. Elwell noted that the original draft report mentions hypertrophy in the pituitary and hyperplasia, but that it was not mentioned in the oral presentation. He asked if hypertrophy was included in the pituitary findings.

- Dr. Mark Cesta explained that NTP relooked at the data about the pituitary and hyperplasia. Hyperplasia often occurs with hypertrophy lesions, so they wanted to explain the observed hyperplasia. However, after a closer look, NTP could not make that conclusion and decided to remove it from the abstract.

Dr. Ludewig noted that page 78 of the draft report explains the concentrations found in the amniotic fluid. When humans and rats both take up 1 mg/kg/day, a historic report showed that there were 22 ng/mL of the metabolite in the amniotic fluid of humans. However, NTP only found 5 ng/mL in rats. Dr. Ludewig said she was initially surprised until she noticed that NTP did not measure the glucuronic acid conjugate. She wondered if that would also cross the placenta or if conjugation would protect the fetus. She suggested adding a statement that the gluconate conjugate was not measured.

- Dr. Huang agreed that there are differences in distribution and metabolism between rodents and humans. She was unsure if glucuronidation affects its ability to cross the placenta and said that was something NTP would look into.

5.4. Vote on NTP Conclusions

5.4.1. Male Hsd:Sprague Dawley® SD® rats

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Bunton so moved, and Dr. Mahrt seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

5.4.2. Female Hsd:Sprague Dawley® SD® rats

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. John Pierce Wise so moved, and Dr. Elwell seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

5.4.3. Male B6C3F1/N mice

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Bunton so moved, and Dr. Wise seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

5.4.4. Female B6C3F1/N mice

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Wise so moved, and Dr. Bunton seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

6. Toxicology and Carcinogenesis Studies of Di(2-ethylhexyl) Phthalate

6.1. Presentation and Clarifying Questions

Dr. Chad Blystone summarized the studies and conclusions reported in the *Draft NTP Technical Report on the Toxicology and Carcinogenesis Studies of Di(2-ethylhexyl) Phthalate (CASRN 117-81-7) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats*.

Di(2-ethylhexyl) phthalate (DEHP) is a phthalate ester that was widely used in manufacturing of PVC polymers and corresponding products, such as cosmetics and toys. Over the years DEHP use has declined due to toxicity concerns, but chronic exposure throughout multiple life stages still occurs. The literature suggests that exposure to DEHP during an early life stage may result in chronic or carcinogenic health outcomes. However, previous DEHP chronic rodent studies did not include exposure during the gestational period up to weaning in rodents. To address this, NTP conducted two comparative DEHP carcinogenesis studies in rats to determine if including early life exposure would alter chronic toxicity or carcinogenicity outcomes.

Dr. Blystone presented a summary of results from the perinatal and postweaning toxicity/carcinogenicity study in Hsd:Sprague Dawley® SD® rats. In the perinatal and postweaning study, time-mated female rats were fed diets containing 0, 300, 1,000, 3,000, or 10,000 ppm DEHP from gestational day (GD) 6 through postnatal day (PND) 21 (n=45/group). Select dams were removed on GD 18 to quantify internal concentrations of a metabolite of DEHP, mono(2-ethylhexyl) phthalate, in plasma and tissue samples. NTP provided F_1 generation rats with the same respective DEHP concentration in feed as their dam for 2 years (n=2/sex/litter; n=50 total/sex/group).

Dr. Blystone then presented a summary of results from the postweaning toxicity/carcinogenicity study in Hsd:Sprague Dawley® SD® rats. In the postweaning only study, rats were fed diets containing 0, 300, 1,000, 3,000, or 10,000 ppm DEHP for 2 years (n=50/sex/group).

Under the conditions of these 2-year studies, NTP's draft conclusions were:

- Perinatal and Postweaning Feed Study:
    - ***Clear evidence of carcinogenic activity*** in male Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular adenoma or carcinoma (combined) and acinar adenoma or carcinoma (combined) neoplasms (predominantly adenomas) of the pancreas.
    - Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, heart, pituitary gland, testis, and epididymis and increased incidences of gross lesions of the reproductive tract, bone marrow, and kidney in male rats.
    - ***Clear evidence of carcinogenic activity*** in female Hsd:Sprague Dawley® SD® rats based on the increased incidence of hepatocellular adenoma or carcinoma (combined).
    - The occurrence of pancreatic acinar adenoma or carcinoma (combined) was considered to be related to exposure. (*Some evidence*)

  - The occurrence of uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined) in female rats may have been related to exposure. (*Equivocal evidence*)
  - Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver and increased incidences of gross lesions of the kidney in female rats.

- Postweaning-only Feed Study
  - ***Clear evidence of carcinogenic activity*** in male Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular adenoma or carcinoma (combined) and increased incidences of acinar adenoma or carcinoma (combined) neoplasms (predominantly adenomas) of the pancreas.
  - The occurrence of testicular interstitial cell adenoma in male rats may have been related to exposure. (*Equivocal evidence*)
  - Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, pancreas, bone marrow, heart, pituitary gland, testis, and epididymis.
  - ***Clear evidence of carcinogenic activity*** in female Hsd:Sprague Dawley® SD® rats based on the incidences of hepatocellular adenoma or carcinoma (combined) and uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined).
  - The occurrence of pancreatic acinar adenoma or carcinoma (combined) in female rats was considered to be related to exposure. (*Some evidence*)
  - Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, pancreas, bone marrow, and uterus in female rats.

- Comparative Carcinogenic Benchmark Dose Analyses
  - No consistent pattern indicating that perinatal and postweaning exposure was more sensitive compared to postweaning-only exposure and modeled responses were within threefold of each other.
  - However, there was a stronger carcinogenic response in the reproductive organs (uterus and testis) in the postweaning-only exposure study compared to the perinatal and postweaning exposure study.

Dr. Gabriele Ludewig asked a clarifying question about a shift in male to female fetus ratios that was not mentioned during Dr. Blystone's talk. Dr. Blystone responded that female fetuses were lost at the highest concentration. The reduction in litter size was due to this but was inconsistent and not considered related to DEHP exposure.

6.2. Public Comments

Dr. Ludewig acknowledged that there were no written public comments submitted on the draft technical report. She noted that the panel also did not receive requests for oral public comments on the draft technical report.

6.3. Peer-Review Comments and Panel Discussion

6.3.1. First Reviewer – Dr. John Pierce Wise

- Dr. Wise indicated that the study was well-designed, well-conducted, and clear in its data presentation.
- He suggested that NTP clarify that pinworm infections were not treated with medication.
    - Dr. Blystone said that NTP can add this clarification.
- Dr. Wise also reiterated his comment from sodium tungstate dihydrate that NTP should explain that the choice of dose was deliberate and not intended to test the most environmentally relevant level of exposure, but rather to test whether the substance is carcinogenic.
    - Dr. Blystone agreed that the point of these studies is hazard characterization and noted that NTP can clarify that in the report and include it in the lay summary.
    - Dr. Wise suggested that NTP could add this in a dose selection rationale section.
    - Dr. Blystone responded that the reports have a section covering the technical aspects of the exposure concentration selection and that a sentence can be added to highlight rationale for exposure concentrations.

6.3.2. Second Reviewer – Dr. Daniel Spade

- Dr. Spade stated that the report was thorough and clear; it makes a massive amount of work easily understandable.
- He asked for clarification in how the reduction in litter size was presented, and asked whether there was an effort to address post-implantation loss as part of the reduced litter size. Reduced litter size could not be due to exposure related pre-implantation loss since the dosing window did not begin before implantation. However, Dr. Spade argued that with exposures beginning on GD 6, this overlaps with organogenesis and poses a risk for post-implantation loss which has been reported in the phthalate literature.
    - Dr. Blystone said that NTP only evaluated post-implantation loss in females which did not deliver, so there are no more data available on post-implantation loss and litter size.
- Dr. Spade asked why pup survival data for PND 1-4 and PND 5-21 on page 29 of the draft report were analyzed separately. Dr. Spade thought that there could have been a trend in mortality if PND 1-21 were analyzed together.
    - Dr. Blystone explained that NTP typically standardizes the litter size on PND 4, so the analysis looks at early (PND 1-4) and later (PND 5-21) mortality. Dr. Blystone acknowledged that based on Dr. Spade's written comment NTP reanalyzed the data after combining the two periods and there was still no significant trend or pairwise comparison.
- Dr. Spade also noted that it was unclear why some lesions of unknown biological significance on page 61 of the draft report were classified as such. Of note were the

adrenal gland lesions, because of the known antiandrogenic effect of DEHP. Also, he questioned the ovarian atrophy as classified as unknown biological significance, because published data from academic studies indicate that phthalates change the rates of follicle maturation which could be related.

  - Dr. Blystone indicated that NTP can clarify the language and focus more on toxicological significance.

- Dr. Spade commented that 24 months is reproductively aged, so for certain findings such as seminiferous epithelium degeneration, the control levels are very high which makes it less likely that there will be a significant pairwise test. This limits the ability to know with certainty what the dose response would look like for an endpoint such as epithelial degeneration. If 4-month-old males were tested, you might see a significant response at lower levels. The study supports the conclusions within the constraints of the study, but this is a limitation.
  - Dr. Blystone agreed that at this age, the model is not very sensitive. NTP can add a statement about that to the report.
- Dr. Spade noted that for gestational transfer, as discussed on page 33 of the draft report, one limitation is that DEHP has many secondary metabolites. He noted that without measuring these secondary metabolites it is difficult to determine the total transfer.
  - Dr. Blystone agreed and said that NTP can clarify that metabolites can be transferred at different rates.

Dr. Ludewig stated that it was interesting that mono(2-ethylhexyl) phthalate was found in the amniotic fluid and the fetus of the control animals, but nothing in the serum of dams.

- Dr. Blystone agreed that this was unusual and noted that sometimes sample preparations can lead to irregularities.
- Dr. Suramya Waidyanatha said NTP concluded it was probably due to contamination of samples during collection or preparation and that this is likely due to the small volume of these samples.

6.3.3. Third Reviewer – Dr. Michael Elwell

- Dr. Elwell agreed with previous reviewers that the results are clearly presented and discussed.
- He noted that at the highest dose, body weights were reduced by approximately 30% in the perinatal postweaning and approximately 20% in the postweaning study. Although this did not affect survival, the decreased incidence of several neoplastic and nonneoplastic findings (especially for the high dose group of each study) might be attributable to significantly lower body weights. In the report (pages 61 and 77) these are indicated to be of unknown biological significance. If these findings are due to lower body weight, they should be addressed as such rather than reported as unknown significance. For example, neoplasms including the c-cell, pituitary, and mammary gland tumors can be affected by body weight. In females, a single pituitary gland neoplasm reported at the high dose is unexpected and notable in comparison to the control group

where 16 animals were reported to have this tumor. The decreased incidence of the nonneoplastic lesions of testis polyarteritis and parathyroid hyperplasia could also be related to the lowered severity of chronic progressive nephropathy (CPN). In both studies, CPN was decreased in severity at the high dose in both studies and indicated as the cause of death in 0 and 1 animal while 16-18 animals in the control groups list CPN as the cause of death. This report cites other DEHP studies that attribute an increase in CPN to DEHP while this report suggests the opposite effect on CPN. This may be because the highest doses in this study were lower, the decreased body weight, or that the NTP 2000 diet was not used in earlier studies.

  - Dr. Blystone indicated that NTP can clarify biological significance versus toxicological significance. Dr. Blystone agreed that some of the effects could be related to decreased body weight and stated that NTP can clarify.

- Dr. Elwell asked if page 87 of the draft report should state that it is unclear if any difference corresponded to developmental mechanisms when comparing the two studies, or if it is a typo. It appeared the kidney was affected by a developmental mechanism and DEHP was presumed to interfere with proper development.
  - Dr. Blystone stated that NTP can fix the typo.
- Dr. Elwell noted that for the nonneoplastic conclusions, there are dozens of neoplastic and nonneoplastic findings clearly related to DEHP. He questioned why the acute inflammation in the uterus in the perinatal/postnatal study was considered an effect. He also asked why the bone marrow in females in the postweaning-only study was included as a finding and if it was possibly a false positive.
  - Dr. Blystone said that although the response was not strong, NTP considered the bone marrow lesion exposure related since it was observed in males and females. The acute inflammation in the uterus was considered exposure related. NTP can review this and clarify.
  - Dr. Susan Elmore stated that she can see Dr. Elwell's point about the acute inflammation of the uterus possibly not being related to exposure, but that this was something NTP would need to discuss further.

6.3.4. Panel Discussion

Dr. Tracie Bunton said she wanted to see the presentation of the benchmark dose analysis, but beyond that, just had minor edits submitted in writing.

Dr. Spade asked if Dr. Elwell's comments about bone marrow and uterus related to one of the conclusions on which the panel would vote. He wondered if it was about including it in the abstract rather than the conclusion that there was a finding.

- Dr. Elwell said while the other findings listed in the abstract were convincing effects, the rationale to include the non-dose-related difference of acute uterus inflammation in the perinatal-post weaning study was not clear although there may have been reason to include it as chronic uterus inflammation was increased in the postweaning-only study. The question on including the bone marrow finding in females from the postweaning-

only study in the abstract was based on very small group differences in incidence with no apparent effect on severity.

Dr. Bunton noted that a number of genetic toxicity tests were conducted and asked if they were related to this particular report or conducted over time.

- Dr. Blystone said they were not related to this report itself. They were accumulated over time and not published previously, so they were published with this report.

6.4. Vote on NTP Conclusions

6.4.1. Male Hsd:Sprague Dawley® SD® rats (perinatal and postweaning feed study)

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Bunton so moved, and Dr. Wise seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

6.4.2. Female Hsd:Sprague Dawley® SD® rats (perinatal and postweaning feed study)

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Wise so moved, and Dr. Elwell seconded the motion. Dr. Elwell asked to amend the motion to vote and moved that NTP delete "and increased incidences of gross lesions of the" from the conclusion and add "and uterus." Dr. Wise motioned to accepted revisions to the conclusion and Dr. Spade seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the new conclusion.

In a second round of revisions, Dr. Blystone noted that the end of a sentence was cut off. He added "and gross observations in the female reproductive tract" to the end of the final conclusion. Dr. Ludewig called for a motion from the panel to approve the second round of revised conclusions. Dr. Wise so moved, and Dr. Elwell seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the new conclusion, below.

Revised Conclusion:

- *Clear evidence of carcinogenic activity*
  - Increased incidence of hepatocellular adenoma or carcinoma (combined).
- The occurrence of pancreatic acinar adenoma or carcinoma (combined) was considered to be related to exposure. (*Some evidence*)
- The occurrence of uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined) in female rats may have been related to exposure. (*Equivocal evidence*)
- Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, ~~and increased incidences of gross lesions of the~~ kidney, and uterus in female rats and gross observations in the female reproductive tract.

6.4.3. Male Hsd:Sprague Dawley® SD® rats (postweaning-only study)

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Wise so moved, and Dr. Elwell seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

6.4.4. Female Hsd:Sprague Dawley® SD® rats (postweaning-only study)

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. The panel did not offer a motion. Dr. Elwell moved that NTP delete the reference to increased incidences of nonneoplastic lesions in the bone marrow from the conclusion. Dr. Wise seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the new conclusion, below.

Revised Conclusion:

- *Clear evidence of carcinogenic activity*
    - Increased incidences of hepatocellular adenoma or carcinoma (combined) and uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined).
- The occurrence of pancreatic acinar adenoma or carcinoma (combined) in female rats was considered to be related to exposure. (*Some evidence*)
- Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, pancreas, ~~bone marrow,~~ and uterus in female rats.

6.4.5. Hsd:Sprague Dawley® SD® rats (comparative benchmark dose analyses)

Dr. Ludewig called for a motion from the panel to approve the conclusions as written. Dr. Wise so moved, and Dr. Elwell seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the conclusions as written.

6.5. Final Conclusions

Because revisions were proposed and approved during the meeting, the final approved conclusions are presented below:

- Perinatal and Postweaning Feed Study:
    - ***Clear evidence of carcinogenic activity*** in male Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular adenoma or carcinoma (combined) and acinar adenoma or carcinoma (combined) neoplasms (predominantly adenomas) of the pancreas.
    - Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, kidney, bone marrow, heart, pituitary gland, testis, and epididymis and increased incidences of gross lesions of the reproductive tract
    - ***Clear evidence of carcinogenic activity*** in female Hsd:Sprague Dawley® SD® rats based on the increased incidence of hepatocellular adenoma or carcinoma (combined).

  - The occurrence of pancreatic acinar adenoma or carcinoma (combined) was considered to be related to exposure. (*Some evidence*)
  - The occurrence of uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined) in female rats may have been related to exposure. (*Equivocal evidence*)
  - Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, kidney, and uterus in female rats and gross observations in the female reproductive tract.
- Postweaning-only Feed Study
  - ***Clear evidence of carcinogenic activity*** in male Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular adenoma or carcinoma (combined) and increased incidences of acinar adenoma or carcinoma (combined) neoplasms (predominantly adenomas) of the pancreas.
  - The occurrence of testicular interstitial cell adenoma in male rats may have been related to exposure. (*Equivocal evidence*)
  - Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, pancreas, bone marrow, heart, pituitary gland, testis, and epididymis.
  - ***Clear evidence of carcinogenic activity*** in female Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular adenoma or carcinoma (combined) and uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined).
  - The occurrence of pancreatic acinar adenoma or carcinoma (combined) in female rats was considered to be related to exposure. (*Some evidence*)
  - Exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver, pancreas, and uterus in female rats.
- Comparative Carcinogenic Benchmark Dose Analyses
  - No consistent pattern indicating that perinatal and postweaning exposure was more sensitive compared to postweaning-only exposure and modeled responses were within threefold of each other.
  - However, there was a stronger carcinogenic response in the reproductive organs (uterus and testis) in the postweaning-only exposure study compared to the perinatal and postweaning exposure study.

7. Closing Remarks on the Draft Reports

Dr. Gabriele Ludewig welcomed additional panel comments on the draft report. There were no additional comments.

Closing the meeting, Dr. Sheena Scruggs thanked all the peer-review panelists.

Dr. Ludewig added her thanks to the NTP staff and the panel members for their efforts.

Dr. Ludewig adjourned the meeting at 2:00 p.m. EDT on April 2, 2021.

8. Approval of the Peer-Review Report by the Chair of the Peer-Review Panel

The peer-review panel chair read this peer-review report and approved of the April 2, 2021 Peer Review of the *Draft NTP Technical Reports on the Toxicology and Carcinogenesis Studies of Sodium Tungstate Dihydrate, Di-n-butyl Phthalate, and Di(2-ethylhexyl) Phthalate.*

Gabriele Ludewig, Ph.D.

Peer-Review Panel Chair

Date: 6/28/21