National Toxicology Program

Peer Review of the Draft NTP Developmental and Reproductive Toxicity Technical Reports on 2-Hydroxy-4-methoxybenzophenone and 2-Ethylhexyl p-Methoxycinnamate

October 14, 2021

National Institute of Environmental Health Sciences
Research Triangle Park, NC

Peer-review Report

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1. Attendees[1]

Peer-review Panel

Chair: Rebecca Fry, University of North Carolina at Chapel Hill

Brian Enright, AbbVie, Inc.

Bethany Hannas, Corteva Agriscience

Linda Roberts, NapaTox Consulting LLC

Mary Alice Smith, Retired, formerly with University of Georgia

National Toxicology Program Board of Scientific Counselors Liaison

Susan Tilton, Oregon State University

National Institute of Environmental Health Sciences Staff

Brian Berridge

Chad Blystone

Mark Cesta

Brad Collins

Angela King-Herbert

Barry McIntyre

Georgia Roberts

Sheena Scruggs, Designated Federal Official

Kelly Shipkowski

Keith Shockley

Vicki Sutherland

Suramya Waidyanatha

Nigel Walker

Mary Wolfe

Other Federal Agency Staff

Christina Lawson, National Institute for Occupational Safety and Health

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

Contract Support Staff

Canden Byrd, ICF

Cary Haver, ICF

Elizabeth Maull, Kelly Government Services

Megan Rooney, ICF

Karen Setty, ICF

Samantha Snow, ICF

Sam Whately, ICF

Jess Wignall, ICF

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

2. Introductions and Welcome

The National Toxicology Program (NTP) convened a peer-review panel for the Draft NTP Developmental and Reproductive Toxicity Technical Reports on 2-Hydroxy-4-methoxybenzophenone and 2-Ethylhexyl p-Methoxycinnamate on October 14, 2021, via webcast.

- Dr. Rebecca Fry, panel chair, called the meeting to order at 10:00 a.m. EDT 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. Brian Berridge, Associate Director for NTP and Scientific Director for the National Institute of Environmental Health Sciences (NIEHS)/Division of the NTP (DNTP), welcomed all participants to the meeting.
- Dr. Sheena Scruggs, Designated Federal Official, read the conflict-of-interest policy statement and briefed the attendees on meeting logistics.
- Dr. Susan Tilton attended as the liaison to the NTP Board of Scientific Counselors.
- Dr. Christina Lawson 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 developmental and reproductive toxicity (DART) report objectives, including a review of the levels of evidence for the potential developmental and reproductive toxicity and factors considered for tested chemicals. He also described the modified one-generation (MOG) study design to provide context for the report findings. Dr. Blystone provided the charge 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 under the conditions of each study.

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

4. Modified One-Generation Study of 2-Hydroxy-4-Methoxybenzophenone

4.1. Presentation and Clarifying Questions

Dr. Barry McIntyre summarized the studies and conclusions reported in the *Draft NTP Developmental and Reproductive Toxicity Technical Report on the Modified One-Generation Study of 2-Hydroxy-4-methoxybenzophenone (CASRN 131-57-7) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats with Prenatal and Reproductive Performance Assessments in F_1 Offspring*.

2-Hydroxy-4-methoxybenzophenone (2H4MBP) is a common synthetic ultraviolet (UV) filtering ingredient in sunscreens. It was nominated for study due to concerns about potential widespread human exposure via dermal application of sunscreen products and possible endocrine activity. Diet was selected as a sustained route of exposure, since dermal exposure was not feasible given group housing and grooming behaviors of the animals.

Dr. McIntyre presented a summary of results from the MOG study in Hsd:Sprague Dawley® SD® rats. Time-mated female rats were continually exposed to 0, 3,000, 10,000, or 30,000 ppm 2H4MBP or 0.05 ppm ethinyl estradiol ([EE]; as a positive control) in feed from gestation day (GD) 6 through postnatal day (PND) 28. At weaning, F_1 offspring were assigned to reproductive performance (2/sex/litter), prenatal (1/sex/litter), or biological sampling (1/sex/litter) cohorts. The F_1 and F_2 generation rats from all cohorts were continually exposed to the same respective 2H4MBP concentrations in feed as to their dams.

Under the conditions of this MOG study, NTP's draft conclusions were:

- ***Equivocal evidence of reproductive toxicity*** of 2H4MBP in Hsd:Sprague Dawley® SD® rats based on a decrease in F_2 litter size in both the prenatal and reproductive performance cohorts.
- ***Some evidence of developmental toxicity*** of 2H4MBP in Hsd:Sprague Dawley® SD® rats based on the observed postnatal growth retardation. The relationship of the increased occurrence of diaphragmatic and hepatodiaphragmatic hernias in F_1 adults and F_2 pups to 2H4MBP exposure is unclear.
- Exposure to 2H4MBP was not associated with signals consistent with alterations in estrogenic, androgenic, or antiandrogenic action. Exposure to 2H4MBP was associated with lower F_1 and F_2 mean body weights; this effect on body weight contributed to the apparent 2H4MBP-related decreases in male reproductive organ weights. Mating and littering were not significantly affected by 2H4MBP exposure. Exposure to 2H4MBP was associated with nonneoplastic kidney lesions in the F_0, F_1, and F_2 generations. Expected estrogenic responses were observed in the EE group.

Dr. Fry asked whether any of the panelists had clarifying questions or comments about the presentation.

- Dr. Brian Enright asked whether gestational exposure was assessed. Dr. McIntyre indicated that no samples had been taken from pregnant animals to assess maternal plasma concentrations of 2H4MBP.
- Dr. Linda Roberts asked several clarifying questions about feed consumption interval data and feed spillage, the use of the no-observed-effect level (NOEL) versus no-observed-adverse-effect level (NOAEL) in the report, and the criteria for classifying a liver as enlarged.
    - Dr. McIntyre provided the following responses:
        - Feed spillage was recorded in the raw room data. When animals were missing data for a particular day or days within an interval, data would have been excluded from the interval calculations.

  - DNTP staff will clarify the use of NOEL and NOAEL in the report.
  - The criteria for classifying a liver as enlarged was a doubling in the expected size of a fetal liver.

- Dr. Mary Alice Smith asked whether DNTP staff considered feed wastage in calculating the doses and if they studied palatability. Dr. McIntyre commented that feed consumption (palatability) was similar among dose groups in the preliminary dose range-finding study. In the case of feed spillage, it was generally documented (e.g., as a laboratory weighing error) and affected data were excluded from statistical calculations. Given the data, DNTP staff were fairly confident that feed spillage was not a driver of changes in body weights.
- Dr. Bethany Hannas asked how DNTP staff distinguished between "catch-up" feeding and feed wastage as the reasons for apparent increasing feed consumption. Dr. McIntyre noted that increased consumption was seen in both the dose range-finding study and sporadically in the MOG study. Data were handled in a similar manner in both cases.
- Dr. Hannas next asked whether the vaginal cytology findings were attributable to 2H4MBP treatment or biological variability. Given the magnitude of the response, Dr. McIntyre considered that natural variability was more likely.
- Referring to a written public comment, Dr. Roberts asked whether thyroid weights were collected. Dr. McIntyre indicated that some organ weights were collected and that DNTP staff would correct this as appropriate in the report.

4.2. Public Comments

Dr. Fry acknowledged the receipt of written public comments from Mr. Joe C. DiNardo, a private citizen, and Jette Rud Heltved on behalf of the Danish Environmental Protection Agency. These comments were distributed to the panelists and DNTP staff before the meeting. Dr. Fry noted that the panel did not receive requests for oral public comments on the draft DART report.

4.3. Peer-review Comments and Panel Discussion

4.3.1. First Reviewer – Dr. Linda Roberts

- Dr. Roberts indicated that her comments were primarily minor. She complimented DNTP staff on the robust study design and writing and referencing of the report.
- Regarding her concerns about the interval data and feed spillage, she noted that a fourfold difference between rat and human exposure was not very large. Thus, it is important to make sure feed intake data are as accurate as possible.
  - Dr. McIntyre thanked Dr. Roberts for her comments and indicated that they would be useful in revising the report.
- Regarding liver enlargement, she posed a question to DNTP staff: did they want to consider this an unclear finding, along the lines of the diaphragmatic hernia findings, or was it below the threshold for including it with the conclusions? Kidney weight changes

were explained clearly, and Dr. Roberts was mainly interested in clarifying whether a NOEL or NOAEL was intended.

  - Dr. McIntyre said that DNTP staff felt liver enlargement was likely a secondary effect, while growth retardation was again considered the primary evidence to make a robust developmental toxicity determination.

- Dr. Roberts asked whether the finding of decreased corpora lutea in the prenatal cohort at 30,000 ppm was a contributor to the equivocal evidence call for reproductive toxicity.
  - Dr. McIntyre explained that the determination oscillated between some evidence of reproductive toxicity and equivocal evidence of reproductive toxicity. Growth retardation was considered the major driver of the call.

4.3.2. Second Reviewer – Dr. Brian Enright

- Dr. Enright concurred with Dr. Roberts that the report was easy to follow and accurately represented the data and conclusions.
  - Dr. McIntyre thanked Dr. Enright for his feedback.

4.3.3. Third Reviewer – Dr. Mary Alice Smith

- Dr. Smith agreed with the comments of the previous reviewers and indicated that the study was well designed and carried out. She felt inclusion of the positive control group (EE) was a strength, but it could be helpful to separate this positive control data more clearly in figures to differentiate from the highest exposed group. She had minor concerns about the presentation of figures and tables but did not feel these affected the overall conclusions. She requested that the palatability assessment be more clearly discussed in the text. Given issues of feed spillage and palatability, she would hesitate to use these data for a NOAEL calculation. Dr. Smith felt this should be addressed in the text.
  - Dr. McIntyre thanked Dr. Smith for her feedback and agreed that DNTP staff would address reviewer comments in the report text.

4.3.4. Panel Discussion

- Dr. Hannas indicated that it would be useful to add historical control data if available and relevant across studies, cohorts, and life stages (e.g., F_1 vs. F_2 generations). This addition could put the data into context, given natural variability in litter sizes.
  - Dr. McIntyre agreed that DNTP staff would add this information to the report.

4.4. Vote on NTP Conclusions

4.4.1. Reproductive Toxicity

Dr. Fry called for a motion from the panel to approve the conclusions as written. Dr. Roberts so moved, and Dr. Enright seconded the motion. The panel voted unanimously (4 yes, 0 no, 0 abstentions) to approve the conclusions as written.

4.4.2. Developmental Toxicity

Dr. Fry called for a motion from the panel to approve the conclusions as written. Dr. Smith so moved, and Dr. Roberts seconded the motion. The panel voted unanimously (4 yes, 0 no, 0 abstentions) to approve the conclusions as written.

4.4.3. Other Effects

Dr. Fry called for a motion from the panel to approve the conclusions as written. Dr. Hannas so moved, and Dr. Roberts seconded the motion. The panel voted unanimously (4 yes, 0 no, 0 abstentions) to approve the conclusions as written.

4.5. Final Conclusions

Because no revisions were proposed or approved during the meeting, the final approved conclusions are presented below:

- ***Equivocal evidence of reproductive toxicity*** of 2H4MBP in Hsd:Sprague Dawley® SD® rats based on a decrease in F_2 litter size in both the prenatal and reproductive performance cohorts.
- ***Some evidence of developmental toxicity*** of 2H4MBP in Hsd:Sprague Dawley® SD® rats based on the observed postnatal growth retardation. The relationship of the increased occurrence of diaphragmatic and hepatodiaphragmatic hernias in F_1 adults and F_2 pups to 2H4MBP exposure is unclear.
- Exposure to 2H4MBP was not associated with signals consistent with alterations in estrogenic, androgenic, or antiandrogenic action. Exposure to 2H4MBP was associated with lower F_1 and F_2 mean body weights; this effect on body weight contributed to the apparent 2H4MBP-related decreases in male reproductive organ weights. Mating and littering were not significantly affected by 2H4MBP exposure. Exposure to 2H4MBP was associated with nonneoplastic kidney lesions in the F_0, F_1, and F_2 generations. Expected estrogenic responses were observed in the EE group.

5. Modified One-Generation Study of 2-Ethylhexyl p-Methoxycinnamate

5.1. Presentation and Clarifying Questions

Dr. McIntyre summarized the studies and conclusions reported in the *Draft NTP Developmental and Reproductive Toxicity Technical Report on the Modified One-Generation Study of 2-Ethylhexyl p-Methoxycinnamate (CASRN 5466-77-3) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats with Prenatal, Reproductive Performance, and Subchronic Assessments in F_1 Offspring*.

2-Ethylhexyl p-methoxycinnamate (EHMC) is a synthetic UV filtering ingredient in sunscreens. It was nominated for study due to concerns about potential widespread human exposure via dermal application of sunscreen products and possible endocrine activity. Diet was selected as a sustained route of exposure, since dermal exposure was not feasible given group housing and grooming behaviors of the animals.

Dr. McIntyre presented a summary of results from the MOG study in Hsd:Sprague Dawley® SD® rats. Time-mated female rats were continually fed diets containing 0, 1,000, 3,000, or 6,000 ppm EHMC from GD 6 through PND 28. At weaning, F_1 offspring were assigned to reproductive performance (2/sex/litter), prenatal (1/sex/litter), or subchronic (1/sex from 10 litters) cohorts. The F_1 and F_2 generation rats from all cohorts were continually exposed to the same respective EHMC concentrations in feed as to their dams.

Under the conditions of this MOG study, NTP's draft conclusions were:

- ***No evidence of reproductive toxicity*** of EHMC in Hsd:Sprague Dawley® SD® rats at exposure concentrations of 1,000, 3,000, or 6,000 ppm. Mating and littering were not affected significantly by EHMC exposure.
- ***Equivocal evidence of developmental toxicity*** of EHMC in Hsd:Sprague Dawley® SD® rats based on the observed postnatal effects on body weight that showed some indication of recovery by study end, delays in postnatal day 28-adjusted vaginal opening and balanopreputial separation, which could have influenced the apparent transient effects on body weight, and time in estrus was slightly longer in EHMC-exposed females relative to that of the control group.
- No other signals consistent with alterations in estrogenic, androgenic, or antiandrogenic action were observed. EHMC exposure did not induce any specific fetal malformations.

Dr. Fry asked for clarifying questions or comments about the presentation.

- Dr. Smith asked about changes to the conclusions statement, from "which could have influenced" to "which could have been influenced by." Dr. McIntyre confirmed that this should be edited because body weights were suspected to have contributed to the delay in vaginal opening and balanopreputial separation.
- Dr. Enright asked whether findings such as skeletal variations were considered evidence of teratogenic effects. Dr. McIntyre explained that this was a limitation of the study design. It is possible that the skeletal findings were related to exposure, but the level of evidence was considered "little to none" because the finding is common. It could also have been related to maternal toxicity to some extent, reflecting the change in body weight.
- Dr. Enright also asked about the time spent in estrous, suggesting it was not biologically relevant even though it was statistically significant. Dr. McIntyre commented that the report text will be clarified using the reviewers' input.

5.2. Public Comments

Dr. Fry acknowledged the receipt of one written public comment from Mr. Joe C. DiNardo, a private citizen. These were distributed to the panelists and DNTP staff before the meeting. Dr. Fry noted that the panel did not receive requests for oral public comments on the draft DART report.

5.3. Peer-review Comments and Panel Discussion

5.3.1. First Reviewer – Dr. Mary Alice Smith

- Dr. Smith commented that the dose range-finding study and MOG study were appropriately designed and executed well.
- She found the changes in mean body weight, vaginal opening, and balanopreputial separation of greatest interest. She agreed androgenic effects and reproductive toxicity were not supported by the study.
- She was concerned about the ability to adequately predict dose given feed spillage and encouraged DNTP staff to pursue calculations of internal dose for this type of study in general.
    - Dr. McIntyre agreed that DNTP staff will clarify the text to make the treatment of feed spillage data in calculating interval summary statistics more explicit.
- She thanked DNTP staff for addressing the text change related to body weight, which addressed her main concern about the conclusions.

5.3.2. Second Reviewer – Dr. Bethany Hannas

- Dr. Hannas agreed with Dr. Smith's comments and noted that the study was well designed and conducted and the report was well written. She appreciated the number of endpoints evaluated. Most of her comments were minor and requesting clarification.
    - First, she recommended comparing data to historical controls (e.g., for estrous length, which had the same magnitude of change across dosed groups).
    - Second, she asked about the dose level selection and justification, as the report mentioned spacing was chosen to enable identification of a NOAEL. The dams may have increased feed consumption during lactation, which appears to be reflected in the data. One option to address this is to reduce the fixed concentration in feed. A NOAEL did not appear to be identified.
        - Dr. McIntyre indicated that adjusting feed concentrations was considered, but the challenges overrode the possibility. He added that this could be clarified in the dose selection justification of the report.
    - Third, Dr. Hannas noted the absence of an assessment of gestational implantation sites to improve observations about littering.
    - Fourth, she requested more information in the report on possible variability in anogenital distance, areola and nipple retention, and vaginal opening as related to timing and data collection procedures.
        - Dr. McIntyre noted that a small pool of individuals was trained with confirmation of consistency among researchers. He suggested that increased detail could be added to the report methods.

5.3.3. Third Reviewer – Dr. Linda Roberts

- Dr. Roberts indicated that the study was well designed and conducted. She generally agreed with the interpretations. She also noted that the historical control data were sparse. Dr. Roberts agreed that the correct call was made to not consider skeletal findings abnormal in the absence of other indications.
    - Dr. McIntyre thanked Dr. Roberts for her comments.

5.3.4. Panel Discussion

- Dr. Enright asked whether the rationale for the dosing route could be explained in the report text.
    - Dr. McIntyre commented that this clarification could be added.

5.4. Vote on NTP Conclusions

5.4.1. Reproductive Toxicity

Dr. Fry called for a motion from the panel to approve the conclusions as written. Dr. Smith so moved, and Dr. Hannas seconded the motion. The panel voted unanimously (4 yes, 0 no, 0 abstentions) to approve the conclusions as written.

5.4.2. Developmental Toxicity

Dr. Fry called for a motion from the panel to approve the conclusions as written. Dr. Smith so moved, and Dr. Roberts seconded the motion. The panel voted unanimously (4 yes, 0 no, 0 abstentions) to approve the conclusions as written.

5.4.3. Other Effects

Dr. Fry called for a motion from the panel to approve the conclusions as written. Dr. Hannas so moved, and Dr. Enright seconded the motion. The panel voted unanimously (4 yes, 0 no, 0 abstentions) to approve the conclusions as written.

5.5. Final Conclusions

DNTP staff acknowledged to the panel that an error was identified in the report draft conclusions and presented revisions to the draft conclusions (underlined) to the panel for consideration and voting:

- ***No evidence of reproductive toxicity*** of EHMC in Hsd:Sprague Dawley® SD® rats at exposure concentrations of 1,000, 3,000, or 6,000 ppm. Mating and littering were not affected significantly by EHMC exposure.
- ***Equivocal evidence of developmental toxicity*** of EHMC in Hsd:Sprague Dawley® SD® rats based on the observed postnatal effects on body weight that showed some indication of recovery by study end, delays in postnatal day 28-adjusted vaginal opening and balanopreputial separation, which could have <u>been</u> influenced <u>by</u> the apparent transient

effects on body weight, and time in estrus was slightly longer in EHMC-exposed females relative to that of the control group.

- No other signals consistent with alterations in estrogenic, androgenic, or antiandrogenic action were observed. EHMC exposure did not induce any specific fetal malformations.

6. Closing Remarks on the Draft Reports

Dr. Fry welcomed additional panel comments on the draft report.

- Dr. Roberts had one additional question about what was meant by kidney amputation.
    - Dr. McIntyre explained that this was likely an entry error from the pathology data.
- Dr. Smith mentioned she agreed with Dr. Hannas' recommendation to incorporate historical data if possible.

Dr. Berridge thanked all the peer-review panelists and DNTP staff.

Closing the meeting, Dr. Scruggs added her thanks for everyone's participation in the meeting. She announced the slides from the meeting and report materials would be posted publicly.

Dr. Fry added her thanks to all participants for their efforts. Dr. Fry then adjourned the meeting at 11:52 a.m. EDT on October 14, 2021.

7. 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 October 14, 2021, Peer Review of the Draft NTP Developmental and Reproductive Toxicity Technical Reports on 2-Hydroxy-4-methoxybenzophenone and 2-Ethylhexyl p-Methoxycinnamate.

Rebecca Fry, Ph.D.

Peer-review Panel Chair

Date: January 12, 2022