National Toxicology Program

Peer Review of the Draft NTP Technical Reports on the Toxicology and Carcinogenesis Studies of 2-Hydroxy-4-methoxybenzophenone and Perfluorooctanoic Acid

December 12, 2019

National Institute of Environmental Health Sciences
Research Triangle Park, NC

Peer-Review Report

Table of Contents

1. Attendees[1]

Peer-Review Panel[2]

Chair: Russell Cattley, Auburn University

Michael Elwell, APEX TOXPATH, LLC

Wendy Halpern, Genentech, Inc.

Gabriele Ludewig, University of Iowa

Kristini Miles, Venture Chemical Consulting, LLC

Karen Regan, Regan Pathology/Toxicology Services, Inc. (2-hydroxy-4-methoxybenzophenone only)

National Toxicology Program Board of Scientific Counselors Liaison

Anne Ryan, Act 5 Ventures LLC (by webcast)

National Institute of Environmental Health Sciences Staff

Brian Berridge

Chad Blystone

Michelle Cora

Stephen Ferguson

Shawn Harris

Angela King-Herbert

Ron Herbert

Dave Malarkey

Elizabeth Maull, Designated Federal Official

Barry McIntyre

Georgia Roberts

Sheena Scruggs

Keith Shockley

Robert Sills

Matthew Stout

Suramya Waidyanatha

Nigel Walker

AtLee Watson

Kristine Witt

Mary Wolfe

Other Federal Agency Staff

Gonçalo Gamboa, FDA (by WebEx)

Contract Support Staff

Amy Brix, EPL, Inc.

Canden Byrd, ICF

Josh Cleland, ICF

Katherine Duke, ICF

[1]The meeting was webcast. Individuals who viewed the webcast are not listed except as noted.
[2] With the exception of the peer-review panel chair, all panel members participated remotely via WebEx.

Sophie Hearn, ICF
Ernie Hood, Bridport Services
Kyathanahalli Janardhan, ILS
Kelly Shipkowski, ICF
Samantha Snow, ICF
Jessica Wignall, ICF

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 2-Hydroxy-4-methoxybenzophenone* and *Perfluorooctanoic Acid* on December 12, 2019 via webcast.

- Dr. Russell Cattley, panel chair, called the meeting to order at 10:00 a.m., welcomed everyone to the meeting, asked all attendees to introduce themselves, and reviewed the peer-review meeting format for the panel and audience.
- Dr. Brian Berridge, NTP Associate Director, welcomed all participants to the meeting.
- Dr. Elizabeth Maull read the conflict of interest policy statement and briefed the attendees on meeting logistics.

3. 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 the potential carcinogenic activity of the chemicals tested. He also described the NTP's historical controls, which are categorized by route of exposure and rodent strains. He then gave the charge to the panel 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 regarding the hypothesis under the conditions of this study. The peer-review meeting materials can be found on the NTP website.

4. Toxicology and Carcinogenesis Studies of 2-Hydroxy-4-methoxybenzophenone

4.1. Presentation and Clarifying Questions

Dr. Barry McIntyre summarized the studies and conclusions reported in the *Draft NTP Technical Report on the Toxicology and Carcinogenesis Studies of 2-Hydroxy-4-methoxybenzophenone (CAS No. 131-57-7) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice*.

2-Hydroxy-4-methoxybenzophenone is an ultraviolet (UV) filter used in sunscreens and cosmetics to protect the wearer from solar erythema. 2-Hydroxy-4-methoxybenzophenone is also an indirect food additive as it is added to acrylic and modified acrylic plastics that encounter food to prevent UV-mediated degradation. NTP chose to study 2-hydroxy-4-methoxybenzophenone due to widespread human exposure and lack of carcinogenicity data.

Dr. McIntyre first presented a summary of results from the 2-hydroxy-4-methoxybenzophenone Endocrine Disruptor Screening Panel studies reported in Appendix F of the technical report.

Genetic toxicology studies conducted in *Salmonella typhimurium* strains TA98 and TA100 as well as *Escherichia coli* strain *uvr*A pKM101 with and without S9 were negative.

NTP conducted a perinatal toxicity/carcinogenicity study in time-mated female Hsd:Sprague Dawley® SD® rats, with exposure concentrations of 0, 1,000, 3,000, and 10,000 ppm in the diet. Exposure began on gestation day (GD) 6 through lactation in the dams. In the offspring, exposure continued after weaning on postnatal day (PND) 21 (n = 50/dose) for 2 years. In addition, there was a 14-week interim necropsy for the 0 and 10,000 ppm groups (n = 10/sex/dose). Dr. McIntyre presented a summary of results from the 2-hydroxy-4-methoxybenzophenone perinatal toxicity/carcinogenicity study.

NTP also conducted a standard chronic bioassay in male and female B6C3F1/N mice, with exposure concentrations of 0, 1,000, 3,000, and 10,000 ppm in the diet for 2 years. Dr. McIntyre presented a summary of findings from the standard chronic bioassay.

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

- ***Equivocal evidence of carcinogenic activity*** of 2-hydroxy-4-methoxybenzophenone exposure in male Hsd:Sprague Dawley® SD® rats based on the occurrence of brain and spinal cord malignant meningiomas.
- Exposure to 2-hydroxy-4-methoxybenzophenone resulted in increased incidences of nonneoplastic lesions of the testis and pancreas in male rats.
- ***Equivocal evidence of carcinogenic activity*** of 2-hydroxy-4-methoxybenzophenone exposure in female Hsd:Sprague Dawley® SD® rats based on the increased incidence of thyroid C-cell adenomas and the increased incidence of uterine stromal polyps.
- Exposure to 2-hydroxy-4-methoxybenzophenone resulted in increased incidences of nonneoplastic lesions of the uterus and adrenal cortex in female rats.
- ***No evidence of carcinogenic activity*** in male or female B6C3F1/N mice at exposure concentrations of 1,000, 3,000, and 10,000 ppm 2-hydroxy-4-methoxybenzophenone.
- Exposure to 2-hydroxy-4-methoxybenzophenone resulted in increased incidences of nonneoplastic lesions of the bone marrow, spleen, and kidney in male and female mice, and liver in male mice.

There were no clarifying questions or comments about the presentation.

4.2. Public Comments

Dr. Cattley acknowledged the receipt of written public comments from private citizen Mr. Joe DiNardo. He 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. Karen Regan

Dr. Karen Regan said that the studies were well-written and well-conducted. She requested clarification on how the dose used in the studies compared to human exposure. She asked for more information on the historical control data used for this study and whether there were any data available from the same strain of rat exposed from GD 6. Dr. Regan questioned why NTP

did not evaluate spinal cords for all animals in the study. Dr. Regan asked whether there were any tables that showed the overall incidence of multiplicity for thyroid tumors in female rats, and whether there were any animals with multiple tumors.

- Dr. McIntyre indicated that the plasma levels found in the rodents following exposure were comparable to levels recently reported in human blood (JAMA 2019).
- Dr. Blystone reported that the historical controls used in this study include all exposure start times with most of these studies starting at GD 6.
- Dr. Amy Brix stated that only animals with clinical neuropathological signs had their spinal cords examined and indicated that NTP would consider adding a sentence to the report to clarify this point. She noted that it is possible that they did not observe additional occurrences of malignant meningiomas in the spinal cords. Dr. Brix noted that there were no occurrences of these type of tumors in the historical controls or control group. Even without the single occurrence in the spinal cord, NTP considered the occurrence of malignant meningioma in the brain of adult rats to be equivocal evidence of carcinogenicity.
- Dr. Brix noted that only one female in the highest dose group had bilateral C-cell adenomas in the thyroid, with the rest of the adenomas being unilateral. She remarked that NTP only counted an animal once if it had both a thyroid adenoma and carcinoma, which occurred in only one rat. She stated that they will explain these methods further in the report.

4.3.2. Second Reviewer – Dr. Kristini Miles

Dr. Kristini Miles stated that the study was well-designed and executed. She noted that NTP chose sufficient doses for the rats and mice as they were based on previously conducted studies. Dr. Miles noted that 2-hydroxy-4-methoxybenzophenone has been reported in the literature to be a persistent environmental contaminant, specifically in water sources, and there are reports that traditional wastewater treatment processes may not eliminate the contaminant. She asked whether NTP tested 2-hydroxy-4-methoxybenzophenone in the municipal tap water used in the study. Dr. Miles inquired whether there was any information available on 2-hydroxy-4-methoxybenzophenone concentrations in synthetic resins and plastics, and how it might leach out of such containers. She asked that NTP include the information in the report. Regarding Table 8 in the report, Dr. Miles asked whether the trend test referred to historical controls or something else.

- Dr. McIntyre stated that they did not examine the municipal water used for the presence of 2-hydroxy-4-methoxybenzophenone; however, all rodents received the same water supply.
- Dr. McIntyre indicated that NTP provided information on leachates in plastics, and they would consider adding this information to the report.
- Dr. Keith Shockley noted that they based the trend test on experimental data, not historical controls.

4.3.3. Third Reviewer – Dr. Michael Elwell

Dr. Michael Elwell indicated that he had no comments on the study design or dose selection and the results and figures were clearly presented by the NTP. He concurred with the neoplastic and nonneoplastic findings listed in the draft report for the male rats. Regarding the data and discussions of the meningioma and interstitial cell hyperplasia, Dr. Elwell requested confirmation that NTP examined spinal cords from only one or two animals per group. He asked whether it was correct to combine the spinal cord and brain data as the study staff did in Table 11 to perform statistical analysis on the occurrence of these tumors. Dr. Elwell noted that there was one meningioma present in the spinal cord but three in the brain in male rats exposed to 1,000 ppm. He remarked that one of these animals (Animal 151) also had a meningioma recorded in the trigeminal nerve. He asked whether NTP should report that meningioma separately and include it in the discussion. He questioned whether NTP should mention the occurrence of granular cell tumors in the meninges in the report as part of the assessment of the meningiomas since NTP Technical Report 573 discussed the relationship between granular cell tumors and meningiomas, with an indication that they had similar morphologies and a common progenitor cell type. Dr. Elwell recommended that the NTP report the increased incidence of interstitial cell hyperplasia of the testes in the discussion section since they included it in the abstract and results section. Dr. Elwell agreed in principle with the conclusion of no evidence of carcinogenicity in the male mice. He suggested clarifying the wording that described the cytoplasmic alteration of the kidneys that occurred. Dr. Elwell stated that the incidence of lymphocyte infiltrates and nephropathy in the kidney listed in the abstract appeared to be very minor given the minimal severity and common background occurrence of these findings. Dr. Elwell remarked that prior studies with ethylbenzene reported no association of syncytial cell alterations in the liver with hepatocellular neoplasia. He suggested this would be worth mentioning in the discussion since ethylbenzene has a similar structure to 2-hydroxy-4-methoxybenzophenone.

In response to Dr. Elwell's comments, Dr. Brix indicated that:

- While NTP examined the brains of all animals, only animals with clinical neurological signs had their spinal cords examined. NTP will update the table to reflect this. NTP will also consider removing the spinal cord data from this analysis.
- There was an error in the table reporting two meningiomas in Animal 151 (one should have been recorded as a metastasis of the other) and they would update the table to reflect this. NTP will also consider adding language to the discussion section regarding malignant meningiomas.
- NTP will consider adding to the discussion section that meningiomas and granular cell tumors may have a common progenitor.
- NTP will consider adding text to the report that there was no effect on interstitial cell adenomas reported in male rats. NTP will also consider adding text to the discussion regarding interstitial cell hyperplasia.
- There was an error in describing the cytoplasmic alteration in the kidneys in male mice. NTP will update the report to reflect this.

- NTP will consider adding language to the results section downplaying the importance of the chronic progressive nephropathy and lymphocytic infiltrates in the kidneys of male mice and will remove mentions of these findings from the discussion.
- NTP would consider adding language to the report regarding the lack of association between syncytial cell alteration in the liver and exposure to ethylbenzene.

In a follow up question, Dr. Elwell described his confusion regarding the statistical approaches for the non-neoplastic kidney findings in this report, which seemed to take severity into account.

- Dr. Shockley replied that the statistical analysis did not take severity into account but was instead based on incidence. However, this method did account for differential survival.

4.3.4. Panel Discussion

Dr. Gabriele Ludewig stated that the report was clear and well-written. She wondered whether the number of incidences of meningiomas indicated a relationship to exposure and whether NTP should report this as clear evidence instead of equivocal. Dr. Ludewig asked for comment on why they used rats and not hamsters since they metabolize this compound differently, which may make it genotoxic. Dr. Ludewig noted that ER and AR binding is only one way a compound can be an endocrine disruptor. She noticed in the report that NTP reported findings in the adrenal gland which could lead to serotonin disruption and suggested adding that point to the report. Dr. Ludewig observed that only the highest exposure group had female rats that were not pregnant and suggested adding a statement regarding this point.

- Dr. Brix stated that it is always challenging to make a call on uncommon tumors without statistical support, dose response, a change in latency, or supporting evidence from other sex, species, or preneoplastic lesions. Therefore, NTP decided there was equivocal evidence based on the presence of meningiomas.
- Dr. McIntyre noted that NTP no longer used the hamster S9 assay under Organisation for Economic Co-operation and Development (OECD) guidance and that performing those tests would be outside the scope of this report.
- Dr. McIntyre indicated that NTP has done extensive work in relation to testing for hormonal activity and is in the process of reporting on reproductive performance as well as markers of altered endocrine action in another technical report.
- Dr. McIntyre noted that they examined the animals that did not deliver for implantation sites and they were not pregnant. Since exposure began after implantation, they were not concerned that this was an exposure-related effect.

In a follow up statement, Dr. Ludewig concluded that she was between some and equivocal evidence regarding the presence of meningiomas.

Dr. Wendy Halpern noted that other reviewers addressed most of her comments. She asked the panel to comment on whether it was a common observation that there were fewer potentially exposure-related tumors identified at the highest dose where the animals had lower body weight.

- Dr. Blystone reported that the influence of lower body weight on the cancer response has been characterized for only a few tumor types (e.g., mammary gland tumors) and that we do not have the information for the other tumor types to include in the report.

Dr. Cattley questioned why there had been a separate peer review conducted on the uterus in the rats.

- Dr. Brix reported that over the past several years, NTP had changed its procedure from doing cross sectional evaluations to more comprehensive analyses of the uterus.
- Dr. Brix reported that NTP would consider adding language to the report to clarify this point.

4.4. Vote on NTP Conclusions

4.4.1. Male Hsd:Sprague Dawley® SD® rats

Dr. Cattley called for a motion from the panel to approve the conclusions as written. The panel did not offer a motion. Dr. Regan moved that NTP delete the reference to spinal cord malignant meningiomas from the conclusion. Dr. Ludewig seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to approve the new conclusion.

4.4.2. Female Hsd:Sprague Dawley® SD® rats

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

Following the voting, Dr. Cattley noted that Dr. Regan would sign off from the session.

5. Toxicology and Carcinogenesis Studies of Perfluorooctanoic Acid

5.1. Presentation and Clarifying Questions

Dr. Blystone summarized the studies and conclusions reported in the *Draft NTP Technical Report on the Toxicology and Carcinogenesis Studies of Perfluorooctanoic Acid (CAS No. 335-67-1) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats.*

Perfluorooctanoic acid is a perfluoroalkyl substance (PFAS) used for decades in creating non-stick properties in a variety of products. Manufacturers agreed to discontinue use due to widespread exposure and health concerns. Due to a long half-life measured in years and resistance to environmental degradation, exposure has continued but declined. Perfluorooctanoic acid is the second most abundant PFAS measured in the human population, including children and pregnant women.

Human exposure to perfluorooctanoic acid can occur during early development. It is unknown whether exposure during gestation and lactation alters the carcinogenic response induced by perfluorooctanoic acid. NTP tested the hypothesis that including perinatal exposure with postweaning exposure would quantitatively or qualitatively alter the perfluorooctanoic acid response compared to postweaning exposure only.

NTP conducted a perinatal and postweaning toxicity/carcinogenicity study in Hsd:Sprague Dawley® SD® rats. In Study #1, they exposed time-mated female rats to 0, 150, or 300 ppm perfluorooctanoic acid during the perinatal period. NTP provided F_1 female rats with 0, 300, or 1,000 ppm perfluorooctanoic acid during the postweaning period (i.e., perinatal/postweaning exposures of 0/0, 0/300, 0/1,000, 150/300, or 300/1,000 ppm) while they provided F_1 male rats with 0, 150, or 300 ppm perfluorooctanoic acid during the postweaning period (i.e., perinatal/postweaning exposures of 0/0, 0/150, 0/300, 150/150, or 300/300 ppm) (n = 50/sex/dose). Female rats have a lower systemic exposure than males due to a faster perfluorooctanoic acid elimination rate, so NTP provided a higher feed exposure concentration to female rats postweaning. In addition, they conducted a 16-week (19 weeks of age) interim necropsy (n = 10/sex/dose).

Due to observed unanticipated toxicity in males during the interim necropsy, NTP removed males from Study #1 at week 21. In Study #2, they exposed time-mated female rats to 0 or 300 ppm perfluorooctanoic acid during the perinatal period. They provided F_1 male rats 0, 20, 40, or 80 ppm perfluorooctanoic acid during the postweaning period (i.e., perinatal/postweaning exposures of 0/0, 0/20, 0/40, 0/80, 300/0, 300/20, 300/40, or 300/80 ppm) (n = 50/sex/dose). Dr. Blystone presented a summary of results from the perinatal and postweaning toxicity/carcinogenicity study.

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

- ***Clear evidence of carcinogenic activity*** of perfluorooctanoic acid in male Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular neoplasms (predominately hepatocellular adenomas) and increased incidences of acinar cell neoplasms (predominantly acinar cell adenomas) of the pancreas.
- Exposure to perfluorooctanoic acid resulted in increased incidences of nonneoplastic lesions in the liver and pancreas of male rats. The additional effect of combined perinatal and postweaning exposure was limited to a higher incidence of hepatocellular carcinomas compared to postweaning exposure alone.
- ***Some evidence of carcinogenic activity*** of perfluorooctanoic acid in female Hsd:Sprague Dawley® SD® rats based on the increased incidences of pancreatic acinar cell adenoma or adenocarcinoma (combined) neoplasms.

- The higher incidence of hepatocellular carcinomas and higher incidence of adenocarcinomas of the uterus may have been related to perfluorooctanoic acid exposure.
- Exposure to perfluorooctanoic acid resulted in increased incidences of nonneoplastic lesions in the liver, kidney, forestomach, and thyroid gland.
- The combined perinatal and postweaning exposure was not observed to change the neoplastic or nonneoplastic response compared to postweaning exposure alone.

There were no clarifying questions or comments about the presentation.

5.2. Public Comments

Dr. Cattley acknowledged the receipt of three written public comments from Dr. Oyebode A. Taiwo on behalf of the 3M Company, Dr. Alexis Temkin on behalf of the Environmental Working Group, and Mr. Jason Dadakis from the Orange County Water District. Dr. Cattley noted that there was one oral public comment from Mr. Steve Risotto on behalf of the American Chemistry Council (ACC).

Mr. Risotto said the ACC believed the peer review committee should carefully consider NTP's conclusion that there is "some evidence of carcinogenic activity" in female rats, requested additional analysis of the pancreatic tumor data for male rats, and asked whether a conclusion could be reached about the sensitivity of fetal rats to perfluorooctanoic acid exposure. Regarding the evidence for carcinogenicity in female rats, Mr. Risotto observed that there was a non-significant increase of combined acinar cell adenomas and adenocarcinomas at 1,000 ppm. He noted that while the increased incidence of acinar cell neoplasms in males increased NTP's confidence that neoplasms in females were related to perfluorooctanoic acid exposure, NTP did not observe acinus hyperplasia in the female rats, which was significantly increased in the male rats. If this hyperplasia is a potentially preneoplastic lesion as NTP suggests, Mr. Risotto stated that this finding should also have been observed in the female rats. Mr. Risotto also noted that the survival rate in the female rats was quite low, which might raise concerns about the general animal husbandry practices of the study since survival was depressed in both the control and exposed animals. Regarding the incidence of acinar cell neoplasms in the male rats, Mr. Risotto noted that the control group had significantly elevated acinus hyperplasia, a possible preneoplastic lesion, affecting nearly 40% of the control animals. The high background rate observed in the study confirmed the higher sensitivity of the Hsd:Sprague Dawley® SD® rats compared to other rat strains, and more significantly, to humans. Mr. Risotto also observed that NTP used a smaller size criterion for classifying pancreatic acinar cell neoplasms than previous perfluorooctanoic acid studies. He stated that the draft report does not provide an explanation for why NTP reduced the lesion criteria, or the potential impact such a reduction may have on the findings. Mr. Risotto stated that given the flat dose-response for acinar cell neoplasms and high rate of preneoplastic hyperplasia in the control group, NTP should further consider the pancreatic results in the male rats, particularly given the likely contribution of peroxisome proliferator-activated receptor alpha (PPARα) to tumor formation. Mr. Risotto questioned the relevance of the male rat findings to human risk assessment. He said that ACC was unable to find additional information in the draft report on the nature of the toxicity that caused NTP to restart the male portion of the study. He added that it is critically important that the committee understand the nature of the unanticipated toxicity and consider its potential significance to the findings in the

draft report. Regarding the conclusion about fetal sensitivity to perfluorooctanoic acid exposure, Mr. Risotto observed that the draft report indicates that there were very few significant differences between the groups of animals exposed postweaning only versus groups with perinatal and postweaning exposure. He noted that the differences observed were sporadic and that the report does not prove a conclusion regarding the potential impact of perinatal exposure yet that is the stated hypothesis for conducting the study. Given that NTP rarely conducts combined perinatal and postweaning chronic studies, Mr. Risotto said that ACC believes the committee should evaluate whether the study results support NTP's central hypothesis. He stated that the report summary suggests NTP does not support the hypothesis.

5.3. Peer-Review Comments and Panel Discussion

5.3.1. First Reviewer – Dr. Michael Elwell

Dr. Elwell said that both studies were well-designed, nicely presented, and NTP clearly stated the results. Dr. Elwell requested further discussion of why the proposed level of carcinogenic activity for pancreatic neoplasms in females does not also apply to the liver carcinoma findings. He said the rationale for the pancreas acinar neoplasm increases as "some evidence" in females appears to be based on the current and historical control data, and association with the finding of "clear evidence" in males. He felt that the rationale could apply to the female liver neoplasm conclusion as well. Dr. Elwell requested discussion of why NTP did not consider the uterine carcinomas "equivocal." Dr. Elwell asked about the relationship of the extended and standard evaluations on the incidence of adenoma and adenocarcinoma in the uterus.

In response to Dr. Elwell's comments, Dr. Blystone indicated that:

- NTP applied a call of *some evidence of carcinogenic activity* to the adenomas and adenocarcinomas in the pancreas in females since they were present in the highest dose group and considered a rare lesion. However, in the liver, there was only a marginal increase in the incidence of hepatocellular carcinomas in females and no change in the incidence of adenomas.
- NTP used a weight of evidence approach to determine that uterine adenocarcinomas might have been related to exposure.
- NTP found most of the uterine adenocarcinomas after the new sectioning and extended evaluation.

5.3.2. Second Reviewer – Dr. Gabriele Ludewig

Dr. Ludewig agreed that NTP executed and described the studies very well. She noted that due to the interest in the results for human risk assessment, the introduction should be perfectly clear as to the limitations of the study. Dr. Ludewig suggested that NTP should clearly state the reference to the perfluorooctanoic acid half-life in female rats, which is measured in hours, versus the half-life in humans, which is measured in years, in the introduction to prevent a false assumption of safety from the high dose that was used. Dr. Ludewig recommended clarifying what type of "overt toxicity" NTP observed in males in Study #1. Dr. Ludewig suggested they add a statement to the discussion as to whether they considered/analyzed ossification and changes in bone morphology since regulatory values in the United States are based on these findings.

Dr. Ludewig recommended emphasizing in the report that the PPARα pathway, which NTP mentions in the report, is not relevant to humans. The CAR pathway and any observation of its activation should be discussed. Dr. Ludewig said NTP should clearly state that the adult exposure included some developmental exposure during sexual maturation since the rats do not fully develop until PND 90. She noted this is important since the hypothesis was to test whether there was an influence of developmental exposure. Dr. Ludewig pointed out that NTP did not mention fecal excretion. Dr. Ludewig stressed that they clearly state the study limitations, especially since the short half-life in rodents is thought to be due to specific transporters present in the kidneys and other organs which may have influenced organ-specific concentrations of PFOA. Dr. Ludewig reported that NTP should cite recently published studies on perfluorooctanoic acid, glucose levels, and liver toxicity to support the findings that there is some liver toxicity.

- Dr. Blystone indicated that NTP would consider clarifying some of the language and adding statements to the report based on her suggestions. This may include additional information on the differences in perfluorooctanoic acid elimination between rodents and humans, a statement about bone morphology findings, and highlighting the point that exposure occurred during sexual maturation.

5.3.3. Third Reviewer – Dr. Wendy Halpern

Dr. Halpern indicated that NTP clearly presented and described the findings in the draft report. Dr. Halpern suggested adding more information to the discussion section, in a similar manner to the introduction, that highlights the potential mechanisms of these findings. For instance, the discussion could address the fact that previous carcinogenicity studies identified interstitial cell tumors in male rats as potentially related to perfluorooctanoic acid exposure, which NTP did not identify in this study. Dr. Halpern requested more discussion on the potential immunomodulatory activity as indicated by the decreased spleen and thymus weights. Dr. Halpern suggested expansion of the interim necropsy results, particularly the effect on liver weights. She noted that the biggest challenge in interpreting the study results is the exposure differences, both between rats and humans and between male and female rats. For instance, if testosterone is the driver for exposure differences in males, it is hard to interpret the exposure relationship during development when testosterone is still low in male pups and where female pups were given a higher dose based on exposure differences in adult rats. Dr. Halpern agreed that the decreased body weight and hepatic findings in the males in the initial 16-week necropsy warranted a change in dose administration. However, it is difficult to distinguish the sex-related from potential study-related differences in hepatocellular carcinoma incidence, especially with a small absolute magnitude of difference. Dr. Halpern noted that in almost every parameter evaluated, perinatal exposure seemed to have minimal or no effect, with the singular exception of a numerical increase in hepatocellular carcinomas in male rats exposed to 300/80 ppm in Study #2. She did not think the overall data supports that perinatal exposure drives this finding since there was a numerically similar incidence of hepatocellular carcinomas in the female rats and because of the potential linkage of these neoplasms to PPARα. She noted that she did not see a difference with perinatal exposure in either liver weights, acyl CoA activation, or any other parameter that would suggest that PPARα induction was different. She did not think this finding was clearly related or dependent on perinatal exposure, which goes back to the hypothesis presented. Dr. Halpern indicated that her main question was whether this was a developmentally sensitive effect

given that male rats had the same exposure as female rats based on the kinetic data and these findings were not present in female rats.

In response to Dr. Halpern's comments, Dr. Blystone indicated that:

- NTP phrased the conclusion statements as they were because they limited the response to that one finding and, since perinatal exposure may by an influence, it was not an outright rejection of the hypothesis.
- NTP did not observe differences in liver weights or acyl CoA oxidase activity. He noted that there might be other mechanisms at play besides increased PPARα via acyl CoA activation, such as the constitutive androstane receptor (CAR) pathway. While CAR activity has been shown in other studies, NTP did not evaluate it in this study.
- NTP would consider clarifying various points in the report based on Dr. Halpern's suggestions such as the exposure concentration differences between this study and concentrations used previously, the role sexual maturation may play in the exposure differences, and details regarding spleen and thyroid weights.

In a follow up statement, Dr. Halpern said it was her impression from reading the report that NTP did not expect that male and female perinatal exposures would have differed, yet they only saw hepatocellular carcinomas in the males, not the females.

- Dr. Blystone confirmed Dr. Halpern's statement.

5.3.4. Panel Discussion

Dr. Cattley expressed concern that there was not sufficient evidence to conclude that there was an influence of perinatal exposure. For instance, the hepatocellular carcinoma response was so weak that there was not much of a window to detect any increase that might occur without any perinatal exposure. In addition, there was no effect on the incidence of hepatocellular adenomas with perinatal exposure. He noted that the report appropriately combined the incidence of hepatocellular adenomas and hepatocellular carcinomas since NTP considered adenomas to be the precursor lesion and can potentially progress to carcinomas. He indicated that he was struggling with the hypothesis about the impact of perinatal exposure on the incidence of hepatocellular neoplasia in the study.

- Dr. Blystone confirmed that the incidence of adenomas did not appear different between animals with and without perinatal exposure. He remarked that although the strength of response was not strong, the rarity of the lesion and the fact that the liver responded to the chemical led NTP to make this conclusion.

In a follow up statement, Dr. Cattley indicated that he was still hesitant to conclude that there was a perinatal effect in this study.

5.4. Vote on NTP Conclusions

5.4.1. Male Hsd:Sprague Dawley® SD® rats

Dr. Cattley called for a motion from the panel to approve the conclusions as written.
Dr. Ludewig so moved, but there was no second to the motion. Dr. Cattley called for a motion

from the panel to approve the first two bullets as written and delete the third bullet, and the panel would consider adding a replacement third bullet. Dr. Halpern so moved and Dr. Ludewig seconded the motion. The panel voted unanimously (4 yes, 0 no, 0 abstentions) to approve the first two bullets of the conclusion. Dr. Cattley called for a motion to add a third bullet. Following an extensive discussion, the panel proposed the third bullet read, "The additional effect of perinatal exposure in combination with postnatal exposure was uncertain and limited to the observation of hepatocellular carcinomas." Dr. Halpern moved for the approval of the third bullet. Dr. Ludewig seconded the motion. The panel voted unanimously (4 yes, 0 no, 0 abstentions) to approve the proposed third bullet.

5.4.2. Female Hsd:Sprague Dawley® SD® rats

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

6. Closing Remarks on the Draft Reports

Dr. Cattley welcomed additional panel comments on the draft reports. There were no additional comments.

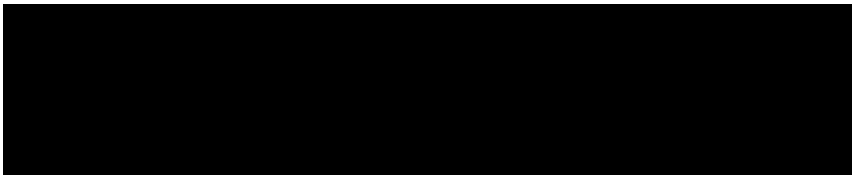
Closing the meeting, Dr. Maull thanked all the peer review panelists.

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

Dr. Cattley adjourned the meeting at 1:15 p.m. EDT on December 12, 2019.

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 December 12, 2019 Peer Review of the *Draft NTP Technical Reports on the Toxicology and Carcinogenesis Studies of 2-Hydroxy-4-methoxybenzophenone and Perfluorooctanoic Acid.*



Russell Cattley, V.M.D., Ph.D.

Peer-Review Panel Chair

Date: March 2, 2020