

**National Toxicology Program**

**Technical Reports Peer Review Panel Meeting**

**July 13, 2017**

**National Institute of Environmental Health Sciences**

**Research Triangle Park, NC**

***Peer Review Report***

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*Peer Review Report*

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## **I. Attendees**

### **Members in Attendance:**

Russell Cattley (Panel Chair)  
Michael Conner  
Noël Dybdal  
Terry Gordon  
Gabriele Ludewig  
Kristini Miles  
Richard Peterson

### **NTP Board of Scientific Counselors Representative:**

Jennifer Sass (via Webcast)

### **National Institute of Environmental Health Sciences (NIEHS) Staff:**

Chad Blystone	Daven Jackson-Humbles	Keith Shockley
John Bucher	Gloria Jahnke	Robert Sills
Sheba Churchill	Angela King-Herbert	Stephanie Smith-Roe
Michael DeVito	Grace Kissling	Diane Spencer
Darlene Dixon	Ramesh Kovi	Matt Stout
June Dunnick	David Malarkey	Vicki Sutherland
Anika Dzierlenga	Barry McIntyre	Molly Vallant
Gordon Flake	Dan Morgan	Suramya Waidyanatha
Gopi Gadupudi	Esra Mutlu	Nigel Walker
Dori Germolec	Arun Pandiri	Kristine Witt
Virginia Guidry	Georgia Roberts	Mary Wolfe
Robbin Guy	Veronica Robinson	Rick Woychik
Ron Herbert	Kristen Ryan	Michael Wyde
Michelle Hooth	Kelly Shipkowski	

### **Contract Staff to NIEHS**

Susan Blaine, ICF  
Amy Brix, Experimental Pathology Labs, Inc.  
Canden Byrd, ICF  
Kyathanahalli Janardhan, Integrated Laboratory Systems  
Ramesh Kovi, Experimental Pathology Labs, Inc.  
Anna Stamatogiannakis, ICF  
Cynthia Wilson, ILS

### **Public Attendees**

Denice Aleman, University of Florida  
Susan Borghoff, ToxStrategies  
Ernie Hood, Bridport Services

## **II. Welcome and Introductions**

The National Toxicology Program (NTP) Technical Reports Peer Review Panel Meeting convened on July 13, 2017, in Rodbell Auditorium, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Russell Cattley served as chair. The other panel members in attendance were Drs. Michael Conner, Noël Dybdal, Terry Gordon, Gabriele Ludewig, Kristini Miles, and Richard Peterson. Dr. Jennifer Sass attended by webcast as the NTP Board of Scientific Counselors representative. Interested public attended the meeting in person or watched the proceedings via webcast.

Dr. Cattley welcomed everyone to the meeting and asked all attendees to introduce themselves. Dr. Bucher welcomed participants and thanked the board members and staff for their work. Designated Federal Officer Dr. Mary Wolfe read the conflict of interest statement.

## **III. Peer Review of Draft NTP Technical Reports: Charge**

Dr. Chad Blystone, toxicologist in the Toxicology Branch of the Division of NTP (DNTP) briefly reviewed the Levels of Evidence of Carcinogenic Activity guidelines used to express the draft conclusions. He also stated the panel's charge.

## **IV. Draft NTP Technical Report on *p*-chloro- $\alpha,\alpha,\alpha$ -trifluorotoluene (TR594)**

### **A. Presentation**

NTP study scientist Dr. Georgia Roberts briefed the panel on the draft NTP Technical Report on *p*-chloro- $\alpha,\alpha,\alpha$ -trifluorotoluene (PCTFT). PCTFT is a non-ozone-depleting solvent used in automobile body coatings and parts cleaning and as an intermediate in the synthesis of other chemicals such as herbicides. In 2006, a representative from the Kowa American Corporation (a large importer of PCTFT) nominated PCTFT because of its expanding use and lack of occupational exposure limits. The nomination included several other benzotrifluoride compounds. Based on the production volume and use pattern, NTP selected PCTFT for further evaluation.

NTP conducted 3-month and 2-year whole-body inhalation studies in male and female Hsd:Sprague Dawley SD rats and male and female B6C3F1/N mice. The 3-month studies showed higher body weights, higher liver weights and incidence of nonneoplastic lesions in the liver of all sexes and species, and increased severity of nonneoplastic lesions of the kidney in male rats, and suggested that PCTFT via inhalation exposure exhibits the potential to be a reproductive toxicant in the model animals.

In the 2-year studies, the highest concentrations administered were 1,000 ppm in rats and 400 ppm in mice.

Based on the 2-year studies, the draft NTP reports conclusions on PCTFT were:

Male Hsd:Sprague Dawley SD rats

- ***Some evidence of carcinogenic activity***
  - Increased incidences of C-cell adenoma in the thyroid gland
- ***May have been related to treatment*** (equivocal evidence)
  - Combined occurrences of alveolar/bronchiolar adenoma or carcinoma in the lung

Female Hsd:Sprague Dawley SD rats

- ***Some evidence of carcinogenic activity***
  - Increased incidences of C-cell adenoma in the thyroid gland
  - Increased incidences of benign pheochromocytoma in the adrenal medulla
  - Increased incidences of adenocarcinoma in the uterus
  - Increased incidences of stromal polyp in the uterus

Male B6C3F1/N mice

- ***Clear evidence of carcinogenic activity***
  - Increased incidences of hepatocellular carcinoma and hepatoblastoma in the liver

Female B6C3F1/N mice

- ***Clear evidence of carcinogenic activity***
  - Increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma in the liver
- ***Related to treatment*** (some evidence)
  - Combined incidences of adenoma or adenocarcinoma in the Harderian gland

Exposure to PCTFT caused increased incidences of nonneoplastic lesions in the lung and liver of male and female rats and mice, in the nose of male rats, in the adrenal medulla and uterus of female rats, in the forestomach of male and female mice, and in the larynx in male mice. Exposure to PCTFT caused increased severity of nonneoplastic lesions in the kidney of male rats.

## **B. Peer Reviewer Comments**

Dr. Gordon, the first reviewer, stated that the study design was excellent, and that based on the 3-month studies, the appropriate choices were made for the 2-year exposure concentrations for both rodent species. He also approved of the generation and monitoring of the exposure chamber concentrations, and the pathological survey of neoplastic and non-neoplastic changes. His only methodological concern was why the sperm endpoints were not included in the 2-year studies, since quite positive findings were observed for male reproductive endpoints in the 3-month rat studies. He found the data presentation very clear in both text and tables. He felt that appropriate conclusions were delineated, except that the correct conclusion for carcinogenesis in the female rats was somewhere between some and clear evidence. He suggested several additions to the report.

Dr. Brix, the study pathologist, said that in the 2-year studies, the animals were too old and there were so many background changes, it would not be possible to detect subtle changes in male reproductive endpoints.

Dr. Conner, the second reviewer, stated that the designs and conduct of the studies were appropriate for the questions raised. He recommended the inclusion of an estimate of dose, even if it is not precise. He felt that the conclusion for C-cell adenomas in male and female rats should have been *equivocal evidence* rather than *some evidence*. He also suggested that the conclusion for increased incidences of stromal polyp in the uterus of female rats should have been *equivocal evidence* rather than *some evidence*.

Dr. Roberts said it was not feasible to take accurate measurements during exposure, which would be needed for an accurate estimate of dose, due to several factors. Dr. Conner reiterated that that information is important in the regulatory world, and an estimate of dose, even with caveats, would be useful. Dr. Roberts discussed the reasons for the conclusions Dr. Conner mentioned.

Dr. Ludewig, the third reviewer, said that the studies were very well-planned and well-executed. She agreed with the NTP findings with respect to neoplasms. She said she would like to see if there were any difference in the control animals from this (inhalation) study and controls from other types of studies, since the housing conditions for the inhalation studies were different. She noted a decrease in mammary adenomas and carcinomas in the treated animals compared to controls, indicating a hormonal aspect. She suggested further discussion of mode of action parameters in the report. She noted that the word “obesogen” was not used in the report, despite the clear evidence of weight gain. She suggested some reference as a trigger for future research.

Dr. Brix said that before labeling PCTFT an obesogen, further studies would need to be conducted to determine whether the weight gain was due to fat versus increased hypertrophy or liver tumors. Dr. Roberts noted that PCTFT was included in Tox21 evaluations. Regarding the housing used, she said that differences in controls from inhalation studies compared to other routes it was one reason there had been so much focus on the concurrent controls. Dr. Walker added that it was always an issue in inhalation studies.

Dr. Peterson, the fourth reviewer, concurred that the study was well-conducted, with appropriate study design and clear reasoning for the concentrations used in the 2-year study. Overall, he agreed with the conclusions.

### **C. Panel Discussion and Vote**

Dr. Cattley called for the conclusions to be projected.

Dr. Gordon asked Dr. Conner which conclusions he had felt should be changed from *some evidence* to *equivocal evidence*. Dr. Conner reiterated changing the conclusion from *some evidence* to *equivocal evidence* for the thyroid gland C-cell tumors and

uterine stromal polyps, but said he would defer to the NTP calls and would not present a motion to change them.

Dr. Cattley asked for a motion to accept the NTP conclusions as written. Dr. Gordon so moved, with Dr. Peterson seconding the motion.

The panel voted (6 yes, 0 no, 0 abstentions) to accept the conclusions as written.

## **V. Draft NTP Technical Report on 2,3-Butanedione (TR593)**

### **A. Presentation**

NTP study scientist Dr. Daniel Morgan briefed the panel on the draft NTP Technical Report on 2,3-butanedione. It is the compound that gives butter its characteristic flavor, and is naturally present in butter, coffee beans, honey, and some fruits. It is a fermentation product in dairy products, beer, and wine, and is a component of artificial flavorings used in food, cooking oil, and beverages. Occupational exposure is the main concern. The potential toxicity of butanedione was first reported when there was a cluster of workers in a microwave popcorn packaging plant who developed an unusual lung disease called bronchiolitis obliterans. NIOSH determined that there was a strong correlation between exposure to the butter flavoring vapors and the occurrence of the rare, irreversible, and progressive disease. Based on the prevalence of the disease in exposed workers and the lack of chronic inhalation toxicity data, the compound was nominated by the United Food and Commercial Workers union for long-term inhalation studies.

NTP first conducted short-term studies of the compound to characterize its toxicity. Exposure to  $\geq 150$  ppm for 10 days produced bronchiolitis obliterans-like lesions in rats, similar to what had been seen in humans. As expected (since rodents are nose breathers), the most severe toxicity occurred in the nasal cavity, with decreasing toxicity in more distal regions of the respiratory tract. NTP conducted 3-month and 2-year whole body inhalation studies in male and female Wistar Han rats and male and female B6C3F1/N mice. In the 3-month studies, exposure-related significantly increased incidences of nonneoplastic lesions occurred in the respiratory tract of male and female rats and mice, with the highest number of lesions occurring in the nose. Effects were also seen in the larynx, trachea, and lung. Bronchiolitis obliterans was not present in rats or mice in the 3-month and 2-year studies.

Based on the 2-year studies, the draft NTP reports conclusions on 2,3-butanedione were:

Male Wistar Han rats

- **Some evidence of carcinogenic activity**
  - Combined incidences of squamous cell papilloma and squamous cell carcinoma of the nose

Female Wistar Han rats

- ***Some evidence of carcinogenic activity***
  - Incidences of squamous cell carcinoma of the nose

Male B6C3F1/N mice

- ***No evidence of carcinogenic activity*** exposed to 12.5, 25, or 50 ppm

Female B6C3F1/N mice

- ***Equivocal evidence of carcinogenic activity***
  - Occurrences of adenocarcinoma of the nose

Exposure to 2,3-butanedione resulted in increased incidences of nonneoplastic lesions of the nose, larynx, trachea, lung, and eye in male and female rats and mice.

## **B. Questions for Clarification**

Dr. Gordon asked about the 10-day study where bronchiolitis obliterans had been found at 150 ppm. He found it surprising that the disease was not observed at the slightly lower doses used in the 3-month studies. Dr. Morgan said that the compound has a very steep dose-response curve, and it took some time to find the breaking point in terms of dosage. Dr. Gordon asked about the choice to use the vapor phase, as in the popcorn workers it seemed there was considerable dust. Dr. Morgan agreed, but noted that dust would be an almost totally separate test agent, and would represent a totally different study.

## **C. Peer Reviewer Comments**

Dr. Conner, the first reviewer, noted that the studies were complicated, and reminiscent of formaldehyde studies, in which dosimetry was affected by breathing patterns. He suggested adding an estimate of dose to the report. He said the studies were well-conducted. He recommended upgrading the conclusion for combined incidences of squamous cell papilloma and squamous cell carcinoma of the nose in male rats from *some evidence* to *clear evidence*, based on the number of tumors seen. Similarly, he recommended upgrading the conclusion for adenocarcinoma of the nose in female mice from *equivocal evidence* to *some evidence*.

Dr. Morgan said that adding an estimate of dose would potentially be misleading in this instance. He described the thinking behind the conclusions mentioned by Dr. Conner. Dr. Conner felt that the number of instances of such a rare tumor would justify the upgrade he suggested. Dr. Morgan agreed that interpretation of the incidences of rare tumors is challenging.

Dr. Gordon, the second reviewer, said the study design was excellent, with appropriate choices for the 2-year exposure concentrations. He felt that the data presentation was clear in both the text and the tables, with statistical comparisons appropriate as described. He agreed with the *some evidence* conclusion for the rare tumors.

Dr. Dybdal, the third reviewer, also felt that the study design was well done, with the experimental results presented fully and clearly. She said she was concerned whether the rodent model was the correct model to look at the disease, given the compound's



background and its effect on the lung. She noted that although bronchiolitis obliterans was not seen, a very clear impact on respiratory tract damage was seen, along with fibrosis. Thus, it is a shame within the confines of the reporting system that that point could not be emphasized more in the report, she observed, “Because I think there is a smoking gun in this data that supports that this compound could well be problematic to the workers.” She agreed with the level of evidence conclusions.

Dr. Morgan noted that issues associated with use of rodents are inherent in all inhalation studies, but modelers have done well in extrapolation efforts. Dr. Flake said that in the report there had been an effort to highlight the fibrosis that had developed, in that it was anticipated that the animals would not develop bronchiolitis obliterans at the dosages used. The finding of fibrosis in the nose was unusual in itself, he added. Dr. Walker asked Dr. Dybdal if she was recommending adding detail to the discussion of the fibrotic responses. She said that would be advantageous.

Dr. Miles, the fourth reviewer, also thought that the study was well-designed and comprehensive, and evaluated doses relevant to human exposures. She found interesting the sex difference seen in the studies, and wondered if there were any reports of sex differences in the development of bronchiolitis obliterans in the literature. She suggested investigating whether there are any reports in the literature of nasal or respiratory cancer in the workers. She asked why the specific strains of rats and mice were chosen, and why data for female spleen was not included in one of the tables. She noted that she agreed with the conclusions stated in the report.

Dr. Morgan said there are no data available on nasal cancer in workers, nor is there reference to sex difference in bronchiolitis obliterans literature. He explained that the Wistar Han rat was the strain being used at the time, and the mouse strain was the standard. Dr. Flake said that no alterations in the spleen were seen in the females.

Dr. Miles mentioned that it would be helpful to include concentrations reported from other types of processing facilities, such as coffee roasters. Dr. Morgan said that type of data should be available through NIOSH, and he would work to include it.

#### **D. Panel Discussion and Vote**

With respect to sex differences, Dr. Ludewig said the animals were very heavy at the end of the studies, especially the males, which could result in very shallow breathing. Day/night activity patterns with exposure during the resting phase could also play a role, she observed. Both could result in lower/less deep exposure by inhalation than expected. She said she was surprised to see that the ulcers and inflammation in the skin were rated as not related to exposure, since those issues had only occurred in the exposed group. She noted that there were also skin lesions in exposed workers, indicating that the compound damages the skin.

Dr. Morgan said the differences between day and night activity in the animals were slight, and noted that those parameters had been taken into account when extrapolating the doses. Regarding the skin lesions, he said they had been seen in control animals

as well, so it is more likely that exposure exacerbated the lesions, rather than causing them.

Dr. Ludewig recommended adding citations about recommendations to use more butanedione in food production due to its antimicrobial activity, a factor adding to the timeliness of this report.

Dr. Peterson supported Dr. Dybdal's comments about addressing the fibrosis in the nonneoplastic lesions in the report, noting that this may represent a rodent surrogate of bronchiolitis obliterans, due to the difference between species.

Dr. Cattley called for the conclusions to be projected.

Dr. Miles moved to accept the conclusions as written. Dr. Dybdal seconded the motion.

The panel voted to accept the conclusions as written. The vote was 4 in favor, 2 opposed, 0 abstentions. Dr. Conner said he had voted no based on his recommendation for upgrades in the conclusions from *some evidence* to *clear evidence* in male rats and *equivocal evidence* to *some evidence* in female mice. Dr. Ludewig concurred.

## VI. Draft NTP Technical Report on Dietary Zinc (TR592)

### A. Presentation

NTP study scientist Dr. Michael Wyde briefed the panel on the draft NTP Technical Report on dietary zinc. Zinc is an essential trace element with various other critical biological functions. Zinc intake in many men over 18 and women over 14 is below the estimated average requirement. With the increased popularity of zinc as a dietary supplement, many are also ingesting excess zinc. Dietary zinc deficiency was nominated by private individuals. Excess zinc exposure was nominated by the Agency for Toxic Substances and Disease Registry.

Zinc carbonate was selected to be the test article in 2-year feed studies in Hsd:Sprague Dawley SD rats. Genotoxicity testing was also conducted. Management of zinc levels was a critical element of the study design, particularly elimination of extraneous sources of zinc.

Based on the 2-year feed studies, the draft NTP reports conclusions on dietary zinc were:

Male Hsd:Sprague Dawley SD rats

- ***Equivocal evidence of carcinogenic activity*** of diets deficient in zinc
  - Higher incidences of adenoma of the pancreas and increased incidences of animals with multiple pancreatic adenomas

Female Hsd:Sprague Dawley SD rats

- ***No evidence of carcinogenic activity*** of diets deficient in zinc (3.5 or 7 ppm)

Male Hsd:Sprague Dawley SD rats

- **No evidence of carcinogenic activity** of diets containing excess zinc (250 or 500 ppm)

Female Hsd:Sprague Dawley SD rats

- **No evidence of carcinogenic activity** of diets containing excess zinc (250 or 500 ppm)

Exposure to diets containing excess zinc resulted in increased incidences of nonneoplastic lesions of the pancreas in male and female rats. Exposure to diets deficient in zinc resulted in increased incidences of nonneoplastic lesions of the testes in male rats.

## B. Peer Reviewer Comments

Dr. Ludewig, the first reviewer, found the study design and conduct appropriate, with the data well-described and analyzed. She said the study was interesting in that it involved both an excess and deficiency of dietary zinc. She agreed with the findings included in the draft technical report. She questioned references to *blood levels* of the metals, in that metals are usually measured in plasma or serum, not whole blood. She also requested clarification of LOD and LOQ with respect to measured copper levels. She said she would have added selenium to the analysis, and would have liked to see metal determinations in the organs. She also would have liked to have seen a discussion about kinetics with respect to the reference to “no effects on Zn levels in blood,” especially for the Zn deficient dietary group. She asked for further explanation of the reference to adenomas in the pituitary gland, and the discussion of clear cell foci in the liver. She found the atrophy of the pancreas in the excess zinc exposure to be surprising, since the pancreas is the major organ to get rid of excess zinc – suggesting that excretion is completely changed in those animals. She requested more discussion of the literature on zinc deficiency and Alzheimer’s disease.

Dr. Brix said there were no good historical controls due to the unique diet the animals were fed, and that the pituitary adenomas in the deficiency diet were probably due to the lower survival in controls than in the other two groups. Regarding the clear cell foci, she noted that typically results are not brought forward into the body of the report if they do not attain statistical significance unless they are very unusual or very important. Clear cell foci can be a very common background lesion, she added. She said that historical controls are not kept for nonneoplastic lesions, but the incidences of clear cell foci seen in this study are in line with what we would expect to see. Regarding the atrophy of the pancreas, she observed that it is commonly seen in old rats.

Dr. Wyde noted that metal levels were measured in blood, and the statement in the report pointed out by Dr. Ludewig was a mistake and would be corrected. Regarding the copper data, the staff agreed that more discussion should be added to the report. Regarding the zinc excretion, he said the staff would check the literature to see if there would be further information that could be added to the report on that issue.

Dr. Dybdal, the second reviewer, agreed that the study was well-designed. She was impressed with the efforts that were made to manage and track environmental zinc

exposure. She felt that the report was well-written, including the presentation of the nonneoplastic lesions. She agreed with the level of evidence conclusions.

Dr. Miles, the third reviewer, also felt that the study was comprehensive and well-designed. She said that there appeared to be a sex difference. She recommended inclusion of additional information on phytate in the introduction. She asked why the colon was selected for the Comet assay rather than the duodenum, since that is the area reportedly responsible for the majority of zinc absorption. She agreed with the draft report's conclusions on carcinogenicity.

Dr. Wyde agreed to add more information on phytates to the introduction. He said that several other tissues were considered for the Comet assay, but the colon was one of the few that yielded results.

Dr. Peterson, the fourth reviewer, said the study design was excellent, with a single study evaluating both high and low concentrations. He approved of the care taken to avoid environmental contamination, and agreed with the draft report conclusions.

### **C. Panel Discussion and Vote**

Dr. Conner noted that there is a body of literature about zinc deficiency in animals. He recommended some discussion of cell proliferation in the esophagus associated with zinc deficiency in rodents. He found it interesting that no effect on the esophagus had been observed in the NTP study. He discussed the difficulty of removing zinc from the environment and diet, and questioned how successfully it had been done in the study, given the blood levels seen.

Dr. Dybdal recommended caution about the early literature Dr. Conner had referenced, due to confounding factors.

Dr. Wyde said he was also surprised that nothing was seen in the esophagus.

Dr. Gordon said that there should have been more discussion and emphasis in the report about how the environment and diet were controlled for zinc contamination. Dr. Wyde said that diet batches were analyzed carefully for zinc content throughout the study. He agreed to check the report and add to it as necessary.

Dr. Ludewig felt that the diet had been well-described. She said she was surprised by the statement that zinc carbonate had been chosen due to its bioavailability. Having checked the literature, she felt that the choice was justified, but not due to "greater" bioavailability. Dr. Wyde agreed to change the language in the report to reflect Dr. Ludewig's comment.

Dr. Cattley called for the conclusions to be projected.

Dr. Dybdal moved to accept the conclusions as written. Dr. Conner seconded the motion.

The panel voted (6 yes, 0 no, 0 abstentions) to accept the conclusions as written.

Dr. Bucher thanked the panel for their deliberations, particularly regarding the equivocal calls, where its input is especially useful. He thanked the staff for its efforts, and Dr. Cattley for chairing the meeting. Dr. Wolfe thanked the panel for its contributions.

Dr. Cattley added his thanks to everyone, and adjourned the meeting at 11 am, July 13, 2017.

## **VII. Approval of the Peer Review Report by the Chair of the Peer Review Panel**

This peer review report has been read and approved by the Chair of the July 13, 2017 National Toxicology Program Technical Reports Peer Review Panel.

# Russell Cattley

Digitally signed by Russell Cattley  
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Russel C. Cattley, D.V.M., Ph.D., D.A.C.V.P.

Peer Review Panel Chair

Date: