Comments on NTP Technical Report on the Toxicology And Carcinogenesis Studies of 2,3-Butanedione (CAS NO. 431-03-8) in Wistar Han [Crl:WI (HAN)] Rats and B6C3F1/N Mice (Inhalation Studies)

I, Anders Abelmann, an employee of Cardno ChemRisk, am submitting comments as a response to the National Toxicology Program (NTP) notice of request for comments regarding the *NTP Technical Report on on the Toxicology And Carcinogenesis Studies of 2,3 Butanedione (CAS NO. 431-03-8) in Wistar Han [Crl:WI(Han)] Rats and B6C3F1/N Mice (Inhalation Studies)*, as published on the NTP website on or around June 5, 2017.

Cardno ChemRisk is a scientific consulting firm focused on occupational and environmental health issues, particularly as they pertain to human health risk. As such, we believe we have a professional responsibility to share information with government bodies as they explore matters germane to our expertise. Over the last decade, our firm has been engaged by several manufacturers and suppliers of diacetyl and diacetyl-containing flavorings in various litigation and non-litigation matters; some of our employees have provided expert deposition and trial testimony on behalf of these firms. As such, we have carefully studied diacetyl and other flavorings compounds, performed original research, as well as detailed reviews and commentaries, and have developed a substantial body of knowledge with respect to flavorings compounds and human health risk assessment. We have published several articles and letters-to-the-editor on the medical, epidemiological, and toxicological aspects of this family of chemicals, and have also presented our research findings at numerous scientific conferences for example the annual meetings of the American Industrial Hygiene Association (AIHA) and the Society of Toxicology (SOT); several of these presentations were awarded with "Best Of" awards. Some of our research efforts have received partial funding from the private sector or firms involved in diacetyl litigation.

Thank you for your time and consideration of these comments, which are submitted on behalf of Cardno ChemRisk *in lieu* of in-person statements. I am registered and will attend the webcast.

General Comments

As stated above, our firm has been involved in research related to diacetyl for more than 10 years, during which we have become intimately familiar with the available scientific data and knowledge. We have followed the progression of NTP's diacetyl research since the early 2000s, including the publication of several papers in the 2002 to 2008 time period. We note that the 90-day study was concluded in 2008 and the 2-year study in 2011, but that, to date, these findings have not been published in the peer-reviewed literature. Notably, we presented analyses of NTP inhalation data at the SOT Annual Meetings, including the 90-day study in 2015 and 2016 and the 2-year study in 2017 (i.e., the datasets that comprise the vast foundation of the NTP technical report under review) (Beckett et al. 2015; Finley et al. 2017; Glynn et al. 2015; Glynn et al. 2016). As such, we are not only very familiar with diacetyl in general, but also with NTP diacetyl data in particular, including those data that have not yet been analyzed and published in the peer-reviewed literature. Although, we have performed our own independent analyses of these data, we believe that the publication of NTP's findings would be an important contribution to the scientific literature and would make the results of these studies available to a wider audience.

We would like to make the following comments to NTP's draft report:

Comments on Non-Neoplastic Findings

As noted by NTP, inhalation toxicology studies of diacetyl were initiated in part as a result of "outbreaks of bronchiolitis obliterans in workers exposed to its vapors" (p. 5). The draft NTP report, therefore,

seems "incomplete", as there are no observations regarding this endpoint presented anywhere in the results. Several chemicals are known to cause *bronchiolitis obliterans* in mice and rats following inhalation (e.g., nitrogen oxides, phosgene), and the respiratory toxicity profiles of those agents are consistent and well understood. We do not believe there is any valid rationale for omitting the fact that *bronchiolitis obliterans* was not observed in any of the tested animals, and we suggest that the NTP report explicitly state that there was no evidence to suggest that diacetyl causes *bronchiolitis obliterans* in animals; this observation would seemingly fit in well, both in the conclusions on page 12, as well as in the related discussion on page 103.

Comments on Neoplastic Findings

In the "Conclusions" section of the draft report, the NTP notes that:

> "Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity** of 2,3-butanedione in male Wistar Han rats based on the combined incidences of squamous cell papilloma and squamous cell carcinoma of the nose. There was *some evidence of carcinogenic activity* of 2,3-butanedione in female Wistar Han rats based on the incidences of squamous cell carcinoma of the nose. There was *no evidence of carcinogenic activity* of 2,3-butanedione in male B6C3F1/N mice exposed to 12.5, 25, or 50 ppm. There was *equivocal evidence of carcinogenic activity* of 2,3-butanedione in female B6C3F1/N mice based on the occurrences of adenocarcinoma of the nose" (p. 11-12).

Table I below shows the relevant tumor incidence results from the NTP 2-year study, as it relates to rats:

Table I – Tumor Incidence Data for Nasal Squamous Cell Carcinoma (adapted from Table 9 of NTP report)

Endpoint (all rats)	0 ppm	12.5 ppm	25 ppm	50 ppm
Nose (male): squamous cell carcinoma	0/50	0/50	0/50	3/50
Nose (male): squamous cell papilloma/carcinoma[*]	0/50	0/50	0/50	4/50
Nose (female): squamous cell carcinoma	0/50	0/50	0/50	3/50

[*] 1 animal in 50 ppm group showed SC papilloma (potential precursor to carcinoma), so these were combined by NTP

Our analysis of these data suggests that there are at least three reasons why it is highly questionable whether these results are actually indicative of "some evidence" of carcinogenicity in rats:

1) **It is not appropriate to combine the nasal carcinoma and papilloma endpoints.**

As can be seen in Table I, the NTP chose to combine papillomas and carcinomas and treat the summed incidence as "tumors". Although benign neoplasms are used within NTP's definition of support for the "some evidence" category (p. 16), our review of accepted guidelines on carcinogenic risk assessment suggests it is inappropriate to combine these incidences.

Specifically, when evaluating the carcinogenicity of a chemical, it may be useful to consider precursors to malignant endpoints, even if the precursor itself is not adverse. The U.S. EPA states in its guidelines for risk assessment of carcinogens that:

> "*When good quality precursor data are available and are clearly tied to the mode of action of the compound of interest* [U.S. EPA emphasis], models that include both tumors and their precursors may be advantageous for deriving a POD. Such models can provide insight into quantitative relationships between tumors and precursors (see Section 3.2.2), possibly suggesting the precursor response level that is associated with a particular tumor response level. The goal is to

> use precursor data to extend the observed range below what can be observed in tumor studies" (U.S. EPA 2005: p. 3-16 – 3-17).

While this guidance suggests consideration of precursors as a means for assessing the carcinogenicity of a compound, it does not suggest that precursors should be combined with carcinogenic endpoints. In the case of the NTP study, a papilloma is a ***benign*** growth that ***may or may not*** lead to carcinoma. Combining the incidences of these endpoints suggests that a papilloma and carcinoma have "equal weight", which does not make biological sense. Additionally, ***if*** diacetyl was truly a carcinogen via this pathway, a higher incidence of papilloma than carcinoma would be expected. However, that did not occur in this study (papillomas occurred far less than carcinomas), which suggests that papilloma formation, *which, it must be emphasized, occurred in only one animal in the entire study*, may not be a precursor to carcinoma formation and may not even be related to diacetyl exposure.

2) **The nasal tumor incidence observed in the 50 ppm exposure group is not statistically different compared to control rats.**

Results of pairwise comparison tests, as reported in the draft report, indicate that the 3/50 incidence of squamous cell carcinoma in males and females in the 50 ppm exposure group is not statistically significantly increased above controls. A significant increase is only found when the papilloma and carcinoma incidences were combined in the male rats, which, as noted above, is not a standard method for interpreting cancer bioassay data. Hence, there is no evidence of an actual dose-response relationship for any cancer-related endpoint in the entire NTP study, and we believe the NTP report should state that explicitly.

3) **Nasal tumors only occurred at 50 ppm, a concentration at which significant chronic toxic effects were observed.**

It has previously been noted that, chronic toxicity and tissue damage is a potential confounding factor in rodent carcinogenesis assays, particularly in the nasal cavity (Ward 2007). Ward (2007) stated that "[s]ome scientists have suggested over the years that rodent tumors were, in fact, not tumors but lesions of hyperplasia and reactivity to toxic damage of an organ" (p. 14). In the NTP 2-year study, there was evidence of chronic toxicity and evidence of tissue damage in rats at 50 ppm. We believe the NTP report should discuss the possibility that chronic toxicity was a potential confounding factor of neoplasia at the 50 ppm exposure.

In summary, NTP characterized the tumor incidence results as "some evidence" of carcinogenicity. As stated by NTP, "… the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies" (p. 16). We believe that, if the NTP data were to be used as part of an analysis to classify possible human carcinogenicity of diacetyl, then we believe the only proper weight-of-evidence conclusion would be one of "no evidence". Specifically, based on the data published by NTP, diacetyl's mode of action for carcinogenesis is unclear and may not even exist; thus, there is no evidence to support that these tumor incidences are physiologically relevant or applicable to humans. Even if this were true, to the best of our knowledge, there are no other agents with similar equivocal results that have ever been classified by authoritative agencies. As noted by NTP, "… the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies" (p. 16).

The NTP notes (p. 30) that cancer endpoints have not been evaluated *per se* in the diacetyl-exposed worker cohorts studied to date. However, provided the existing in-depth studies of several of the cohorts,

it would appear reasonable to hypothesize that concerns regarding respiratory toxicity would have triggered the reporting of perceived associated cases of nasal and related cancers. To date, none of the multiple studies of diacetyl-exposed populations, nor any of the several documents addressing occupational exposure limits for diacetyl, have suggested evidence to support that diacetyl is a human carcinogen.

Thank you for the opportunity to comment on this very important issue. The use of proven scientific methodology is critical to enable reproducibility and we believe that the comments and suggestions outlined herein will assist in this process and help move the science forward.

We would be more than happy to provide further insight at your request. Please direct any communication to anders.abelmann@cardno.com.

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