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Cardno ChemRisk

75 Arlington Street
Suite 5071
Boston, MA 02116-3936
USA

www.cardno.com

www.cardnochemrisk.com

Thomas Armitage, Ph.D.
Designated Federal Officer
EPA Science Advisory Board Office
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460

Subject: Comments regarding the August 27, 2019 meeting of the EPA Science Advisory Board

Dear Dr. Armitage:

Thank you for the opportunity to provide additional technical information and published scientific papers for the EPA Science Advisory Board (SAB) to consider. Specifically, I wish to provide context pertaining to research I and others have contributed to a growing body of scientific literature regarding formaldehyde as a possible cause of human leukemia.

The impetus for this submission, in part, is a response to several incomplete or vague statements made by Dr. Bernard Goldstein before the SAB on August 27, 2019. Although his prepared statement appears primarily to address data access and IRB challenges, Dr. Goldstein also used this opportunity to reiterate his opinion that my colleagues and I misused our right and privilege to access and more fully analyze data from taxpayer-funded research. Rather than address Dr. Goldstein's opinions directly, I wish to present here additional scientific evidence and technical evaluations to facilitate the SAB's objective review and interpretations of the scientific evidence pertaining to formaldehyde as being leukemogenic.

Dr. Goldstein's statement points to one government-funded study (i.e., Zhang et al., 2010) that my colleagues and I were able to re-evaluate using the original study data. However, I oversaw extended analyses of the original data from a second publicly funded study (i.e., Beane Freeman et al. (2009)). Both of these studies tremendously influenced the International Agency for Research on Cancer (IARC)'s determination that formaldehyde causes leukemia. Soon after IARC classified formaldehyde as leukemogenic, the National Toxicology Program (NTP) of the US National Institute for Environmental Health Sciences and the US EPA's Integrated Risk Information System (IRIS) (draft) evaluation followed suit, largely relying on the same two epidemiological studies to support their causal conclusions.

The Zhang et al. (2010) and Beane Freeman et al. (2009) studies, as published, did not address some key research questions directly relevant to leukemia. These authors also may not have presented all of the results from their statistical analyses. Zhang et al.

(2010), for example, described their method for evaluating chromosomal aneuploidy (i.e., having an abnormal number of chromosomes – too many or too few – in cells), and, specifically, the required minimum number of cells to examine to obtain valid results. In their publication, however, they did not verify the actual numbers of cells evaluated. Also, the publication specifically mentioned that three formaldehyde measurements were obtained for each formaldehyde-exposed worker. Yet, Zhang et al. (2010) did not present any results that actually used these valuable individual level data. Why would investigators perform industrial hygiene monitoring for formaldehyde exposure for each worker, but not present statistical results using these data to address their primary research questions? Instead, the authors simply compared the average test results for a group of "exposed" workers with those of a separate and notably dissimilar group of "unexposed" workers. We requested the original study data to perform and present the results of the omitted analyses.

Similarly, Beane Freeman et al. (2009) updated the National Cancer Institute's study of industrial formaldehyde workers. They reported that the "myeloid leukemias" (all types combined) were associated with a novel representation of "peak" exposure, but not with conventional cumulative, average, or duration of exposure. More importantly, they did not present results for chronic myeloid leukemia (CML) and acute myeloid leukemia (AML) separately. CML and AML are discrete malignancies with very different etiologies, and AML is the type of leukemia most likely associated with chemical exposures (as seen with benzene at high exposure concentrations). We requested the original study data to allow more in-depth statistical analyses specifically focused on the potential (but unreported) relationship between "peak" formaldehyde exposure and AML.

More than one year after submitting a Freedom of Information Act (FOIA) request, we received part of the data from the Zhang et al. (2010) study, but not any exposure data. Subsequently, we requested the individual exposure data from the National Cancer Institute (NCI) via the Data Transfer Agreement (DTA) process. NCI provided the averages of the three formaldehyde monitoring results for each exposed worker, but not the individual test result values, which we have never seen. We also received all of the data from the Beane Freeman et al. (2009) study via the NCI's DTA. For both studies, the data provided were stripped of all personal identifiers, which are unnecessary for statistical evaluation of the data. The FOIA and DTA are important vehicles for sharing data from publicly funded research, including published studies that have been critical in shaping hazard evaluations, while protecting individually identifiable data. All of the methods and results of the fuller statistical analyses we performed on the original study data have been published in various peer-reviewed scientific journal articles.

Two publications present previously unreported findings from the Zhang et al. (2010) study. Gentry et al. (2013) discovered and reported that the number of cells evaluated by Zhang et al. (2010) for determining rates of aneuploidy were severely inadequate. According to the Organization for Economic Co-operation and Development (OECD) global standard methods for assessing in vitro mammalian chromosomal aberrations: "At least 300 well-spread metaphases should be scored per concentration and control to conclude a test chemical as clearly negative. . . Scoring 300 cells has the advantage of increasing the statistical power of the test and in addition, zero values will be rarely observed (expected to be only 5%)" (OECD, 2016). However, the Zhang et al. (2010) protocol set the minimum number of metaphases at 150 cells, half of the OECD standard. Despite this compromised "standard," Zhang et al. (2010) failed to satisfy it most of the time. Of the 44 total aneuploidy tests performed (22 each for tests of monosomy 7 and trisomy 8), for example, only 22% counted more than 150 cells for monosomy 7, and only 27% counted more than 150 cells for trisomy 8. Clinically, it is common to count up to 400 cells. Quite surprisingly, not only did these investigators fail to follow their own protocol, <u>no single aneuploidy test reported satisfies current global toxicology testing or clinical standards for number of cells counted</u>, which ultimately casts serious doubt on the validity of the test results.

Furthermore, Gentry et al. (2013) noted that based on the kinetics of CFU-GM colony formation, the observed aneuploidies could not have arisen *in vivo*, but most likely occurred *in vitro* during cell culture. Even assuming that some of the test results were valid, our analyses of the blood and aneuploidy test results by individual exposure concentrations, which the investigators acknowledged as "relatively high levels of formaldehyde (mostly between 0.6 and 2.5 ppm)" (Zhang et al. 2010), demonstrated no association with formaldehyde level. In the exposed group, the actual average individual formaldehyde concentrations (based on three measurements) ranged from 0.4 to 5.6 ppm (Mundt et al. 2017).

The new findings based on the original study data led to the following conclusion:

> Therefore, IARC's interpretation of the Zhang et al. (2010) study and the implications on the formaldehyde hazard classification should be reconsidered in light of the fuller evaluation of all of these data, and the updated EPA IRIS report should reflect the limited inferential value of the Zhang et al. (2010) study or any of its progeny (Hosgood et al. 2013; Lan et al. 2015; Bassig et al. 2016) until the scientific validity of each can be demonstrated. In particular, unmeasured factors – including workplace factors – that are distributed differently between the exposed and unexposed workers may explain differences noted in blood parameters and aneuploidies in the original results. IARC (2012) called for the replication of the Zhang et al. (2010) study. We suggest it be replicated using a new study population, actual measured formaldehyde exposures, and valid laboratory tests not subject to methodological problems such as deviation from protocol standards or complicated by questions of the origin (i.e. in vivo versus in vitro) of the effects. (Mundt et al., 2017).

Results comparing individual formaldehyde exposure with all blood measures are presented in Table 1, and with the aneuploidies in Figure 3 in Mundt et al. (2017).

The IARC Working Group noted that Zhang et al. (2010) provided supporting data for their conclusion that formaldehyde causes leukemia, but with the caveat that the study needed to be replicated (IARC, 2012). In our 2018 review, we reiterate this conclusion, but also note that replication would not be straightforward.

> Indeed, proper replication of this study is still needed, because the study protocol was not consistent with adequate cell counting standards, including the authors' earlier descriptions of the OctoChrome FISH method (Zhang et al., 2005; Zhang et al., 2011) and other standards (American Society of Medical Genetics, 2006). One particular challenge is that occupational exposure limits in North America, Europe and in many countries around the world protect workers from the levels of occupational formaldehyde exposures that were studied by Zhang et al. (2010a) in China making replication of the study logistically difficult. Proper replication of this study also will require use of methods to successfully distinguish between aneuploidy arising in vivo from aneuploidy that arises during the period of in vitro culture, as discussed in section 3.3.3 below. (Mundt et al., 2018)

One critic has argued that Zhang et al. (2010) never published findings based on their own directly measured formaldehyde exposure data because these measurements were not made over the "full time of exposure" or "at least one year" prior to blood and aneuploidy tests. Red blood cells, however, are known to turn over about every three months, so the formaldehyde monitoring in the weeks prior to the blood testing was indeed appropriate. Given that none of the blood or genetic anomaly tests showed any correlation across relatively high measured concentrations of formaldehyde exposure, the value of

replicating such a study becomes questionable, both from a scientific and a resource investment perspective.

Based on our ability to obtain and more thoroughly analyze the data used in Beane Freeman et al. (2009), we were able to examine the specific relationship between various estimates of formaldehyde exposure and AML mortality, and publish these more detailed results. Our paper concludes as follows:

> No other clear associations for peak or cumulative formaldehyde exposures were observed in this cohort for any of the specific LHM, including AML. Although our re-analysis using redefined "peak" exposure detected associations similar to those previously reported with the combined MLs [*myeloid leukemias*], our new analyses of AML and CML mortality separately suggest that the observed patterns with peak exposure were confined to CML. Furthermore, when taking into account the timing of peak exposure, no increased risk for AML is seen, as only one AML death occurred within 15 years of first, or even last, peak exposure. Sensitivity analyses assuming all the "unspecified" acute leukemia deaths were AMLs did not change these findings.

> Our re-analysis of the data from the NCI cohort study of workers in the formaldehyde industries provides no support for the hypothesis that formaldehyde causes AML, the LHM [*lympho-hematopoietic malignancy*] of greatest prior concern. (Checkoway et al., 2015)

Obtaining and more fully analyzing the underlying data from these two studies likely was the only available means to address several key research questions and to verify the results as originally presented. Nevertheless, one colleague continues to criticize our decision to obtain and perform fuller analyses of available data from these two pivotal, publically funded studies.

That our re-analyses demonstrated no valid associations between formaldehyde exposure and AML or the blood and chromosomal anomaly test results, notwithstanding the unreliable methodology, also aligns with other relevant and strengthening lines of evidence. Noteworthy are several important animal studies performed at the University of North Carolina demonstrating that exogenous formaldehyde cannot reach the bone marrow, and that formaldehyde DNA adducts (at points distant from the nose) involve endogenous formaldehyde (Lu et al., 2012).

A detailed critical review and synthesis of the latest scientific evidence from all relevant lines of inquiry pertaining to formaldehyde and leukemia risk came to the following conclusion:

> Overall, the quality and amount of evidence relevant to the understanding of a potential causal relationship between formaldehyde inhalation exposure and risk of LHM has increased substantially since the completion of the Draft IRIS Assessment (EPA, 2010) and release of the NRC peer review (NRC, 2011). New evidence has been published in each of the major streams of evidence (i.e., human, animal and mechanistic) that consistently indicates a lack of a causal association between formaldehyde exposure and LHM, and specifically AML. These new studies have addressed many of the NRC (2011) scientific criticisms surrounding the evaluation of a combination of cancer types, as well as increased our understanding of the potential impact of exogenous exposure on endogenous levels, which is critical in attempting to understand the potential hazards or risks from formaldehyde exposure. Regardless of which of the several similar approaches to integrating the available evidence between formaldehyde inhalation exposure and the potential for leukemia risk, there is at most only limited suggestive positive evidence, in contrast with the bulk of evidence

suggesting no such association. Therefore, a conclusion of causation is not justified scientifically. (Mundt, et al., 2018).

The most recent and comprehensive compendium of the evidence reflecting discussion and deliberation of 24 invited experts (and observed by four US EPA scientists) presented the following summary statement:

> Conclusions from looking to both the animal toxicology and proposed human MOA evaluations were (1) the animal data, cited in USEPA (2010) to support a hazard of leukemia, are weak; (2) inhalation of formaldehyde does not increase blood formaldehyde concentrations; (3) biomarkers of formaldehyde exposure (e.g. formaldehyde-DNA adducts) are not detected beyond the portal of entry in exposed animals; (4) in vivo genotoxicity assays following formaldehyde inhalation are negative both systemically and at the portal of entry; and, overall, that (5) animal data have yet to be generated that support a hazard for leukemia or hematotoxicity following formaldehyde exposure. (Andersen, et al., 2019)

For the SAB's consideration and convenience, I provide links below to Checkoaway et al., 2015 and Mundt et al., 2017, the publications based on the fuller analysis of the Zhang et al. (2010) and Beane Freeman et al. (2009) studies, respectively, as well as our synthesis of the evidence (Mundt et al., 2018).

Thank you,

Kenneth Mundt, PhD, FACE
Senior Principal Health Scientist
for Cardno ChemRisk
Direct Line +1 857-444-9369
Email: kenneth.mundt@cardno.com

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