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Formaldehyde, Hematotoxicity, and Chromosomal Changes - Response

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We conducted a cross-sectional study of 43 workers exposed to formaldehyde in the workplace and 51 unexposed controls to examine the biological plausibility that formaldehyde causes myeloid leukemia (1). We published the initial report in Cancer Epidemiology, Biomarkers & Prevention in 2010 (2) and a follow-up report in Carcinogenesis in 2015 (3). Mundt and colleagues have raised several concerns about both publications (4). We welcome the opportunity to clarify our study's findings, respond to their comments, and note that data for these publications are available upon request. Their comments and our responses are as follows:

i. "Lack of evidence that group differences in aneuploidy are significant to leukemogenesis"

- Aneuploidy of specific chromosomes is clearly an important mechanism of leukemia induction based on its presence in many cases of myeloid neoplasms, including acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS; ref. 5). Monosomy of chromosome 7 and trisomy of chromosome 8 are the most common aneuploidies observed in AML and MDS cases (5, 6). There is, however, no direct evidence that higher aneuploidy rates in cultured myeloid progenitor cells are related to future risk of leukemia, as, to the best of our knowledge, no data exist in prospective cohorts that could test this hypothesis given the special procedures needed to process, store, and culture such samples and the intense labor and expense that characterize such analyses. However, we have previously shown that benzene exposure was associated with higher rates of monosomy 7 compared

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with unexposed controls using a similar protocol (i.e., interphase FISH of cultured myeloid progenitor cells) conducted by our same study team (7). As benzene is an established leukemogen (1), a known inducer of aneuploidy (8), and was associated with higher rates of monosomy 7 in the cultured myeloid progenitor cells of exposed workers, we reasoned that showing a similar association for workers exposed to formaldehyde supports the biological plausibility that formaldehyde causes myeloid leukemia (9).

ii. "Personal monitoring data were collected but not analyzed and presented"

- Personal monitoring data were collected and summary data of those data were presented at the group level (2, 3), but we deemed it inappropriate to conduct exposure-response analyses with the individual data. We have noted that there was an insuficient range in exposure to be able to appropriately evaluate exposure-response relationships with biomarker endpoints in this study (3), where almost all workers were relatively highly exposed to formaldehyde, above the OSHA permissible exposure limit (PEL) of 0.75 ppm. The median exposure for the subgroup of workers (n 29 exposed, 23 controls) we reported chromosomal data for was 1.38 ppm, and the 10th to the 90th percentile was 0.78 to 2.61 ppm (a 3.3-fold difference; ref. 3), similar to the range of the entire study population. To give some context to the limitation of this exposure range in the evaluation of an exposure-response relationship with chromosomal aneuploidy and other biomarker endpoints, we previously reported that occupational benzene exposure was associated with an exposure-dependent increase in monosomy 7 in interphase cells from cultured myeloid progenitors in a similar number of workers occupationally exposed to benzene and controls using similar methods by the same study team (7). In that study of a subgroup of workers with data on myeloid progenitor cells, there was an 88-fold difference in exposure range (10th, 90th percentile 0.35, 30.8 ppm benzene, respectively) and included workers exposed to well below the OSHA PEL (of 1 ppm). Furthermore, there was only a 23% increase in monosomy 7 among workers with higher (10 ppm) versus lower (<10 ppm) levels of benzene exposure, even though there was a 9.2-fold difference in mean benzene exposure level between the two groups (7). In contrast, the mean formaldehyde exposure in a similarly constructed higher and lower (based on a median of 1.38 ppm) group of exposed workers with data for monosomy 7 in our formaldehyde study (3) would have differed by only 2.3-fold (mean 1.08 vs. 2.45 ppm).
- As such, although study subjects in our article were highly exposed to formaldehyde, there was neither a suficient range in exposure nor a large enough sample size to have adequate power to be able to appropriately evaluate exposure-response relationships for chromosomal aneu- ploidy or other biomarker endpoints.

iii. Lack of “exposure-response relationships”

- Given (ii) above, one would not expect that an exposure- response relationship with endpoints measured in this study would be present. Such analyses of data from this study are not informative, in our view.

iv. “Failure to adhere to the study protocol”

- Our study methods called for counting all scorable meta- phases on a subject’s slide with a minimum of 150 cells counted per study subject (2, 3).* A reference for the method, called OctoChrome FISH, was provided in reference 45 (10) of that publication (2), and additional details and references were provided in the follow-up article in Carcinogenesis (3, 10–12). In brief, we used an automated metaphase finder to detect metaphases on each subject’s slide. These meta- phases were spread out over 8 squares on each slide in which 3 chromosomes were analyzed in each square. The 3 chromosomes in each square are selected so that combinations facilitate the identification of most specific aneuploidy and chromosomal rearrangements related to human leukemia and lymphoma. Thus, under our protocol, a minimum of 18 to 19 metaphases would be scored on average for each chromosome (i.e., 150 total metaphases counted per slide distributed in 8 squares), although the number of meta- phases actually scored for each set of 3 chromosomes was usually much larger (13). There was no minimum number of metaphase cells required in any given square, just for the slide as a whole. The statistical method used to analyze these data, negative binomial regression, takes the number of meta- phases counted in the denominator into account when calculating the variance and thus statistical signiÔ¨Åcance. We originally analyzed data from chromosomes 7 and 8 in two different squares, using the a priori hypothesis that these two chromosomes are the ones most commonly altered in number in AML and MDS (5, 6). Having subsequently obtained additional funding for a more comprehensive study, we then undertook the analysis of all 24 chromosomes in 8 squares in a larger number of subjects and published our findings in Carcinogenesis (3).

*We note that inclusion criteria for 2 of the original 22 subjects were relaxed to maximize the number of subjects who could be included in the analysis in our initial report (2). For these two subjects (who were controls), only a total of 120 and 132 metaphases could be scored on their slides, rather than 150. When these 2 subjects were excluded from data used in the original report, the results were essentially unchanged. For monosomy 7, reported results: n 10 exposed workers, 12 control workers, mean (SE) 11.10 (2.23) % versus 5.32 (1.05) % of metaphases scored, respectively, P 0.0039 (2); results excluding 2 controls with fewer than 150 total metaphases scored: mean (SE) of monosomy 7 for the 10 exposed versus 10 control workers: 11.10 (2.23) % versus 4.83 (1.16) % of metaphases scored, respectively, P 0.0032. This Ô¨Ånding was replicated with essentially identical results in our follow-up article that included an independent, nonoverlapping sample from the same study of 19 exposed and 13 control workers (3, 13). In that follow-up report, which pooled results from the initial report with the new data (3), the two controls with less than 150 total metaphases scored were removed from the data to strictly adhere to the study protocol (13), and the pooled result was highly statistically signiÔ¨Åcant (3). The results for trisomy 8 were also essentially identical after excluding the 2 controls with less than 150 metaphases scored [reported results: n 10 exposed workers, 12 control workers, mean (SE) 1.21 (0.40) % vs. 0.32 (0.14) % of metaphases scored, respectively, P 0.040 (2); results excluding 2 controls with fewer than 150 total metaphases scored: mean (SE) of trisomy 8 for the 10 exposed vs. 10 control workers: 1.21 (0.40) % vs. 0.39 (0.16) % of metaphases scored, respectively, P 0.042], but the pooled results in the follow-up analysis were not significant (3).

We apologize if some readers may have misunderstood the procedures involved.

v. Need for the “findings properly replicated”

- We discussed the limitations of our study and the need for replication and extension in the first report from this study (2), our response to an initial letter to that report in Cancer Epidemiology, Biomarkers & Prevention (14), and in the follow- up article in Carcinogenesis (3) and agree that there is a need for our findings to be independently confirmed.

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