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LETTER TO THE EDITOR

Response to letter to the editor of Carcinogenesis by Pira *et al.*, 2017

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We conducted a cross-sectional study of 43 workers exposed to formaldehyde in the workplace and 51 unexposed controls ([1]) to examine the biological plausibility that formaldehyde causes myeloid leukemia ([2]). In our article entitled 'Chromosome-wide aneuploidy study of cultured circulating myeloid progenitor cells from workers occupationally exposed to formaldehyde' published in 2015 in Carcinogenesis ([3]), we reported that aneuploidy rates were increased for multiple chromosomes in 29 formaldehyde-exposed workers compared to 23 controls ([3]). Of particular interest was our *a priori* hypothesized increase in monosomy 7, the most frequent autosomal monosomy in acute myeloid leukemia (AML) ([4]) that was elevated in an initial report from a smaller subgroup of this study ([1]). A letter to the editor by Pira *et al.* ([5]) has raised a number of concerns about our article. We welcome the opportunity to respond and further clarify our study's findings.

The key criticisms of our article and our responses are as follows:

(I) 'Failure to conduct an exposure–response analysis'

As noted in our article, there was an insufficient range in individual exposures to be able to appropriately evaluate exposure–response relationships with biomarker endpoints in this study ([3]), in which almost all workers were relatively highly exposed, well above the US OSHA permissible exposure limit (PEL). Among the workers exposed to formaldehyde reported in Lan *et al.* ([3]), the median exposure was 1.38 ppm and the 10th to the 90th percentile was 0.78 to 2.61 ppm (*3.3-fold difference*). To give some context to the limitation of this exposure range in the evaluation of an exposure–response relationship with chromosomal aneuploidy, we previously reported that benzene, which has been causally linked to AML ([2]) and is a known inducer of aneuploidy ([6]), was associated with an exposure-dependent increase in monosomy 7 in cultured interphase cells from myeloid progenitor cells obtained from a similar number of workers occupationally exposed to benzene and controls using similar methods by the same study team ([7]). In that study, however, there was an *88-fold difference* in exposure range (median exposure = 5.99 ppm, 10th, 90th percentile 0.35, 30.8 ppm benzene) and included workers exposed to well below the OSHA PEL of 1 ppm. Further, there was only a 23% increase in monosomy 7 among the workers exposed to higher (≥10 ppm) versus lower (<10 ppm) benzene levels, even though the difference in mean benzene exposure between the two groups was 9.2-fold (mean 2.64 versus 24.2 ppm benzene). In contrast, the difference in mean exposure between a higher vs. lower group of exposed workers in our formaldehyde study based on a median of 1.38 ppm would have been only 2.3-fold (mean 1.08 versus 2.45 ppm formaldehyde). Although all study subjects were highly exposed to formaldehyde, there was an insufficient range of exposure to have adequate power, given the expected effect size for the endpoints of interest and sample size of the study, to be able to appropriately evaluate exposure–response relationships. Such analyses of data from this study are not informative in our view.

(II) 'Failure to adhere to the study protocol for counting 150 cells per subject for *FISH* analysis'

Our study protocol called for counting all scorable metaphases on a subject's slide with a minimum of 150 cells counted per study subject ([1,3]). As described in our article, the method we used is called OctoChrome FISH and 3 references were provided for this approach ([8–10]). We used an automated metaphase finder to find at least 150 metaphases on each subject's slide. These metaphases were spread out over eight squares on

each slide in which three chromosomes were analysed in each square. The three 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 18–19 metaphases (i.e. 150 total metaphases counted per slide distributed in eight squares) would be scored on average for each chromosome, although the number of metaphases actually scored for each set of three chromosomes was usually much larger. There was no minimum number of metaphase cells required in any given square, just for the slide as a whole. In our report (3), there was a median of 121 cells examined per subject for chromosome 7, with a range of 24–670. There was a median of 1029 total metaphases counted, range 164–4949, which represent the total number of metaphases scored summed across all eight squares (which included a total of 24 chromosomes) on the OctoChrome FISH slide. Thus, data used in this report were fully adherent to our metaphase counting protocol. The statistical method used to analyse these data, negative binomial regression, takes the number of metaphases counted in the denominator into account when calculating the variance and thus statistical significance.

(III) 'Use of an "ecological" design and never having explored other explanations to account for the differences between formaldehyde-exposed and unexposed workers'

We employed a cross-sectional design to study biologic effects of formaldehyde exposure using an exposed and control population. Unlike an ecological study, where data are not collected on individuals, we collected data on potential confounding exposures on individuals and explored those in the analysis (i.e. age, sex, alcohol use, tobacco use, recent infections, BMI, medication use). None of these explained the differences in effects we report between workers exposed to formaldehyde and unexposed controls (3). Further, extensive industrial hygiene analysis showed that there were no other additional toxic exposures present in any of the study factories that could account for differences (1). And, study subjects were initially screened to exclude those who had been employed previously in industries with exposure to known or suspected hematotoxic or genotoxic agents, as well as those with a history of cancer, chemotherapy, or radiotherapy (1). Finally, exposed and control workers lived in the same area and came from the same general population that works in manufacturing in this region (1). Although the study was not able to evaluate exposure–response relationships, as described in (I) previously, the selection of study factories, characterization of exposures in manufacturing processes, and collection and analysis of data obtained from individual subjects were used to minimize the possibility that the reported group differences were due to aspects of the study populations other than exposure to formaldehyde.

(IV) "Use of overlapping data between the current report and a previous report"

We previously reported that monosomy 7 was statistically significantly elevated in workers exposed to formaldehyde versus controls in the initial article from this study (1) and that we had confirmed this finding in our expanded report in Carcinogenesis (3). Blood was cultured from these additional subjects at the same time as from the subjects initially reported

and myeloid progenitor cells were stored on OctoChrome FISH slides allowed to air dry and stored at –20°C under a nitrogen atmosphere, and analysed using the same FISH methods and devices as in the original study. The results for the new group of workers (*n* = 19 exposed workers, 13 unexposed controls) that we reported only in Carcinogenesis (3), who had not been previously reported on in Zhang *et al*. (1), showed that monosomy 7 was significantly elevated in the exposed vs control workers [mean (SE) 10.11 (1.75)% versus 5.07 (1.16)% of metaphases scored, respectively, *P* = 0.0007], differences that were very similar to what we initially reported [*n* = 10 exposed workers, 12 unexposed controls, mean (SE) 11.10 (2.23)% versus 5.32 (1.05)% of metaphases scored, respectively, *P* = 0.0039 (1)]. In addition, after excluding two of the unexposed controls in the original report who had <150 total metaphases counted, differences in monosomy 7 between the 10 exposed workers and 10 remaining unexposed controls were essentially identical. These two control workers with total number of metaphases scored <150 (*n* = 120 and 132 metaphases) initially reported in Zhang *et al*. (1) were not included in the pooled analysis reported in Lan *et al*. (3) so that our follow-up article in Carcinogenesis strictly adhered to the study protocol for all subjects. At the same time, when those two subjects were excluded from the original report the results were essentially unchanged [mean (SE) of monosomy 7 for the exposed versus control workers: 11.10 (2.23)% versus 4.83 (1.16)% of metaphases scored, respectively, *P* = 0.0032].

(V) 'Data unavailable to determine number of metaphases scored for monosomy 7 and trisomy 8'

Data for monosomy 7 were provided in (II). For trisomy 8, the median and range of metaphase cells counted were 137 (22–548). We also note that data for this publication are available upon request.

Finally, we have discussed the limitations of our study and the need for replication and extension (1,3,11) and agree that there is a need for our findings to be independently confirmed in a separate study.

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