

Occupational Exposure to Formaldehyde, Hematotoxicity and Leukemia-Specific Chromosome Changes in Cultured Myeloid Progenitor Cells – Letter

We read with interest the article by Zhang and coworkers (1). This study was performed to determine whether formaldehyde exposure disrupts the hematopoietic function and produces leukemia-related chromosome changes in exposed humans. The results might point to a yet unknown carcinogenic mode of action of formaldehyde leading to leukemia. Indeed, a working group at the IARC referred to this study when recently re-evaluating the carcinogenicity of formaldehyde (2). However, we are concerned about the reliability of the results because we identified several important shortcomings in the study setup, the evaluation and interpretation of the results, and selection bias in the references given.

Was The Study Appropriately Designed and Set Up?

Study participants, especially those of the unexposed study group, were insufficiently characterized. The only information on the type of work of this group was that they “were engaged primarily in manufacturing.” The basis of the participation rates is unclear: no information is given on the number and on the representativeness of workers monitored for formaldehyde exposure. However, this was a condition for recruitment of the exposed group. The authors reportedly requested information on medical history and current medications but no specific information was presented. Medication might well have affected the results: there was a clearly higher percentage in exposed versus unexposed regarding recent respiratory infections (40% versus 29%). There was also no information given on differences between the five different plants of the study participants and how information varied across plants, e.g., it is unclear whether subsets of exposed and unexposed used for fluorescence *in situ* hybridization analyses were from two, three, four, or five different plants. Obviously, there are uncertainties regarding recruitment, characterization, and exchangeability of study subjects which limits the reliability of this study.

Are The Results Presented Adequately?

Only unadjusted summary measures were reported, no parameter estimates were given from the multiple regression analyses. However, the latter is the really interesting information if any causal conclusions should be drawn (3). Whether the effects are relevantly smaller after adjustment for important variables (e.g., age, gender, smoking, and workplace) was not reported.

Does Formaldehyde Disrupt Hematopoiesis?

A systemic effect of formaldehyde is inferred from blood cell counts. Statistically significant reduced blood cell counts were measured in exposed workers. However, all blood cell counts reported were in the reference range. Therefore, the biological relevance of this finding is questionable. For an independent assessment of the data, the raw data should be published as a supplement on the web site of the journal. Furthermore, important covariates that potentially influence blood cell counts were not taken into account by the authors: physical activity (4) and time of day of blood sampling (5, 6). Type of work was not characterized for the control group: if physical activity level between exposed and controls differed, the comparison might be distorted. Collection and processing time of blood samples may have varied between the different work places where samples were taken. Ignoring these covariates may have limited the reliability of the differences in blood cell counts.

Are The Detected Chromosome Aberrations Exposure-related and Leukemia-specific?

The authors report that they found an increased incidence of leukemia-specific chromosome aberrations in cultured hematopoietic progenitor cells of formaldehyde-exposed workers. They measured the frequencies of monosomy 7 and trisomy 8 in granulocyte-macrophage colony-forming units (CFU-GM) after 14 days of *in vitro* culture. However, the origin of these aneuploidies remains unclear. It is not possible to determine whether these aberrations were already formed in the bone marrow *in vivo* or whether they occurred during proliferation *in vitro*. Nothing is known about the frequency of aneuploidy that occurs *in vitro* during the cultivation of CFU-GM. However, the frequency of aneuploid mitoses in controls (i.e., in the absence of any specific induction) is very high. This finding suggests that mitotic malsegregation is unusually frequent in these cells or that the preparation of metaphases led to a high incidence of artifacts. Surprisingly, the corresponding aneuploid effects (i.e., trisomy 7 and monosomy 8) were neither seen/reported in exposed workers nor in controls.

Obviously, monosomy 7 and trisomy 8 were scored in pooled cultures from CFU-GM cells but the number of colonies that were isolated from each blood sample and the number of colonies analyzed were not stated. For a critical assessment of the results, it would be helpful to have these data published as supplementary information on the journal's web site. Because the incidence of aneuploidy was not determined separately in the single colonies, it is not possible to conclude how often malsegregation of chromosomes had actually happened. It cannot be excluded that the cells with aneuploidy scored originated from a few colonies with different degrees of expansion during the cultivation period. Thus,

a difference in the total number of aneuploid cells in exposed and controls must not be taken as an indication for a difference in the formation of aneuploid colonies. Because monosomy 7 and trisomy 8 were also found in cells of unexposed workers and other aneuploidies were not investigated, a statement regarding the specificity of the types of aberrations found in formaldehyde-exposed workers cannot be made.

It is not clear why monosomy 7 and trisomy 8 were selectively investigated. The authors state that monosomy 7 and trisomy 8 are leukemia-specific chromosome aberrations. However, according to the WHO/IARC classification of myeloid leukemia (7), the typical and most frequent chromosome aberrations are translocations, whereas deletions and aneuploidies belong to the secondary cytogenetic abnormalities. Interestingly, the reference given by the authors (8) for the statement that monosomy 7 and trisomy 8 “are among the most frequent cytogenetic changes observed in myeloid leukemia and myelodysplastic syndromes”, does not mention these aneuploidies at all.

Taken together, the article does not provide convincing evidence that the differences measured in the frequencies of monosomy 7 and trisomy 8 in CFU-GM colonies has any significance for the formation of leukemia or any relationship with the exposure of workers to formaldehyde.

Does Formaldehyde Induce Aneuploidy?

Formaldehyde's mutagenic mode of action is quite well understood and it has been shown that formaldehyde predominantly acts by a clastogenic and not by an aneugenic mode of action (9-11). It is generally accepted that the induction of aneuploidy is a so-called indirect mechanism of genotoxicity due to an interaction with redundant non-DNA targets (e.g., tubulin; ref. 12). In contrast with clastogenic effects, the induction of aneuploidy is expected to show threshold concentration-effect curves. Formaldehyde-induced aneuploidy in the absence of a clear clastogenic effect is highly unlikely and an association between formaldehyde exposure and aneuploidy is most likely a chance finding. A negative *in vivo* micronucleus test with rats exposed to formaldehyde by inhalation for 4 weeks with target concentrations up to 15 ppm supports the view that formaldehyde does not induce aneuploidy in bone marrow cells (13).

Are Systemic Mutagenic Effects of Formaldehyde Likely?

The question of systemic genotoxicity is inadequately addressed by the authors because they provide a biased selection of animal studies with positive results: one study in Russian language (14) and one in Chinese language (15). It is not possible for us to critically eval-

uate these studies, which were not published in internationally recognized journals. However, reliable and comprehensive *in vivo* animal inhalation studies done according to international guidelines for genotoxicity testing are published and clearly indicate that formaldehyde does not have a systemic genotoxic effect on blood and bone marrow (13, 16). Human biomonitoring studies have given inconsistent and conflicting results and the likelihood that cytogenetic effects measured in cultured peripheral lymphocytes of formaldehyde-exposed subjects are actually related to formaldehyde exposure has been critically discussed (9).

In the context of possible systemic effects of formaldehyde, it is important to understand how such a highly reactive substance as formaldehyde could reach distant sites. The authors present the hypothesis that “methanediol... may readily penetrate into tissues, may travel to the marrow through the blood where it is in equilibrium with reactive formaldehyde... (that) can react with cellular macromolecules” referring to Fox et al. (17) and Matubayasi et al. (18). However, these articles investigated specific questions (i.e., the process of tissue fixation and the formaldehyde/methanediol equilibrium under artificial conditions) and the results were not directly related to biological situations. Apart from the well-known fact that the equilibrium at 25°C is far on the side of methanediol, no further information regarding possible physiologic effects could be derived. At present, the proposed role of methanediol in formaldehyde's genotoxicity is merely speculative and the abundance of evidence suggests that there is no delivery of inhaled formaldehyde to distant sites (13, 19).

Finally, the authors showed that CFU-GM progenitor cells are sensitive to formaldehyde exposure *in vitro* and use this finding as an argument to support their hypothesis that “formaldehyde can have an adverse impact on the hematopoietic system and that leukemia induction by formaldehyde is biologically plausible.” However, this conclusion is scientifically unjustified. There is no doubt that formaldehyde damages cells directly exposed *in vitro*. This has been shown for several cell types including human lymphocytes (9, 20), and the effective formaldehyde concentration clearly depends on the method used (20). Therefore, it is not at all surprising that human myeloid progenitor cells are damaged by formaldehyde when they are directly exposed *in vitro* and the effect shown by Zhang and coworkers is certainly not specific for myeloid progenitor cells.

In summary, we have identified some methodologic insufficiencies and scientifically unjustified conclusions that clearly limit the reliability of the publication and its usefulness with regard to supporting a leukemogenic potential from occupational and environmental exposures to formaldehyde. Because this study is too preliminary and has too many shortcomings, it is not suited to demonstrate a systemic (geno-)toxic mode of action of inhaled formaldehyde.

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No potential conflicts of interest were disclosed.

References

- Zhang L, Tang X, Rothman N, et al. Occupational exposure to formaldehyde, hematotoxicity, and leukemia-specific chromosome changes in cultured myeloid progenitor cells. *Cancer Epidemiol Biomarkers Prev* 2010;19:80–8.
- Baan R, Grosse Y, Straif K, et al. A review of human carcinogens—part F: chemical agents and related occupations. *Lancet Oncol* 2009;10:1143–4.
- Rothman KJ, Greenland S, Lash TL. *Modern epidemiology*. Philadelphia: Lippincott Williams & Wilkins; 2008.
- Natale VM, Brenner IK, Moldoveanu AI, Vasiliou P, Shek P, Shephard RJ. Effects of three different types of exercise on blood leukocyte count during and following exercise. *Sao Paulo Med J* 2003;121:9–14.
- Haus E, Nicolau GY, Lakatua D, Sackett-Lundeen L. Reference values for chronopharmacology. *Annu Rev Chronopharmacol* 1988;4:333–424.
- Melchart D, Martin P, Hallek M, Holzmann M, Jurcic X, Wagner H. Circadian variation of the phagocytic activity of polymorphonuclear leukocytes and of various other parameters in 13 healthy male adults. *Chronobiol Int* 1992;9:35–45.
- Swerdlow SH, Campo E, Harris NL, et al. WHO classification of tumors of haematopoietic and lymphoid tissues. 4. Geneva, Switzerland: WHO Press; 2008.
- Rowley JD. Molecular genetics in acute leukemia. *Leukemia* 2000;14:513–7.
- Schmid O, Speit G. Genotoxic effects induced by formaldehyde in human blood and implications for the interpretation of biomonitoring studies. *Mutagenesis* 2007;22:69–74.
- Speit G, Neuss S, Schutz P, Frohler-Keller M, Schmid O. The genotoxic potential of glutaraldehyde in mammalian cells *in vitro* in comparison with formaldehyde. *Mutat Res* 2008;649:146–54.
- Titenko-Holland N, Levine AJ, Smith MT, et al. Quantification of epithelial cell micronuclei by fluorescence *in situ* hybridization (FISH) in mortuary science students exposed to formaldehyde. *Mutat Res* 1996;371:237–48.
- Kirsch-Volders M, Vanhauwaert A, Eichenlaub-Ritter U, Decordier I. Indirect mechanisms of genotoxicity. *Toxicol Lett* 2003;140–1:63–74.
- Speit G, Zeller J, Schmid O, Elhajouji A, Ma-Hock L, Neuss S. Inhalation of formaldehyde does not induce systemic genotoxic effects in rats. *Mutat Res* 2009;677:76–85.
- Kitaeva LV, Kitaev EM, Pimenova MN. The cytopathic and cytogenetic sequelae of chronic inhalational exposure to formaldehyde on female germ cells and bone marrow cells in rats [Russian]. *Tsitologiya* 1990;32:1212–6.
- Tao XY, Yu SY, Kang L, Huang HX, Wei AY. Study on the genetic damage in mice induced by the volatile organic compounds of decoration materials [Chinese]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 2004;22:194–6.
- Kligerman AD, Phelps MC, Erexson GL. Cytogenetic analysis of lymphocytes from rats following formaldehyde inhalation. *Toxicol Lett* 1984;21:241–6.
- Fox CH, Johnson FB, Whiting J, Roller PP. Formaldehyde fixation. *J Histochem Cytochem* 1985;33:845–53.
- Matubayasi N, Morooka S, Nakahara M, Takahashi H. Chemical equilibrium of formaldehyde and methanediol in hot water: free-energy analysis of the solvent effect. *J Mol Liq* 2007;134:58–63.
- Pyatt D, Natelson E, Golden R. Is inhalation exposure to formaldehyde a biologically plausible cause of lymphohematopoietic malignancies? *Regul Toxicol Pharmacol* 2008;51:119–33.
- Speit G, Schmid O, Neuss S, Schutz P. Genotoxic effects of formaldehyde in the human lung cell line A549 and in primary human nasal epithelial cells. *Environ Mol Mutagen* 2008;49:300–7.

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