Misuse of Genomics in Assigning Causation in Relation to Benzene Exposure

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Benzene is an established cause of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), and may also cause lymphocytic leukemias and non-Hodgkin lymphoma (NHL) in humans. Additionally, changes in blood and bone marrow consistent with hematotoxicity are recognized in humans and experimental animals. Despite extensive research, questions remain regarding the exact mechanisms by which benzene and/or its metabolites exert their observed health effects; novel biomarkers of exposure and relevant early biologic effects are needed. Biomarkers or medical tests which could demonstrate past exposures responsible for benzene-induced cancers would be especially valuable, since hematopoietic cancers have a latency period of several months to years. The critical carcinogenic exposures to benzene will therefore take place more than three months before diagnosis. Unfortunately, at present no test exists which can measure benzene exposure more than 3 months in the past, because measurement of benzene-related adducts in the blood is limited by the lifespan of the adducted proteins (less than 1 month for albumin and 3 months for hemoglobin). This makes the assignment of specific causation in courtroom cases of cancers purportedly associated with benzene exposure a complex process relying on expert judgment and historical exposure assessment.

Recently, an article appeared on the BBC website that described a new test developed by a company called the Cytokine Institute.[13] The test "named msds1" could, according to the BBC article, "read the specific pattern of changes to DNA triggered by exposure to a chemical."[13,14] The article went on to state that

> civil courts in California have already heard more than 20 cases that used evidence from the technique. In one case, a worker at a company selling tyres sued his employers alleging that he had suffered illness as a result of exposure to benzene. Liberty Mutual, the employer's insurers, paid for the test to be carried out which proved the illness was not caused by the chemical, saving an estimated $1million in damages.

Having studied the toxicology of benzene for many years, I was not sure how such a technique could possibly work, so I investigated further. I soon learnt that the basis for the test was an article in the journal *Genomics* by Gillis and co-workers.[15] This article makes some absurd claims that must have escaped the peer-review process. For example, the authors state that "the information presented in this study not only offers clarity for determining injurious exposures, but it also provides a methodology for eliminating incorrect diagnoses and conclusions following such an exposure [to benzene]."[15] This is simply not true, as I will explain below.

Unfortunately, several newspapers and business newsletters soon followed the BBC report,[16] and a recent article in the Daily Journal expanded on the claims above, quoting Dr. Gillis, CEO of the Cytokine Institute and developer of the msds1™ test.[17] Dr. Gillis is quoted as saying "If a litigant says, 'I was exposed for 20 years to benzene and I think that's where I got my leukemia,' we can test their cells and see if indeed benzene played a role in their leukemia." Unfortunately, the Gillis et al. *Genomics* article provides no data to support such a claim.

Gillis et al. studied mitogen-stimulated human peripheral blood mononuclear cells (PBMC) from several people exposed to phenolic metabolites of benzene *in vitro* in tissue culture flasks and examined the mRNA gene expression profile using Affymetrix microarrays. They also examined cytokine release into the cell culture media. The paper states that "By detecting early changes in gene expression one can also identify the 'molecular signature' of acute benzene poisoning." This may be true if one were to study the PBMC of a person suffering from acute benzene poisoning who was exposed to benzene very recently, but one

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cannot directly relate findings in a tissue culture flask from exposures to high concentrations of benzene metabolites to the human *in vivo* situation, especially if the benzene exposure was in the past. The effects of benzene on PBMC gene expression are transient and will last only a few hours or days, and the expression profile will change soon after the benzene exposure stops. Further, the study is of stimulated PBMC that will be in G1/S/G2 phase, whereas in the living human they will be resting in G0 which will greatly alter their expression profile.

Microarrays and cytokine measurements are commonly used techniques in toxicology research,[14,15,18] and they have been applied in the study of humans,[19] rats,[20] and mice[21] exposed to benzene. Several articles from my laboratory are referred to in the *Genomics* paper, and Gillis et al. note that we have performed gene expression profiling of PBMC from humans exposed to benzene,[19] using the same Affymetrix arrays that were used in the Gillis et al study. The difference between our findings in humans exposed to benzene and those of Gillis et al. in tissue culture in stimulated PBMC is illustrated by the fact the genes *HSPA1A* and *HSPA1B* are strongly up-regulated in response to the benzene metabolite benzenetriol *in vitro* (Gillis et al. Table 3), whereas in our studies both genes are significantly downregulated. There are many other differences between our *in vivo* findings and the *in vitro* ones, and few commonly altered genes. Thus, chronic exposures to benzene in human PBMC *in vivo* produces a very different gene expression profile (or molecular signature as Gillis et al. call it) than a single high exposure to one minor benzene metabolite, 1,2,4-benzenetriol, would produce in stimulated PBMC in cell culture. Further, the changes in gene expression induced by benzenetriol are likely to be produced by oxidative stress, a phenomenon caused by many chemical agents, so the gene expression profile produced will not be unique to benzene or its metabolite benzenetriol. Similarly, many chemicals and disease conditions change cytokine expression, as it plays a key role in the inflammatory response. It is very unlikely a unique change in common cytokines will be induced by benzene. The Gillis et al. study is of interest in helping us understand the mechanisms of lymphocyte cell death induced by benzenetriol, but it has little or no relevance to actual human exposure to benzene and its health consequences. It certainly does not present a unique "molecular or DNA signature" for benzene toxicity in man. Claiming that the study actually presented such a signature is scientifically indefensible.

This brings us to the use of genomics tests in the courtroom. Persons claiming illness, such as leukemia, from exposures to benzene will presumably have been exposed chronically and repeatedly to benzene. They will not have had a single acute exposure to benzene or benzenetriol. Further, since leukemia has a latency period of at least several months, and more often years, the critical exposures to benzene will have been in the past.[4,22] Testing the current gene expression profile or cytokine profile will therefore reflect only the patient's current medical condition. There is no evidence that these profiles would reflect past exposures to benzene, radiation or any other chemical. I therefore cannot see any possible way one can currently use microarrays or cytokine profiling to help assign causation.

Details of the msds1™ test are unclear but the Cytokine Institute web site claims it "relies on no less than 22,000 DNA-based parameters," so it seems probable that the test is a gene expression profile of PBMC on Affymetrix arrays and perhaps a serum cytokine measurement. If so, as documented above, there is no possibility that it can reliably help us assign causation in relation to benzene exposure. If the only basis for saying so is the Gillis et al. paper in *Genomics*, then the msds1™ test is clearly junk science. Apparently, this supposed genomic technology has been uncritically accepted by both the workers' compensation bar and has been used to deny workers compensation benefits without scientific challenge. This acceptance has occurred despite the fact that the msds1™ test has never been subject to an analysis of sensitivity, specificity or positive predictive value. No knowledgeable scientist would accept the msds1™ test as useful information in attributing disease causation. Unfortunately the msds1™ test, though not a scientific breakthrough, may represent a new advance in "blinding people with science," a colloquial British expression meaning to deliberately confuse someone by giving the impression of highly complex knowledge.

References

1. Aksoy M. Benzene carcinogenicity. Boca Raton, Florida: CRC Press, Inc.; 1988.
2. Glass DC, Gray CN, Jolley DJ, et al. Leukemia risk associated with low-level benzene exposure. Epidemiology. 2003;14:569-577.
3. Hayes RB, Yin SN, Dosemeci M, et al. Benzene and the dose-related incidence of hematologic neoplasms in China: Chinese Academy of Preventive Medicine—National Cancer Institute Benzene Study Group. J Natl Cancer Inst. 1997;89:1065-1071.
4. Rinsky RA, Hornung RW, Silver SR, et al. Benzene exposure and hematopoietic mortality: A long-term epidemiologic risk assessment. Am J Ind Med. 2002;42:474-480.
5. Smith MT, Jones RM, Smith AH. Benzene exposure and risk of non-Hodgkin lymphoma. Cancer Epidemiol Biomarkers Pre.v 2007;16:385-391.
6. Smith MT, Zhang L. Biomarkers of leukemia risk: benzene as a model. Environ Health Perspect.1998;106 Suppl 4:937-946.
7. Strom SS, Gu Y, Gruschkus SK, et al. Risk factors of myelodysplastic syndromes: a case-control study. Leukemia. 2005;19: 1912-1918.
8. Lan Q, Zhang L, Li G, et al. Hematotoxicity in workers exposed to low levels of benzene. Science. 2004;306:1774-1776.
9. Qu Q, Shore R, Li G, et al. Hematological changes among Chinese workers with a broad range of benzene exposures. Am J Ind Med. 2002;42:275-285.
10. Snyder R. Benzene and leukemia. Crit Rev Toxicol. 2002;32:155-210.
11. Lin YS, Vermeulen R, Tsai CH, et al. Albumin adducts of electrophilic benzene metabolites in benzene-exposed and control workers. Environ Health Perspect. 2007;115:28-34.

12. Rappaport SM, Waidyanatha S, Yeowell-O'Connell K, et al. Protein adducts as biomarkers of human benzene metabolism. Chem Biol Interact. 2005;153-154:103-109.
13. DNA test hope over damages claims. BBC News [Internet]. 2007 17 September [Accessed 2007 Dec 22]. Available from: http://news.bbc.co.uk/2/hi/health/6998437.stm
14. The Cytokine Institute [Internet]. Los Angeles; n.d. MSDS1. [Accessed 2007 Dec 22]. Available from: http:// cytokineinstitute.com/msds1.htm
15. Gillis B, Gavin IM, Arbieva Z, et al. Identification of human cell responses to benzene and benzene metabolites. Genomics. 2007;90:324-333.
14. Gant TW. Novel and future applications of microarrays in toxicological research. Expert Opin Drug Metab Toxicol. 2007;3:599-608.
15. Waters M, Stasiewicz S, Merrick BA, et al. CEBS Chemical Effects in Biological Systems: a public data repository integrating study design and toxicity data with microarray and proteomics data. Nucleic Acids Res. 2008; 36 (Database issue): 0892-0900; doi: 10.1093 /nar/gkm 755.
16. The Cytokine Institute [Internet]. Los Angeles; n.d.. News. [Accessed 2007 Dec 22]. (see http://cytokineinstitute.com/news.htm)
17. New DNA Test Could Change Toxic Tort Trials. Daily Journal (Los Angeles) 2007 December 21: front page.
18 Smith MT, Vermeulen R, Li G, et al. Use of 'Omic' technologies to study humans exposed to benzene. Chem Biol Interact. 2005;153-154:123-127.
19. Forrest MS, Lan Q, Hubbard AE, et al. Discovery of novel biomarkers by microarray analysis of peripheral blood mononuclear cell gene expression in benzene-exposed workers. Environ Health Perspect. 2005;113:801-807.
20. Hendriksen PJ, Freidig AP, Jonker D, et al. Transcriptomics analysis of interactive effects of benzene, trichloroethylene and methyl mercury within binary and ternary mixtures on the liver and kidney following subchronic exposure in the rat. Toxicol Appl Pharmacol. 2007;225:171-188.
21. Faiola B, Fuller ES, Wong VA, et al. Gene expression profile in bone marrow and hematopoietic stem cells in mice exposed to inhaled benzene. Mutat Res. 2004;549:195-212.
22. Pyatt D. Benzene and hematopoietic malignancies. Clin Occup Environ Med. 2004;4:529-555, vii.