

Biomarkers in Human Benzene Exposure

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This project team includes investigators who have previously collected samples and data and performed biomarker assays on a large number of subjects. Workers with occupational exposure to benzene that occurred before the current project was started and control groups were enrolled in a previous European Union-supported study. As part of the project, data were collected on urinary benzene metabolites and genotypes at several relevant loci involved with benzene metabolism. Demographic data, benzene exposure data, and biological materials were also available. We examined biomarkers of benzene exposure and the effects in a group with very high occupational exposures to benzene – 158 petrochemical workers from Bulgaria and 50 matched controls. We also measured exposures in a group of workers with much lower exposures from Genoa, Italy. In Bulgaria, the air exposures to benzene among the workers averaged about 1.8 ppm with levels as high as 24 ppm. Controls had an exposure below or equal to 0.023 ppm. The benzene metabolites trans,trans-muconic acid (t,t-MA) and S-phenylmercapturic acid (S-PMA) were measured in the urine, and both showed dose-response relationships with air exposure levels to benzene. The number of single strand breaks in DNA (DNA-SSB) in exposed workers was also significantly higher than in controls, but the dose-response curve showed a supralinear or saturation effect at high exposure. This saturation was not seen for either of the metabolites.

Genotype at the NAD(P)H: quinone oxidoreductase 1 (*NQO1*) locus had no significant effect on the excretion of the two metabolites normalized for benzene exposure. However, individuals with one or two non-functional *NQO1* T alleles showed a significantly higher level of DNA-SSB that was two-fold that of wild type individuals. We conclude from the data on dose response and genotype that the pathways for the metabolites, often used as exposure biomarkers, are distinct from those involved in at least some forms of genotoxic damage by benzene. The saturation findings for the genotoxicity endpoint can be extrapolated to determine the risk from low to high doses. No effect of smoking was seen for the highly exposed Bulgarian workers. However, smoking was the most important determinant of metabolite levels in both controls and workers with low occupational exposures to benzene.

Genotypes at the glutathione S-transferase M1 (*GSTM1*), glutathione S-transferase theta 1 (*GSTT1*), epoxide hydrolase (*EPHX*), and myeloperoxidase (*MPO*) loci have been determined for all samples, allowing us to analyze both metabolic and genotoxic endpoints as a function of the genetic profile for the entire benzene metabolism pathway in each individual. We compared the role of inherited genetic susceptibility factors on the effects of benzene exposure across a large range of exposure levels. The composite genotypes generally produced predicted effects on benzene metabolite levels and genotoxicity based on the benzene metabolism pathway. We found that the genotype at three loci (*NQO1*, *EPHX*, and *GSTT1*) had major effects on the susceptibility to the genotoxic effects of benzene exposure in living human beings. In contrast, the genotype at *MPO* and *GSTM1* had no observable effect. The difference in sensitivity, as measured by the level of DNA-SSB, observed between individuals with the least favorable composite (three gene) genotype and those with the most favorable genotype was more than five-fold.

This study supports the idea that genetic background plays a significant role in individual human susceptibility to the toxic effects of chemicals, and can allow for the prediction of such variations in sensitivity.

Benzene has been shown to produce acute hematotoxicity in people with chronic exposures greater than 10 ppm. A recent study published in *Science* (Lan et al., 2004; *Science* 306:1774-76) using data from

Chinese shoe workers detected evidence for hematotoxicity at exposures below 1 ppm. We have found no evidence for hematotoxicity below 10 ppm of benzene in a group of 208 Bulgarian petrochemical workers. We also found no effect of the *NQO1* polymorphism on benzene hemotoxicity at any dose level. All blood parameters were significantly higher in smokers compared to nonsmokers. When data were stratified by smoking, we found a significant trend for lower eosinophils counts by multiple logistic regression in smokers starting at benzene exposures below 1 ppm. This study population differed from that studied in the Chinese cohort in that the Bulgarian petrochemical workers were mostly smokers, mostly male, and all European, as opposed to the Chinese cohort who were mostly nonsmokers, mostly female, and all Chinese. In conclusion, we cannot confirm the hematotoxicity of benzene at exposure levels below 1 ppm, except for an effect on eosinophil levels in smokers.

Implications: This research clearly shows that the genetic profile of human beings plays an important role in the extent of toxicity caused by exposure to a toxicant such as benzene. The difference between those who are the most genetically susceptible and those who are the least susceptible, when measured by DNA damage, is over five-fold. This implies that risk assessment must take into account not only exposure and hazard data, but also the genetic variability known to exist in the human population. Knowledge of the detailed metabolic pathways by which toxic agents act and are eliminated, as well as of the genes responsible for their effects, can allow for genetic susceptibility testing related to many environmental and occupational toxic chemicals.

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Presentations:

Biochemical susceptibility: An approach to the genetics of environmental carcinogenesis. Presented at the University of Pittsburgh School of Public Health, Department of Environmental and Occupational Health, Pittsburgh, PA, March 29, 2005.

Role of environmental genotoxins in human carcinogenesis evidence from genetic susceptibility. Presented at the 35th Annual Meeting of the European Environmental Mutagen Society Environment and Human Genetic Disease – Causes, Mechanisms and Effects. Kos, Greece, July 4, 2005.

From molecular epidemiology to observational mechanistic analysis. Presented at the UPCI Annual Retreat. Johnstown, PA, June 22, 2006.

Benzene genotoxicity and metabolism in humans: Dose response and effects of composite genotype. Presented at the International Council of Chemical Associations LRI Workshop on Interpretation of Human Biomonitoring Data. Minneapolis, MN, July 26, 2006.

Lack of benzene induced hematotoxicity in Bulgarian petrochemical workers with exposures below 1 ppm. Presented at the International Council of Chemical Associations LRI Workshop on Interpretation of Human Biomonitoring Data. Minneapolis, MN, July 26, 2006.

Environmental toxicology in humans: From molecular epidemiology to mechanism. Presented at the Department of Environmental and Occupational Health, School of Public Health, University of Pittsburgh. November 29, 2007.

Peer-reviewed publications:

Fustinoni, S., Buratti, M., Campo, L., Colombi, A., Consonni, D., Pesatori, A.C., Bonzini, M., Bertazzi, P., Garte, S., Farmer, P.B., Levy, L.S., Pala, M., Valerio, F., Desideri, A., and Merlo, F. (2005). Monitoring low benzene exposure: Comparative evaluation of urinary biomarkers, influence of cigarette smoking and genetic polymorphisms. *Cancer Epidemiology, Biomarkers, and Prevention* 14: 2237-2244.

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Garte, S., Taioli, E., Popov, T., Bolognesi, C., Farmer, P., and Merlo, F. (2008). Genetic susceptibility to benzene toxicity in humans. *Journal of Toxicology and Environmental Health* 71(22): 1482-9.

Pavanello, S., Pulliero, A., and Clonfero, E (2008). Influence of *GSTM1 null* and low repair *XPC PAT+* on *anti-B[a]PDE-DNA* adduct in mononuclear white blood cells of subjects low exposed to PAHs through smoking and diet. *Mutation Research* 638(1-2): 195-204.

Dougherty, D., Garte, S., Barchowsky, A., Zmuda, J., and Taioli, E. NQO1, MPO, CYP2E1, GSTT1 and *Gstm1* polymorphisms and biological effects of benzene exposure – A literature review. *Mutation Research – Genetic Toxicology and Environmental Mutagenesis*. (In Press).

Pesatori, A.C., Garte, S., Popov, T., Georgieva, T., Panev, T., Bonzini, M., Consonni, D., Goldstein, B.D., Taioli, E., Fontana, V., Stagi, E., and Merlo, D.F. Early effects of low benzene exposure on blood cell counts in Bulgarian petrochemical workers. (Submitted).

Other publications: None to date.

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