



STATEMENT

Synopsis of Research Report 115

HEALTH
EFFECTS
INSTITUTE

Validation of Biomarkers in Workers Exposed to Benzene

Human exposure to benzene is widespread because it is a component of gasoline and is also used extensively as an industrial solvent. Exposure to high levels of benzene is associated with development of leukemia and other blood disorders, but the risks of exposure to low levels of benzene are not well understood. In the 1990s the Health Effects Institute initiated a research program designed to study the effects of exposure to toxic air pollutants at ambient levels. As one part of this research program, HEI's Request for Applications (RFA) 93-1 supported studies to develop reliable and sensitive assays for biomarkers of benzene exposure—both recent and longer-term—and of benzene effect. The biomarkers of *recent* exposure were urinary metabolites (measuring responses up to hours after exposure) and adducts of blood proteins (days to weeks after exposure). The biomarkers of *longer-term* exposure were chromosomal changes, integrating exposure over months to years. Because chromosomal changes may be determinants of subsequent health effects, they may also be considered early biomarkers of benzene effect. Chromosomal changes may also be due to causes other than exposure to benzene.

To validate the biomarkers characterized in these studies, another part of the research program, Request for Qualifications (RFQ) 95-3, "Transitional Epidemiology Studies for Benzene or 1,3-Butadiene Biomarkers," solicited applications from investigators with access to suitable human populations exposed to benzene or butadiene. HEI funded a study by Dr Qingshan Qu of New York University School of Medicine to evaluate putative biomarkers in workers occupationally exposed to benzene in China.

APPROACH

Qu and colleagues recruited 181 healthy workers in several factories in the Tianjin region of China. These subjects formed part of a cohort of thousands identified by the US National Cancer Institute (NCI) and the China Academy of Preventive Medicine

for a study to evaluate tumor incidence in benzene-exposed workers (NCI/China study). In phase 1 of their study, Qu and colleagues evaluated the suitability of using urinary metabolites, blood adducts, or chromosomal aberrations in polymorphonuclear leukocytes and lymphocytes as benzene biomarkers in 25 heavily exposed and 25 unexposed workers. The urinary metabolites measured were phenol, catechol, hydroquinone, benzene triol, *S*-phenylmercapturic acid (*S*-PMA), and *trans,trans*-muconic acid (*t,t*-MA). The blood adducts measured were benzene oxide and benzoquinone adducts of albumin.

In phase 2, the investigators used biomarkers validated in phase 1 of the study to evaluate relations between benzene exposures and levels of these biomarkers in another 105 benzene-exposed workers and 26 unexposed workers. The investigators focused on obtaining samples from workers whose current-day exposures to benzene were no more than 5 ppm, representing the low end of occupational exposure. Qu and colleagues also evaluated whether the number and type of blood cells decreased in the exposed subjects because such decreases may be early indicators of a response to occupational benzene exposure. Some biological samples were analyzed in China and some in the United States.

RESULTS AND INTERPRETATION

This study has made important contributions regarding the utility of biomarkers of benzene exposure in occupational settings. It is the first to evaluate multiple possible biomarkers of benzene across a wide range of exposures and to show effects at the lowest end of the range. In addition to using sensitive assays for urinary metabolites and blood adducts, Qu and colleagues made great efforts to accurately measure and monitor personal exposures to a wide range of benzene levels in the workplace—critical features for assessing the accuracy of biomarker information. The investigators also paid careful attention to quality control issues.

The study's most novel finding was that benzene

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exposure was associated with decreases in the numbers of circulating neutrophils and, to a lesser extent, lymphocytes. The decrease in neutrophil numbers is interesting because long-term human exposure to high levels of benzene has been previously associated with the development of cancer in bone marrow precursor cells that give rise to neutrophils. This result—indicating that changes in neutrophil numbers may be a sensitive marker of benzene effects—needs to be corroborated, however, because other studies have found changes in lymphocyte, but not neutrophil, numbers.

A key positive feature of the study design was Qu's 2-step approach to validating possible biomarkers in phase 1 before proceeding to the larger study in phase 2. The phase 1 results indicated that *S*-PMA and *t,t*-MA, minor metabolites of benzene found in urine, might be the most useful markers of recent benzene exposure. Combined analysis of phase 1 and 2 results confirmed the suitability of *S*-PMA and *t,t*-MA as biomarkers for this purpose: both markers had low background levels in unexposed workers and increased levels in exposed workers. *S*-PMA was found to be the most useful biomarker for recent exposure to benzene because of the extent of the change in its level, its sensitivity in correlating with low occupational benzene exposures, and its specificity for benzene exposure. The urinary metabolites phenol, hydroquinone, and catechol were less sensitive to changes in benzene exposure and had higher background levels than *S*-PMA and *t,t*-MA. Therefore, these markers were less suitable for detecting dose-dependent variation across the spectrum of benzene exposures. Benzene triol was found to be unsuitable as a biomarker.

Exposure-dependent changes in blood adduct levels (half-life in blood of approximately 14 days) were found to be suitable measures for evaluating recent exposure although the background levels in unexposed workers were quite high.

Using the fluorescence in situ hybridization (FISH) technique to examine specific chromosomes for effects of longer-term benzene exposure, the investigators did not detect differences between the numbers of chromosomal aberrations in exposed and unexposed workers. In contrast, FISH data in the NCI/China study evaluating the same chromosome (chromosome 7) showed increased numbers of aberrations in exposed

workers. However, differences in cell culture conditions, probes evaluated, and scoring criteria make it difficult to compare the FISH results between the 2 studies. In addition, the overall frequencies of numerical aberrations (hyperdiploidy) reported in the unexposed control subjects participating in the NCI/China study were unusually high, which complicates comparisons. Although the median exposures of workers in the NCI/China and HEI studies were similar, workers in the NCI study with above-median exposures were exposed to much higher benzene levels than those participating in the HEI study. These higher exposures may have also contributed to the differences between the FISH results of the 2 studies. Using conventional cytogenetic techniques to evaluate all chromosomes, Qu and colleagues found some increases in aberrations in exposed workers compared with controls. These increases were difficult to interpret because they were not linear with recent changes in benzene exposure. However, a more consistent exposure-response relationship was seen when the aberration frequencies were categorized by cumulative benzene exposures.

The investigators evaluated exposure-response effects in the phase 2 subjects combined with the subjects who had been evaluated in phase 1, which was conducted in the previous year. Combined analysis of phase 1 and phase 2 results may have introduced unmeasured confounding because exposures in the 2 phases were measured in different years and at different sites. Further, they used different subjects with much lower exposure levels—by design—in phase 2 than phase 1. Although Qu and colleagues amply addressed many aspects of this issue in the report, the validity of combining data from phases 1 and 2 of the study remains uncertain.

In conclusion, Qu and colleagues' study has validated several biomarkers. Urinary levels of *S*-PMA appear to be the most useful measure of exposure to benzene (detecting changes within a few hours). Blood adducts of benzene and albumin may be useful biomarkers of exposure within days to weeks, but background levels of these adducts are quite high in people not exposed to benzene. Finally, the investigators found that changes in neutrophil levels may be a sensitive and early marker of benzene's toxicity, but further research is needed to confirm this last finding.

