Benzene's Adverse Effects

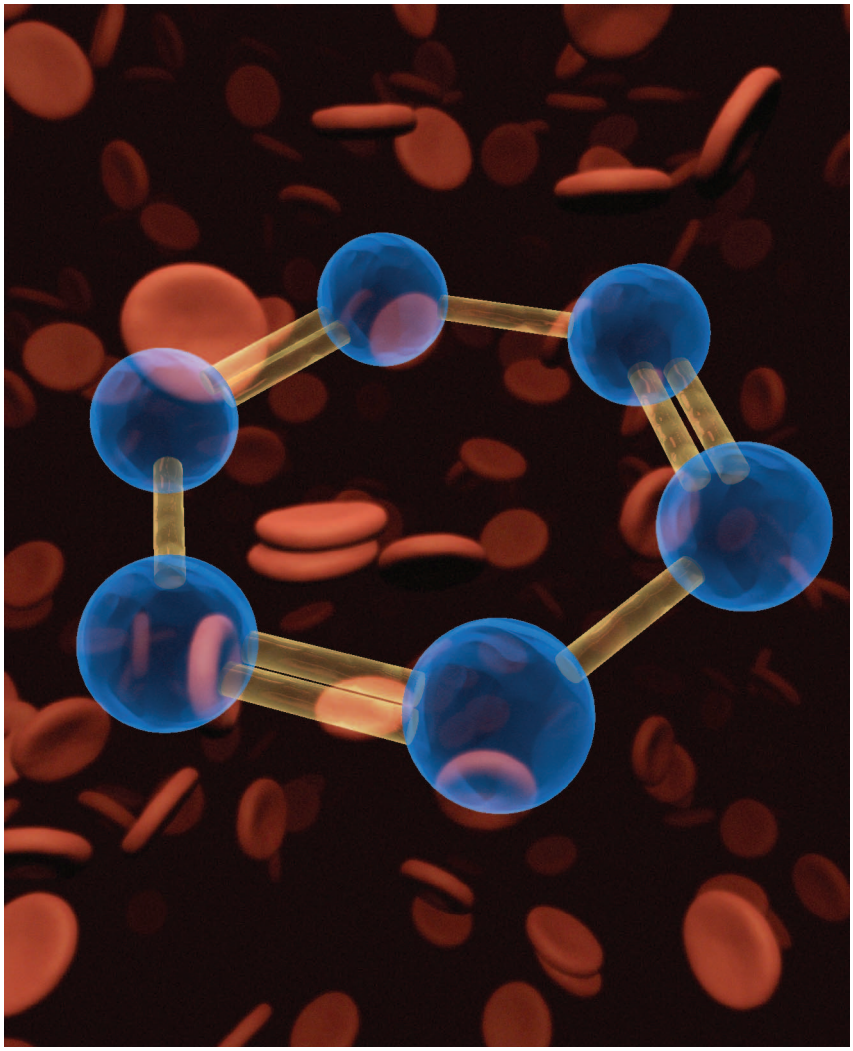
Microarrays Reveal Breadth of Toxicity

Benzene is both widely used and widely studied. Yet, although the chemical is strongly associated with leukemia in humans, questions remain regarding its mechanism of action. Hoping to better understand the genetic mechanisms behind benzene's hematotoxicity and leukemogenicity, a group of researchers from Japan and Korea used cDNA microarrays to analyze mouse bone marrow tissue both during and after a two-week exposure to the compound by inhalation [***EHP* 111:1411–1420**]. The researchers found, among other discoveries, that benzene may perturb cell cycling that is mediated by the gene for the protein p53, triggering a host of fatal problems at the cellular level and thus causing blood cell malignancies epigenetically (that is, without encoding the information in the genetic code).

Benzene is used in fuels, as an industrial solvent, and in other manufacturing applications, and is also found in cigarette smoke. Human populations generally are exposed through polluted ambient air or contaminated water. Benzene is known to cause hematotoxicity and blood tumors in humans and mice. Studies so far have focused on benzene's carcinogenic and genotoxic metabolites, which cause various types of tumors in a number of mouse organ systems. Hepatic enzymes convert inhaled benzene into genotoxic metabolites. Then, to add insult to injury, a number of these benzene metabolites (primarily phenol, hydroquinone, catechol, and *trans-trans* muconic acid) actually intensify the chemical's toxic effect on an organ.

Past studies have suggested that benzene's toxic effects on bone marrow tissue—its major target organ—may be enacted through multiple pathways, including growth factor regulation, oxidative stress reduction, DNA damage repair, cell cycle regulation, and apoptosis. Also, genetic variations may upset the cellular–environmental homeostasis that protects bone marrow cells from toxic effects such as those caused by benzene, resulting in altered gene expression. Therefore, the authors suggest, studying just a few specific genes may not be enough to thoroughly explain the complex molecular mechanisms of benzene-induced hematotoxicity and leukemogenicity.

With this in mind, the research team conducted broad cDNA microarray analyses using multiple gene expression profiling technologies. The team analyzed mouse bone marrow tissue during and after exposure to 300 parts per million benzene over a 2-week period for 6 hours a day, 5 days a week. Two types of C57BL/6 mice were used—standard wild-type mice possessing the gene for p53 and p53-knockout mice. The mice were randomly grouped into control and benzene-exposed groups.



A bad actor in blood. New research shows that benzene's toxic effects—including leukemogenicity and hematotoxicity—are wrought through many pathways. Thus, multiple genes may be implicated.

Photodisc, Christopher G. Reuther/EHP

Twice during the exposure period and then 3 days after the full 2-week exposure, the researchers collected bone marrow from both femurs of each mouse in each group. RNA was extracted from this tissue and used to synthesize cDNA, which was then hybridized onto a microarray chip. The resulting array of gene fragments was scanned as a digital image and analyzed using software that searched for clustering genes specifically expressed and/or suppressed in each group.

The researchers found that benzene caused DNA damage in cells during all phases of the cell cycle. In the benzene-exposed wild-type mice, DNA repair genes were activated, but they were suppressed in the p53-knockout mice. Mice in the latter group were therefore susceptible to benzene's direct genotoxic leukemogenicity, whereas those in the former still experienced epigenetic leukemogenicity via cell-cycle perturbations despite DNA repair.

Besides the p53-mediated pathway, the investigators identified other specific genes that may be involved in G1 cell cycle arrest and apoptosis following benzene exposure, and confirmed that certain repair genes—including the tuberous sclerosis gene and the metallothionein 1 gene—are also triggered by such exposure. They also found that, during benzene exposure, the production of blood cells was arrested due to alterations in the expression of cell cycle checkpoint genes in the wild-type mice. However, production continued in the p53-knockout mice, an important difference that the researchers say could point to mechanisms of benzene's hematotoxicity.

The researchers' cDNA microarray analyses supported the theory that the gene for p53 mediates the effect of benzene on bone marrow tissue by regulating specific genes instrumental in cell cycle arrest, apoptosis, and DNA repair. Because

careful simultaneous screening of different expression patterns of many interrelated genes between the two groups is necessary, the researchers write, toxicogenomics should prove extremely useful for future investigations into the toxicity and leukemogenicity mechanisms of benzene. **–Jennifer Medlin**

Effect of SNPs on OPs

Age and Race Variations Explored

Newborns produce substantially less of the enzyme paraoxonase-1 (PON1)—which detoxifies organophosphate pesticides—than do adults, potentially leaving them more vulnerable to organophosphate exposures. Genetic differences in PON1 activity are also more pronounced in newborns than in adults, according to recent research by Jia Chen and colleagues at the Mount Sinai School of Medicine [***EHP* 111:1403–1409**]. By helping to determine which groups are most susceptible based on age and genetic factors, these results may have implications for setting exposure standards.

Metabolites of organophosphates damage the nervous systems of insects and humans by reducing the ability of the enzyme cholinesterase to regulate the electrochemical signals between neurons. When cholinesterase levels drop, neurons become overstimulated and send repeat signals that can eventually cause muscle weakness, paralysis, and death. PON1 breaks down organophosphates before they can cause nerve damage.

The current study is part of ongoing research on the neurodevelopmental risks posed by exposure to the organophosphate pesticide chlorpyrifos among an inner-city population in New York City. Once among the most commonly used insecticides, chlorpyrifos was banned in 2000 for many residential uses in the United States because of concern over children's health. Like other organophosphates, chlorpyrifos and its toxic metabolite chlorpyrifos oxon can cross the placenta. Therefore, exposures by pregnant women can affect their unborn children. However, little is known about the effects of low-level exposures on children's development, including their ability to learn later in life. Chlorpyrifos is still approved for many agricultural uses, and children in rural areas continue to be exposed.

Researchers have identified five single-nucleotide polymorphisms that affect PON1 production, three in the promoter region of the *PON1* gene (–909, –162, –108) and two in the coding region (L55M, Q192R). *PON1* has also been linked to, or tends to be inherited along with, two other genes, *PON2* and *PON3*.

From March 1998 through March 2002, the Mount Sinai researchers genotyped and measured PON1 activity in the blood of an ethnically diverse group of 402 expectant mothers and 229 newborns. Participants identified themselves, or were identified by their parents, as Caucasian (82 mothers, 56 newborns), African American (117 mothers, 66 newborns), or Caribbean Hispanic (203 mothers, 107 newborns). Blood samples were genotyped for the five *PON1* polymorphisms. Because the researchers are also studying the linkage of *PON* genes, samples were also genotyped for a common *PON2* polymorphism (C311S). The level of PON1 activity in each blood sample was determined by an assay that measures hydrolysis of phenylacetate.

As expected, the results showed that PON1 activity of newborns was less than that of adults, and so newborns are potentially more susceptible to the effects of chlorpyrifos exposure. In addition, the Mount Sinai researchers found that some groups of newborns may be more vulnerable than others. The presence of *PON1* polymorphisms and PON1 activity varied among racial/ethnic groups. PON1 activity in the blood of the expectant mothers was 4.6, 3.6, and 2.6 times greater than in that of newborns for Caucasians, Caribbean Hispanics, and African Americans, respectively. In addition, the impact of genetic variability was greater in the newborns than in the adults. None of the polymorphisms affected PON1 activity in the women by more than 35%. However, among the newborns, several of the polymorphisms affected PON1 activity by as much as 200%. For example, Caucasian infants with the *CC* polymorphism of the –108 *PON1* promoter had, on average, more than twice the PON1 activity of infants with the *TT* polymorphism.

The researchers further found that the polymorphisms tended to be inherited in predictable patterns, a phenomenon referred to as "linkage disequilibrium." There was significant linkage disequilibrium among the three promoter polymorphisms, and among the promoter polymorphisms and the coding polymorphism L55M. These relationships were strongest for Caucasian participants and weakest for African-American participants. In addition, there was significant linkage disequilibrium among the *PON1* promoter polymorphisms and the *PON2* polymorphism C311S. These results may contribute to a better understanding of rates of recombination in genes, as well as provide a basis for future epidemiological studies. **–Kris Freeman**

Separate at birth. New data reveal racial differences in PON1 activity in newborns. This means some infants may be more vulnerable than others to the effects of organophosphates, which are broken down by this enzyme.

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