



STATEMENT

Synopsis of Research Report 113

HEALTH
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Benzene Metabolism in Rodents at Doses Relevant to Human Exposure

Human exposure to high levels of benzene is associated with development of leukemia and other blood disorders, but the effects of exposure to low levels of benzene are not well understood. In the 1990s, HEI initiated its Air Toxics Research Program to address uncertainties about health effects of ambient levels of benzene and other air toxics derived from mobile and other sources. One of the program's goals was to develop methods sensitive enough to measure benzene metabolism at low exposure levels. Such sensitivity is important because one or more benzene metabolites are thought to be responsible for benzene's toxic effects. In addition, understanding benzene metabolism at low exposure levels is critical to benzene risk assessment because the shape of the dose-response curve at low concentrations is not yet resolved.

APPROACH

HEI funded Dr Kenneth Turteltaub to investigate benzene metabolism in rodents over a hundred million-fold dose range. This range encompassed concentrations close to those of human ambient exposure, generally 1 to 10 parts per billion. Turteltaub and his colleague, Chitra Mani, administered radioactive benzene to mice and rats and subsequently analyzed bone marrow, liver, urine, and plasma from these animals. In most experiments, the investigators injected animals intraperitoneally with ^{14}C -labeled benzene, but in some experiments they exposed animals to radioactive benzene via inhalation. After exposure, the investigators coupled high-performance liquid chromatography (HPLC; to separate benzene metabolites) with the novel and sensitive technique accelerator mass spectrometry (to measure ^{14}C) in order to measure low levels of metabolites. Accelerator mass spectrometry was developed by nuclear physicists to measure low levels (10^{-15} to 10^{-18} molar) of long-lived isotopes such as ^{14}C .

RESULTS AND INTERPRETATION

In this innovative study of benzene metabolism, Turteltaub and Mani detected dose-dependent formation of benzene metabolites in plasma, bone marrow, and liver of mice over a wide range of doses (5 ng/kg to 500 mg/kg). Benzene metabolites, including DNA and protein adducts, were detected at levels 100 times lower than had been found in previous studies.

Even at low benzene exposure concentrations, the investigators detected higher levels of benzene metabolites in mouse and rat bone marrow and liver than in plasma. This finding indicates that benzene reaches tissues and is metabolized there, even at levels close to those to which humans are exposed in ambient air. In addition, Turteltaub and Mani found that the levels of DNA and protein adducts detected in bone marrow and liver in different rodents generally correlated well with the ability of benzene to induce tumors in that species or strain. This result suggests that the formation of adducts may be an early marker of benzene carcinogenicity.

All doses of benzene produced a similar pattern of metabolites in mouse urine, suggesting that the pattern of benzene metabolism is similar at widely disparate concentrations. This finding is of interest because other studies have suggested that the pattern of benzene metabolites differs depending on the benzene concentration to which animals are exposed. Such differences in metabolism of benzene could affect the shape of the exposure-response curve. However, Turteltaub and Mani's results are difficult to compare with previous studies: Although the current study has greater intrinsic analytic sensitivity than previous studies, it did not detect a metabolite previously found in the urine of rodents exposed to benzene.

Although results of the current study show the potential of accelerator mass spectrometry coupled with HPLC, they also illustrate the drawbacks. First,

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This Statement, prepared by the Health Effects Institute, summarizes a research project conducted by Drs Kenneth W Turteltaub and Chitra Mani at Lawrence Livermore National Laboratory, Livermore CA, and the University of California, San Francisco. The complete report, *Benzene Metabolism in Rodents at Doses Relevant to Human Exposure from Urban Air*, can be requested from HEI (see reverse side).

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in the current study, urine from mice exposed to radioactive benzene contained a large peak of radioactivity that could not be identified by HPLC. The investigators did not look for this material in plasma or bone marrow; thus, the peak might also have been present in samples from these tissues, with an uncertain impact on the results. This unidentified radioactive material may be a contaminant of the radioactive material used in the assays, a previously unidentified metabolite, or the decomposition product of a known benzene metabolite.

Second, the technique requires administering radiolabeled benzene to the study animals. Although the method uses extremely low levels of radioactive benzene, such an approach is not broadly applicable for controlled exposure studies with humans because benzene is classified as a known human carcinogen. Third, this study indicates the potential influence on results of varying methods of biomarker collection, storage, and processing. In the current study, glucuronidase

inhibitors were not added to urine samples, which possibly resulted in the degradation of a major metabolite, hydroquinone glucuronide, that was detected in other studies of benzene metabolism.

Even given these challenges, Turteltaub and Mani provided important information about benzene metabolism at the lowest end (5–500 ng/kg body weight) of the range of benzene doses tested: the dose-response curve for metabolite formation was flatter than that of higher benzene doses but was above zero. This result indicates that metabolism of benzene to activated metabolites occurs even at very low doses. It further suggests, but does not show conclusively, that the dose-response curve for benzene in mice lacks an obvious threshold at the lowest exposure levels evaluated. This finding may have important ramifications for understanding the human response to low-level benzene exposures. Further studies are required to resolve the shape of the dose-response curve for humans at these low benzene levels.

Benzene Metabolism in Rodents at Doses Relevant to Human Exposure from Urban Air

Kenneth W Turteltaub and Chitra Mani

INVESTIGATORS' REPORT

Specific Aims

Methods and Study Design

- Chemicals and Metabolite Standards
- Animals
- Benzene Administration
- Sample Collection and HPLC Analysis
- Isolation of DNA from Tissue Samples
- Isolation of Protein from Tissue Samples
- AMS Analysis
- Statistical Methods and Data Analysis

Results

- Benzene Metabolism at Low Doses in B6C3F₁ Mice
- Relation Between Dose and Macromolecular Adduct Formation in B6C3F₁ Mice
- Macromolecular Adduct Levels Among Rats and Mice by AUC

- Macromolecular Adducts in Rats and B6C3F₁ Mice After Low-Dose Benzene Inhalation

Discussion

- Metabolism of Benzene to Reactive Intermediates in B6C3F₁ Mice
- Distribution of Reactive Benzene Metabolites Among Liver, Bone Marrow, and Urine in B6C3F₁ Mice
- Relation Between Metabolite Levels and Macromolecular Adduct Formation in B6C3F₁ Mice
- Relation Between Macromolecular Adduct Levels and Toxicity in Rodents
- Inhalation Versus IP Exposure to Benzene
- Summary

Implications of Findings

COMMENTARY Health Review Committee

Scientific Background

- Benzene Metabolism and Mechanism of Toxic Effects
- Assessment of Risk

Technical Evaluation

- Aims
- Methods
- Results

Discussion

Summary and Conclusions

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