STATEMENT

Synopsis of Research Report 144

HEALTH EFFECTS INSTITUTE

Genotoxicity of 1,3-Butadiene and Its Metabolites

BACKGROUND

1,3-Butadiene (BD) is used extensively in the chemical industry (e.g., for synthetic rubber production) and is also part of motor vehicle exhaust and cigarette smoke. BD is listed by the U.S. Environmental Protection Agency (EPA) as a mobile-source air toxic and was recently reclassified by EPA as a human carcinogen via inhalation exposure. This classification was based on occupational exposures of workers in the butadiene rubber industry who showed higher levels of certain leukemias compared with the general population. In 2002, the EPA cited additional evidence for carcinogenicity from chronic inhalation studies with rodents, but the carcinogenic and mutagenic potency of BD is different in mice and rats, which complicates extrapolating results from rodents to humans for risk assessment purposes. Therefore, toxicologic research in the past decade has focused on how the metabolism of BD differs between mice and rats (and how human metabolism compares) and on assessing the mutagenicity of reactive epoxide metabolites of BD in these two species as well as in human cells in vitro. The major metabolites of interest have been the monoepoxide (BDO), the diepoxide (BDO_2), and the epoxydiol (BDO-diol).

Dr. Walker proposed to investigate several unresolved issues surrounding BD metabolites, such as (1) the role of stereoisomerism (differences in the three-dimensional shape of chemical compounds) in the mutagenicity of the metabolites, (2) how much the nonreactive intermediary metabolite BD-diol contributes to the formation of reactive metabolites, and (3) the mutagenic potency of BD in mice and rats exposed to high and low levels of BD. The study would focus on differences in mutagenic potency of BD between rodent species, age groups, and sexes. The HEI Health Research Committee recommended funding the study because it would address many of the outstanding questions regarding the mutagenicity of BD and its metabolites and that information would be important in extrapolating results from animal studies to humans.

APPROACH

The study comprised a series of experiments in which male and female F344 rats and B6C3F1 mice, 4 to 5 weeks or 8 to 9 weeks old, were exposed via inhalation to BD or its metabolites BDO_2 and BD-diol. Exposures lasted for 6 hours/day, 5 days/week, for 2 to 4 weeks; control groups were exposed to filtered air. Upon completion of the exposures, lymphocytes (T cells) were collected from the spleen to determine mutations in the *Hprt* gene. (Rare cells with mutations in the *Hprt* gene can be selected from normal cells by adding 6-thioguanine to the culture medium. It kills normal cells because they incorporate it into their DNA, but does not affect the mutant cells, which are unable to incorporate it.)

First, *Hprt* mutant frequencies were determined in rodents exposed to 3 or 1250 ppm BD to assess sex and age differences in mutagenic response to BD at low and high concentrations. These data were compared with data obtained from earlier studies by Dr. Walker.

Second, rodents were exposed to 2 or 4 ppm BDO_2 of the *meso* form (which does not have optical properties) and killed at different intervals to assess the time course of the formation of *Hprt* mutant cells and to evaluate differences between sexes and species. These data were compared with data from rats exposed to a mixture of + and – forms of BDO_2 (which differ in how they refract light) in earlier studies by Dr. Walker, thereby assessing the role of stereoisomerism of BDO_2 in the mutagenicity of BD.

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Vernon Walker at Lovelace Respiratory Research Institute, Albuquerque, NM, and colleagues. The complete report, *Genotoxicity of 1,3-Butadiene and Its Epoxy Intermediates*, can be obtained from HEI or on our Web site (see reverse side).

Third, rodents were exposed to BD-diol (6, 18, or 36 ppm) to assess the extent and time course of *Hprt* mutant frequencies. (Because BD-diol is converted to BDO-diol, exposure to BD-diol allowed that metabolic pathway to be investigated without interference by BDO_2, which is also converted to BDO-diol.) Additional data were obtained on levels of BD-diol and other metabolites after exposure to BD (200 ppm) or BD-diol (24 or 36 ppm). The investigators developed a sensitive method to analyze BD-diol levels in plasma specifically for this purpose.

Fourth, cells derived from male rodents exposed to BD (1250 ppm), *meso*-BDO_2 (2 or 4 ppm), or BD-diol (6, 18, or 36 ppm) were cloned and propagated for molecular analyses of mutation spectra to assess whether the kinds of mutations (such as point mutations or larger deletions) differ among BD and its metabolites.

RESULTS AND INTERPRETATION

This study has provided important data on the mutagenic potency of BD at low exposure concentrations (3 ppm). In addition, Dr. Walker and colleagues confirmed and extended earlier observations that (1) female rodents are more susceptible than male rodents to BD exposure, (2) mice are more susceptible than rats to inhaled BD, and (3) rodents 4 to 5 weeks old are more sensitive to inhaled BD than animals 8 to 9 weeks old. The investigators attributed this age-related effect to differences in thymus activity and movement of T cells through the body. Dr. Walker and colleagues showed that in both species the contribution of the metabolites BD-diol and BDO-diol to mutagenicity induced by BD exposure is most prominent at high BD concentrations, whereas the metabolite BDO_2 is probably more important at lower concentrations. They found that stereochemistry did not play a role in mutagenicity induced by BDO_2; however, the in vivo role of the stereochemistry of the monoepoxide metabolites (BDO and BDO-diol) remains to be established.

Future studies should include a comprehensive analysis of metabolite concentrations, kinetics, and adduct levels, coupled with in vivo analysis of mutant frequencies. In addition to knowing each metabolite's mutagenic potency, the process of formation and elimination of metabolites in and from the body should be carefully assessed, as well as the extent to which metabolites form DNA adducts that may cause mutations or other genetic damage. In the past, several studies have investigated these issues, but mostly in isolation. A more comprehensive approach could provide the information needed to determine which metabolites are critical to the toxicity of BD and how best to extrapolate results from rodents to humans, especially at low levels of exposure (3 ppm and below).

HEALTH EFFECTS INSTITUTE

101 Federal Street, Suite 500
Boston, MA 02110, USA
+1-617-488-2300 phone
+1-617-488-2335 fax

pubs@healtheffects.org
www.healtheffects.org