



# STATEMENT

Synopsis of Research Report 92

HEALTH  
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## Cancer, Mutations, and Adducts in Rats and Mice Exposed to Butadiene and Its Metabolites

### BACKGROUND

1,3-Butadiene (BD) is an occupational and environmental pollutant that is widely used in the manufacture of resins, plastics, and synthetic rubber; it is also found in combustion emissions from motor vehicles, stationary sources, and cigarette smoke. Ambient exposures to BD (0.3 to 10 parts per billion [ppb]) are orders of magnitude lower than those that occur in occupational settings (10 to 300,000 ppb), but they are a public health concern because BD may be a human carcinogen. In the Clean Air Act Amendments of 1990, BD is listed as a hazardous air pollutant and a mobile-source toxic air pollutant. Moreover, worldwide regulatory interest has evolved in the potential health effects of occupational and ambient exposures to BD.

Epidemiologic studies have suggested that workers occupationally exposed to BD have an increased incidence of cancers of the lymphatic system and of those organs and systems in the body that produce blood cells. Interpretation of these studies has been controversial, however, because of inconsistencies among the results and because some workers may have been exposed to other chemicals in addition to BD. In situations of such uncertainty, data from animal studies are often used to fill information gaps about humans by extrapolating findings from laboratory studies in which animals have been exposed to high doses of a chemical to situations in which human subjects have been exposed to low doses. Consequently, researchers have produced a large range of human cancer risk estimates for BD depending on which animal species and which data they used in their extrapolation models.

One question is: Which species is the best model to use for assessing the human risk of cancer from exposure to BD? Butadiene is known to cause tumors in many organs in rats and mice. These two species differ markedly, however, in their carcinogenic response to BD in that mice have been found to be

about 100 times more sensitive than rats to BD exposure. Some evidence indicates this difference may relate to how rats and mice metabolize BD and convert it either to reactive (and possibly carcinogenic) intermediates or to inactive forms that are excreted from the body. Whether humans metabolize BD more like mice or more like rats is uncertain, as is where the human response to BD exposure fits in the range of rodent sensitivity to BD's cancer-causing effects.

The Health Effects Institute's air toxics research program to reduce uncertainties in estimating the human health risks associated with exposure to BD includes (1) studies of the reactivity of BD and its metabolites in animals and people and (2) the development of methods to measure biomarkers (indicators of exposure or of early biological effects of exposure) to use in cross-species studies and in studies of human populations. Five independent studies from the initial phase of HEI's BD research program are summarized in this Statement and are presented in detail in this Research Report.

### APPROACH

When HEI's program was initiated, scientists knew that BD itself is not carcinogenic. Rather, BD is transformed to reactive metabolites that can bind to DNA (forming adducts), thus causing genetic mutations and possibly initiating the carcinogenic response. The role of individual metabolites in BD-induced carcinogenesis, however, was not known. Furthermore, the metabolites exist in more than one stereochemical (or three-dimensional) form. Because enzymes may react preferentially with a specific form, these stereochemical configurations may be important in species sensitivity. Some of the products from BD reacting with cellular DNA or proteins had been identified and considered for use as biomarkers of exposure or of a biologically effective dose. However, sensitive analytical methods needed

This Statement, prepared by the Health Effects Institute, is a summary of five research projects conducted by Drs. Ian Blair (University of Pennsylvania, Philadelphia), Rogene Henderson (Lovelace Respiratory Research Institute, Albuquerque, NM), Leslie Recio (Chemical Industry Institute of Toxicology, Research Triangle Park, NC), James Swenberg (University of North Carolina at Chapel Hill), and Vernon Walker (New York State Department of Health, Albany). The complete Research Report, *1,3-Butadiene: Cancer, Mutations, and Adducts*, contains the five detailed Investigators' Reports and three Commentaries on the studies prepared by the Institute's Health Review Committee. It can be requested from HEI by phone, fax, or e-mail (see reverse side).

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to be developed and validated if these biomarkers were to be useful in animal or human studies.

The studies reported here were designed to advance our understanding of the roles of different metabolites in BD-induced carcinogenesis and of the differences in sensitivity among species, and to develop methods for identifying and measuring biomarkers. The investigators focused on two BD metabolites (1,2-epoxy-3-butene [BDO] and 1,2,3,4-diepoxybutane [BDO<sub>2</sub>]) that researchers had suspected may play a role in BD carcinogenesis; they also developed information on other metabolites that may be important but had not been extensively studied.

Dr. Rogene Henderson (Lovelace Respiratory Research Institute) exposed mice and rats to BDO<sub>2</sub> to determine whether these species differ in their carcinogenic response to this metabolite. Dr. Leslie Recio (Chemical Industry Institute of Toxicology) and Dr. Vernon Walker (New York State Department of Health) compared the mutagenicity of BD, BDO, and BDO<sub>2</sub> in mice and rats. Dr. Ian Blair (University of Pennsylvania) developed methods for measuring DNA adducts derived from BD metabolites in the tissues and urine of rats and mice with the goal of comparing the levels of adducts in the two species and identifying possible biomarkers. Dr. James Swenberg (University of North Carolina at Chapel Hill) developed a sensitive method for detecting adducts formed between BD metabolites and a blood protein (hemoglobin) and measured these adducts in animals and humans exposed to BD. The investigators shared tissues from animals that were exposed by inhalation to BD or its metabolites at either the Chemical Industry Institute of Toxicology or Lovelace Respiratory Research Institute and, in some cases, developed collaborative ventures.

### RESULTS AND IMPLICATIONS

These five studies have advanced our understanding of BD metabolism, genotoxicity, and carcinogenicity. Some of the most noteworthy accomplishments were

- direct demonstration that BDO<sub>2</sub> is carcinogenic in both rats and mice;
- corroboration of earlier findings that BD and its metabolite BDO have greater mutagenic potencies

and form more adducts in mice than in rats, which agrees with the different sensitivities the two species exhibit in BD-induced carcinogenesis; however, one study found that BDO<sub>2</sub> was more mutagenic in rats than in mice;

- demonstration that each BD metabolite tested produces a characteristic pattern of mutations in rodent DNA; these patterns show potential as biomarkers for human exposure to BD; and
- development of sensitive and specific methods for measuring biomarkers of BD exposure (hemoglobin and DNA adducts derived from BD metabolites) and preliminary validation of using hemoglobin adducts as biomarkers in animals and humans.

The investigators successfully answered many of the questions they set out to address; some unexpected results will require further research to understand the range of their implications. Some avenues of needed research include

- the roles of specific BD metabolites (including their various stereochemical forms) in the carcinogenicity, genotoxicity, and cytotoxicity of BD;
- the relative importance of point mutations and large genetic deletions in BD carcinogenicity, and the forms of the promutagenic lesions;
- the dose-response relations among BD exposure concentrations and the levels of biomarkers (especially the limits of detection relative to ambient exposure conditions and the sources of background or endogenous adducts); and
- if or how the different biomarkers are indicators of cancer risk.

Some of these questions are being addressed in an HEI-supported study led by Dr. Richard Albertini, which is being conducted in the Czech Republic. The goal of that effort is to determine whether a dose-response relation can be established between the level of an individual's exposure and the amount of various biomarkers detected in blood or urine samples from workers exposed to BD.