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A Partnership to Examine Emerging Health Effects: EC/HEI Workshop on 1,3-Butadiene

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Workshop

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INTRODUCTION

As part of its ongoing work to protect health and the environment, Directorate General XI (DG XI) of the European Commission (EC) is charged with proposing legislation to control potentially harmful effects from pollutants. The Commission increasingly has moved to carry out this responsibility by seeking to involve a broad range of stakeholders at different stages of the process.

The Health Effects Institute (HEI) is an independent research organization jointly and equally funded by industry and government to provide independent science on the health effects of air pollution to inform potential regulation.

The materials that follow are the proceedings from a workshop entitled *A Partnership to Examine Emerging Health Effects: EC/HEI Workshop on 1,3-Butadiene,* held in Brussels, Belgium, on 29–30 June 1998. This was the first in a series of collaborative efforts between the Health Effects Institute, the Commission, and others. The workshop brought together leading European and U.S. researchers funded by Directorate General XII and HEI with representatives of the European Parliament, the World Health Organization, the International Agency for Research on Cancer, the U.S. Environmental Protection Agency, member states, local authorities, industry, nongovernmental organizations, and multiple directorates within the Commission in an open and transparent dialogue to examine underlying science relevant to potential regulation.

Although butadiene is not currently on the list of highest priority pollutants, it was chosen as the subject of the workshop because of recent research results concerning the potential carcinogenicity of butadiene and their possible implications for public health, and because of the request from several member states that butadiene have a higher priority in evaluative and regulatory processes. This workshop was intended as one step to help inform DG XI's prioritization decisions, and to help inform future research priorities for the Health Effects Institute and Directorate General XII of the European Commission.

HEI wishes to thank the many diverse interests from within and outside government and the scientific community who contributed to this effort.

EXECUTIVE SUMMARY

EC/HEI Workshop on 1,3-Butadiene

Background

1,3-Butadiene (BD) is a chemical used in the manufacture of rubber. It is detectable in urban and suburban air at concentrations ranging from 0.3 ppb to 10 ppb (1 ppb butadiene is equivalent to 0.451 $\mu g/m^3$), and can be found near industrial sites at concentrations of up to 30 ppb. BD is also found in cigarette smoke and motor vehicle exhaust. A large epidemiologic study found a positive association between employment in the styrene-BD rubber industry and the development of leukemia. In contrast, a study of BD production workers did not show an association with leukemia, although there was evidence for increased incidence of lymphoma. In addition, several studies have shown that BD and its metabolites can cause genetic damage, such as increased mutations and chromosomal aberrations, while some studies have not.

Because of the paucity of data about BD's effects in humans and its possible mechanisms of action, scientists have studied these issues in animals. One complication, however, is that the carcinogenicity of BD in animals appears to vary with species. For example, mice develop cancers at multiple sites, including the lung, liver, and heart, with lung tumors increasing in females after chronic exposures to levels as low as 6.25 ppm BD. Life-shortening lymphocytic lymphomas predominate after exposures to levels of BD of 625 ppm or higher. In contrast, rats develop cancer in endocrine organs and only after chronic exposures to high levels of BD (1,000 or 8,000 ppm). Species differences in BD effects may be the result of differences in metabolizing BD to active intermediates: studies have shown that different animal species have different levels of BD metabolites in blood and urine. Notably, the highly mutagenic metabolite 1,2,3,4-diepoxybutane (BDO_2) is formed in much larger amounts in mice than in rats. **Thus, because it is not clear whether people are more similar to rats or mice in their response to BD, the human risk for cancer from BD cannot easily be extrapolated from information from rodents.**

TERMINOLOGY

Systematic name and abbreviation favored in this document	Other names and abbreviations used in this document
1,3-butadiene (butadiene), BD	—
1,2-epoxy-3-butene, BDO	epoxybutene (EB), 1,2-epoxybutene, butadiene monoepoxide, 1,3-butadiene monoepoxide (BMO)
1,2,3,4-diepoxybutane, BDO_2	butadiene diepoxide, diepoxybutane (DEB)
1,2-dihydroxy-3,4-epoxybutane, BDO-diol	3,4-epoxy-1,2-butanediol, butanediol epoxide (BDE), butadiene monoepoxide diol, epoxybutanediol

Other compounds referred to in the text

M I	=	glutathione conjugate of BDO
M II	=	glutathione conjugate of BDO-diol
Isoprene	=	2-methyl-1,3-butadiene

Human Exposure

Determining the actual level of human exposure to BD is critical to assessing its effects and, ultimately, its risk to people. In the urban atmosphere, the primary source of BD is motor vehicle emissions. There are seasonal patterns of BD exposure; for example, atmospheric concentrations of BD are higher in winter than summer. Most areas of the United Kingdom—one of the few countries in the world with an ambient BD monitoring network—are well below 1 ppb as an annual running average, the level specified in the U.K.'s ambient air quality standard, although areas adjacent to busy roadways occasionally may exceed the standard. **Future emissions of BD from mobile sources are expected to decrease.** Available data suggest diesel engines may emit relatively more BD than gasoline-fueled vehicles. Much of this information is based on older diesel technology and fuels, however, and may not reflect the current fleet or potentially cleaner fuels.

Butadiene Metabolism

In the body, BD is metabolized by a series of enzymes, including cytochrome P450–dependent monooxygenases, to reactive epoxide metabolites. Detoxification of these intermediates occurs through enzymes such as epoxide hydrolase and glutathione S-transferase. **The rates and extent of these enzymatic conversions differ greatly among species.** Epoxide metabolites of BD—including 1,2-epoxy-3-butene (BDO), 1,2-dihydroxy-3,4-epoxybutane (BDO-diol), and BDO_2—can react with DNA and protein such as hemoglobin, and are mutagenic in in vitro assays. BDO_2 is the most potent mutagen of the metabolites examined and is carcinogenic when painted on mouse or rat skin. The carcinogenicity of BDO when applied through skin painting on rodents was equivocal. Mice metabolize BD several times faster than rats. The conversion of BD to BDO, or of BDO to BDO_2, by lung and liver microsomes occurs faster in mice than in humans and rats. Conversely, the elimination of BDO and BDO_2 through action of liver microsomal epoxide hydrolase is fastest in humans and slowest in mice. Detoxification through glutathione S-transferase occurs fastest in mice and slowest in humans. Pharmacokinetic models predict that humans will have lower levels of BDO and BDO_2 as compared with the levels in rodents following equivalent exposures to BD. Furthermore, examining adducts between hemoglobin and BDO indicate there is a low body burden of BDO in humans compared with the level in mice or rats under similar conditions. Thus, it is clear that there are differences in how mice, rats, and humans metabolize BD. The exact nature of these differences is emerging in current research, but is not yet fully understood.

Butadiene Genotoxicity

The three epoxide metabolites of BD—BDO, BDO_2, and BDO-diol—are all able to react covalently with proteins and DNA, and thus have the potential to cause genetic damage. BD is mutagenic in various test systems, including in vitro exposures of prokaryotes and eukaryotes supplied with a metabolism element and in vivo exposures of rats and mice. As with cancer induction and metabolism, there are differences among species in the mutagenicity of BD. For example, inhaled BD is 5-fold more potent in causing mutation in mice than it is in rats. The magnitude of the difference between rats and mice, however, is much lower than would be expected based on metabolism differences. Of the three epoxide metabolites, BDO_2 is the most potent, causing mutations at concentrations 40- to 400-fold lower than mutagenic concentrations of BDO or BDO-diol. BDO_2 has also been shown to cause deletions within the DNA of exposed cells or animals.

When mutations and chromosomal changes are examined in people occupationally exposed to BD, some studies show increases for exposed workers but others do not. These findings may be explained, in part, by methodological differences. Differences in metabolic capability in individual people may influence whether they are susceptible to genetic effects of BD.

In the mouse, BD is a potent clastogenic agent, producing chromosome breaks and aberrations. These aberrations can be seen in cells present in many organs of inhalation-exposed animals, including bone marrow, spleen, lung, and testis. Although the clastogenic effectiveness of BD metabolites varies across organs and species, BDO_2 is the most potent metabolite, followed by BDO and then BDO-diol. The consequences of clastogenic action by BD in the mouse appears to include heritable translocations. BD and metaholites may also interfere with chromosome segregation. In contrast, BD is not clastogenic in the bone marrow of rats, nor does it induce dominant lethal mutations. When cells of humans exposed to BD are examined, evidence of clastogenicity is observed in lymphocytes.

The clastogenic potency of BD and its metabolites is modulated by glutathione S-transferase T1, an enzyme involved in the detoxifying

pathway. Some people do not express this enzyme. These individuals, when exposed to BD, have a greater number of cells showing sister-chromatid exchanges and chromosomal aberrations than BD-exposed people who do express this enzyme.

Epidemiologic Studies of Butadiene

The epidemiologic data on BD are not completely consistent. The most informative data are from a large, carefully conducted study of exposed workers involved in the manufacture of styrene-BD rubber, where an exposure-related increase in the risk of leukemia is observed. The association of BD with increased incidence of other lymphohematopoietic neoplasms is inconsistent among different studies of this cohort. The styrene-BD rubber workers were also exposed to chemicals other than BD. Results from the only other large cohort study, among BD production workers, showed a small but significant increase in lymphoma. The incidence of lymphoma in this cohort was inversely proportional to the length of employment.

Risk Characterization, Risk Assessment, and Regulation of Butadiene

Potential hazards from exposure to BD include cancer, heritable mutation, and reproductive endpoints. There are still questions about whether the appropriate exposure metric should be cumulative or peak exposures, as well as questions about which metabolites are of concern, what the mechanism of toxicity is, what the dose-response relationship is, and whether quantitative extrapolation should start from rat or mouse data.

A crucial issue is whether BD is a human carcinogen. This assessment is the starting point for both regulatory action and further discussion of science and policy issues. Demonstration of carcinogenic activity in a nonhuman species is not necessarily sufficient to establish evidence of human carcinogenicity. Epidemiologic evidence, however, can be sufficient to define an agent as a known human carcinogen. In the absence of definitive epidemiologic data, an agent may still be classified as a known human carcinogen if it has been shown to be carcinogenic in a nonhuman species, and if there is supporting information showing that the biological mechanisms involved may be similar in people.

A number of national and international agencies are currently undertaking risk assessments for BD, attempting to interpret the existing data to determine whether BD is a human carcinogen and how potent it might be.

Within the **European Union (EU)**, there has been a risk assessment under the Existing Substances Regulation with the **U.K. Health and Safety Executive** acting as the rapporteur for the rest of the EU. The U.K. regulatory agency has concluded that, although uncertainties still exist about risk, there is concern, and exposure should be controlled to the lowest practical level. BD is therefore classified as a Category 2 carcinogen (substances that should be regarded as if they are carcinogenic to humans).

The **U.K. Department of Environment** established an air quality standard for BD in the U.K. of 1 ppb as a running annual average. EC Directorate-General V, through their Scientific Committee on Occupational Exposure Limits (SCOEL), are currently determining appropriate workplace exposures for the EU.

The **International Agency for Research on Cancer (IARC)** recently met to discuss the classification of BD, which had previously been classified as a "probable human carcinogen." After reviewing the evidence, IARC has continued to classify BD as a "probable human carcinogen" (IARC Class 2A), and other agencies—such as the EC Directorate-General XI, Health Canada, and the U.S. Environmental Protection Agency (U.S. EPA)—are currently considering the issue.

The U.S. Environmental Protection Agency classified BD as a "probable human carcinogen" in 1985, and a change to "known human carcinogen" is currently being considered. In addition to a quantitative assessment for the risk of cancer, the U.S. EPA has also estimated noncancer risks. Under the U.S. Occupational Safety and Health Act, occupational limits to BD are set at 1 ppm as a permissible exposure limit (time-weighted average), 5 ppm as a short-term exposure limit (15 minutes), and 0.5 ppm as an action level. The U.S. National Institute for Environmental Health Sciences has listed BD as a "known human carcinogen."

Health Canada is in the process of completing a health assessment for BD as required for Priority Substances under the Canadian Environmental Protection Act. The agency is considering a classification of "highly likely to be carcinogenic in humans" in this health assessment, and has included a calculation of cancer potency as derived from human and animal data as well as a benchmark concentration for noncancer health effects.

Research Needs

Additional research is needed in three areas. Studies to clarify the epidemiologic data will help determine the contribution of confounding exposures and allow extension of the results from

the rubber industry to other industries. Further studies on cross-species comparisons should include research aimed at a better understanding of metabolism pathways in different species. Finally, additional research is needed to identify the active carcinogenic metabolite in different species, and to determine whether this metabolite is formed in appreciable amounts in people. **Research in these areas may produce definitive results in a relatively short span of time.**

Conclusions

- Exposure to BD, as measured in the U.K., is generally well below 1 ppb as an annual average and is decreasing.
- BD is species-specific in its actions.
- Human risk for cancer from BD cannot necessarily be extrapolated from rodents without further understanding of the mechanisms involved.
- There is a need to understand how humans metabolize and respond to BD.
- Some people may be similar to rats and some people may be similar to mice in their metabolic response to BD.
- There is epidemiologic evidence for the carcinogenicity of BD, but further studies are needed to determine human variability in response to BD and the effects of confounding exposures on that response.
- With more research, many of the questions raised could be resolved in a few years.

AGENDA

A Partnership to Examine Emerging Health Effects:

EC/HEI Workshop on 1,3-Butadiene

Brussels, 29–30 June 1998

Day One: 29 June 1998
(Morning Chair: Prudencio Perera, European Commission, DG XI)

10:00 am	Welcome Prudencio Perera (European Commission, DG XI) Canice Nolan (European Commission, DG XII) Daniel Greenbaum (Health Effects Institute)
10:15 am	The DG XI Approach to Air Toxics and 1,3-Butadiene Lynne Edwards (European Commission, DG XI)
10:30 am	Introduction to 1,3-Butadiene Rogene Henderson (Lovelace Respiratory Research Institute, USA)
11:00 am	Ambient Concentrations of 1,3-Butadiene in the UK Geoffrey Dollard (AEA Technologies, UK)
11:30 am	Discussion
12:00 pm	Lunch Break

(Afternoon Chairs: Canice Nolan, European Commission, DG XII; and Daniel Greenbaum, HEI)

1:15 pm	Metabolism of 1,3-Butadiene Johannes Filser (GSF Institute of Toxicology, Germany)
2:00 pm	Genotoxicity of 1,3-Butadiene Richard Albertini (University of Vermont, USA) Francesca Pacchierotti (ENEA, CRE Casaccia, Italy)
3:15 pm	Epidemiology of 1,3-Butadiene Paolo Boffetta (IARC, France)
4:00 pm	Introduction to Poster Session Daniel Greenbaum (HEI)
4:10 pm	Break

4:20 pm Poster Session of Butadiene Research and Informal Discussion
European and United States Investigators:
Richard Albertini, University of Vermont, USA
Diana Anderson, BIBRA International, UK
Ian Blair, University of Pennsylvania, USA
James Bond, Chemical Industry Institute for Toxicology, USA
Johannes Filser, GSF Institute of Toxicology, Germany
Rogene Henderson, Lovelace Respiratory Research Institute, USA
Gunter Neumann, University of Würzburg, Germany
Francesca Pacchierotti, ENEA, CRE Casaccia, Italy
Kimmo Peltonen, Finnish Institute of Occupational Health
Leslie Recio, Chemical Industry Institute for Toxicology, USA
Radim Šrám, Academy of Science of the Czech Republic, Czech Republic
James Swenberg, University of North Carolina at Chapel Hill, USA
A. D. Tates, University of Leiden, The Netherlands
Vernon Walker, New York State Dept. of Health, Wadsworth Center, USA

6:15 pm Adjourn for day

Day Two: 30 June 1998
(Chair: Daniel Greenbaum, HEI)

9:30 am Summary of Day One and Issues to Consider for Regulating Risks from 1,3-Butadiene
Seymour Garte (New York University, USA)

10:30 am Panel: Butadiene Risk Assessment in the Regulatory Framework
Michael Penman (Exxon Europe, UK; ECETOC)
Sharon Munn (European Chemicals Bureau, Joint Research Council)
Isla Brooke (UK Health and Safety Executive, UK)
Aparna Koppikar (Environmental Protection Agency, USA)
Kathryn Hughes (Health Canada)

12:00 pm Lunch

1:30 pm Panel: The Broader Picture: Setting Priorities Among Chemicals
Gail Charnley (The Weinberg Group, formerly Presidential/Congressional Commission on Risk Assessment and Risk Management, USA)
Robert Maynard (Department of Health, UK)
Rolaf van Leeuwen (World Health Organization, The Netherlands)

2:30 pm Summary and discussion

3:00 pm Adjourn

EXTENDED ABSTRACTS OF OVERVIEW TALKS ON BUTADIENE

Speakers presented summaries for a number of topics, including exposure to BD, metabolism of BD, and toxicology and epidemiology evidence for genetic damage or cancer caused by BD. These talks provided an overview for the audience, highlighting both what is currently known about BD and where uncertainties lie. The following section includes extended abstracts for these summary talks as well as copies of overheads presented.

1,3-BUTADIENE: INTRODUCTION TO THE PROBLEM

R. F. Henderson, PhD

Lovelace Respiratory Research Institute

Lovelace Respiratory Research Institute
Albuquerque, New Mexico, USA

1,3-BUTADIENE (BD)

A gas at room temperature

$CH_2 = CH - CH = CH_2$

BP –4.4°C

MP –109°C

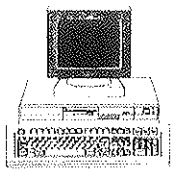
BD is a Monomer Used in Large Tonnage Throughout the World to Produce Polymers for Rubber and Plastics

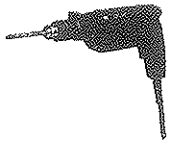
- Butadiene rubber
- Styrene rubber
- Adiponitrile
- Polychloroprene
- Nitrile rubber
- Styrene butadiene latex
- Acrylonitrile-butadiene-styrene polymers

PRODUCTS MADE FROM 1,3-BUTADIENE

Tires

Plastics





DETECTED IN

- Suburban air (0.3–1.0 ppb)
- Urban air (1–10 ppb)
- Air around industrial sites (19-30 ppb)
- Cigarette smoke (~400 µg/cigarette)
- Gasoline engine exhaust (20–60 ppb)

RODENT BIOASSAYS

Because BD was used in such large amounts, rodent bioassays were conducted to determine if chronic (2-yr) exposure to BD would cause cancer or major non-cancer health effects in rats and mice.

RODENT BIOASSAY - RATS

BD is a weak carcinogen in S/D rats. Exposures were to 1,000 or 8,000 ppm BD for 105-111 weeks.

	1,000	8,000
Pancreatic exocrine adenoma (♂)		+
Uterine sarcoma (♀)		+
Zymbal's gland carcinoma (♀)		+
Thyroid follicular tumors		+
Testicular Leydig-cell tumors (♂)		+
Mammary tumors (♀)	+	+

BIOASSAY IN MICE

BD is a potent multiple-site carcinogen in B6C3F$_1$ mice. In first bioassay, mice were exposed for 61 weeks to 625 or 1,250 ppm BD.

- Increased incidence of:
  - Hemangiosarcoma of the heart
  - Malignant lymphoma
  - Alveolar/bronchiolar adenoma and carcinoma
  - Mammary gland and ovary carcinomas
- Stopped assay at 61 weeks due to deaths
- Major cause of death:
  - Lymphocytic lymphoma

SECOND BIOASSAY

Exposed Mice to 6.25, 20, 62.5, 200, and 625 ppm BD

- Increased hemangiosarcoma of the heart in males at 62.5 ppm or higher
- Increased alveolar-bronchiolar neoplasms
  - In males at 62.5 ppm or higher
  - In females at 6.25 ppm or higher
- Lymphocytic lymphomas were major cause of death at 625 ppm

NON-CANCER HEMATOTOXICITY IN RODENT BIOASSAYS

- $B6C3F_1$ Mice
  - Macrocytic-megaloblastic anemia
- S/D Rats
  - None observed

DILEMMA

- In the absence of human data, rodents were used to estimate the health risks for humans exposed to BD.
- The results indicate that rats respond differently to BD exposure than do mice

How can the animal data be used to predict the risk of exposure to BD for humans?

Irons Suggested That Sensitivity of Mice to Lymphocytic Lymphoma was Due to Activation of Endogenous Retrovirus

Exposed B6C3F$_1$ and NIH Swiss mice to 1250 ppm BD for up to 1 year

NIH Swiss Mice: No intact endogenous ecotropic retrovirus sequences, do not express ecotropic retroviruses, and rarely express any type of endogenous retrovirus.

B6C3F$_1$ Mice: Leukemogenic retroviruses isolated from parent strains of this hybrid.

Irons *et al.*, *TAP 101*: 170--176, 1989

RESULTS

	NIH Swiss	B6C3F$_1$
Macrocytic anemia	+	+
Micronuclei	+	+
Chromosomal aberrations in bone marrow cells	+	+
Recovery of ecotropic retrovirus from bone marrow, thymus, spleen	–	+++
Thymic lymphoma/leukemia	14%	57%

Could The Species Differences Be Due To Differences In How The Rats And Mice Metabolize BD?

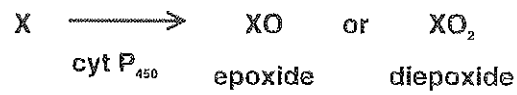
METABOLISM

Food stuff: The body breaks down food to obtain energy and building blocks for tissues

Foreign organic chemical: The body breaks down the organic chemical in a 2-step process that leads to greater water solubility and excretion in the urine.

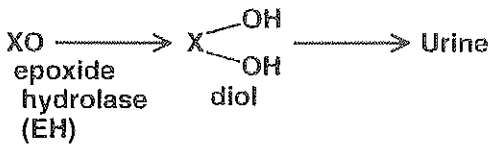
First Step

Oxidize the chemical to make it reactive



Second Step

Hydrolyze the reactive metabolite or bind it to other molecules to make it water soluble and readily excretable in the urine



This process works well except
sometimes the reactive metabolite, before it is made water soluble in the second step, attaches itself to major molecules in the body.

XO + DNA ⟶ altered DNA
(modified DNA may lead to cancer)

XO + protein ⟶ protein adduct

(Modified protein is not a health problem because the body can make more protein. But the protein adducts can serve as indicators of exposure.)

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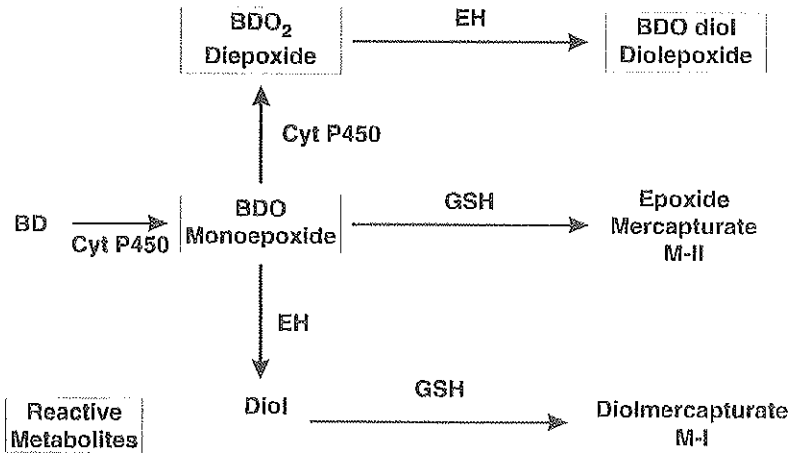
MAJOR METABOLIC PATHWAYS OF 1,3-BUTADIENE (BD)



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MAJOR METABOLIC ENZYMES

Cyt P_{450}	–	Cytochrome P_{450} (activating enzyme)
EH	–	Epoxide hydrolase (inactivating enzyme)
GST	–	Glutathione S-transferase (inactivating enzyme)

GLOSSARY

BD	–	1,3-butadiene
BDO	–	Butadiene monoepoxide
BDO_2	–	Butadiene diepoxide
BDO diol	–	The epoxydiol of 1,3-butadiene

GLOSSARY (Con.)

Epoxide	–	A reactive metabolite
Diol	–	Hydrolyzed or inactivated epoxide
Diepoxide	–	Metabolite with two reactive sites
Epoxy diol	–	Metabolite with one reactive site and one inactivated site

3723W-21

MAJOR METABOLITES IN SPECIES TESTED

Mice	Blood:	BDO, BDO_2, BDO diol (?)
	Urine:	M-I, M-II
Rat	Blood:	BDO, BDO diol (?)
	Urine:	M-I, M-II
Monkey	Urine:	M-I (M-II after exposure to high concentrations of BD)
Humans	Urine:	M-I (occasional person with M-II)

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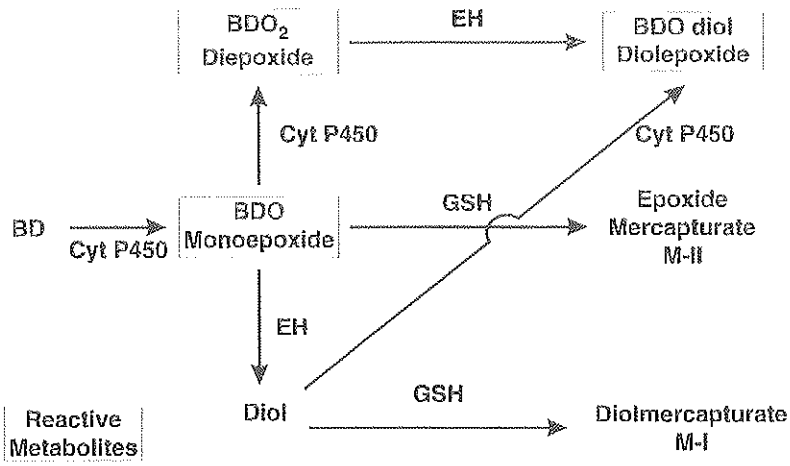
MAJOR METABOLIC PATHWAYS OF 1,3-BUTADIENE (BD)



STUDIES OF GENOTOXICITY IN HUMANS

These studies have led to mixed results. Some studies report increases in mutations, chromosomal aberrations, and sister chromatid exchanges in exposed workers while other studies report no increases.

What Evidence of Toxicity Can Be Found in Workers Exposed to BD, Especially Those Heavily Exposed During World War II?

The most extensive epidemiology study was recently reported by Delzell *et al.* (1995). A positive association was found between employment in the styrene–butadiene rubber industry and all leukemia cell types).

What Additional Information is Needed for Assessing Health Risk Associated with BD Exposure?

- Initiate prospective epidemiology studies
- Determine variability within the human population in response to BD
- Determine extent of human formation of BDO_2
- Determine extent to which the BDO diol is involved in BD toxicity

Ambient Concentrations of 1,3- Butadiene in the UK - Extended Abstract

Geoff Dollard
AEA Technology Environment
National Environmental Technology Centre
Culham
Abingdon
Oxfordshire
United Kingdom

Summary

This presentation contains data on the ambient concentrations of 1,3-butadiene from the UK National Automatic Hydrocarbon Monitoring Network.

Background to the origins of the network is given along with brief details on recent national inventory estimates for emissions of 1,3-butadiene. A brief review of the network configuration and operations is given along with information on the UK Air Quality standard for 1,3-butadiene.

Examples of concentration data and plots are given to illustrate the range and variability of ambient 1,3 butadiene data at rural, urban background and roadside locations.

Introduction

The automatic monitoring network for hydrocarbons began following the publication of a Government White Paper in 1990 - 'This Common Inheritance'. This signalled a great expansion of air pollution monitoring - particularly in urban areas, including inorganic and organic pollutants. The organic measurements included 26 hydrocarbon compounds within the range C_2 to C_8. These were of interest as photochemical ozone precursors and for 2 specified key species - benzene and 1,3-butadiene, for their potential harmful effect on human health.

The 1,3-butadiene emissions inventory for the UK is dominated by motor vehicle sources (Figure 6); the butadiene is generated during the combustion process and 1,3-butadiene does not have any fuel evaporative component. There are localised industrial emissions where it is handled in bulk. Total emissions for 1995 from all sources in the UK were 9570 tonnes with 67% coming from petrol vehicles and 11% diesel.

The Monitoring Network

The network was set up in 1992/93 and now has 13 sites located in major city locations across the UK. The sites are primarily located at urban background locations but included are 2 roadside locations and 1 rural site.

Figure 7 illustrates equipment installed at each site. The main measurement device is a continuous cycling gas chromatograph with a liquid nitrogen - cooled trapping system. Gases are supplied from a hydrogen generator, air compressor and helium cylinder. Liquid nitrogen is held in a large (500 litre container). At each site the system is set to collect and analyse an air sample every hour throughout the year. The air sample is taken from a high volume sample line directly to the analyser. A PC and modem link allow remote access and hourly collection of data remotely. All the chromatographic files are processed on-line to generate provisional concentration data within 1 hour of collection. Data on benzene and 1,3-butadiene are sent out to TV Teletext services and the Internet for supply of information to the public in the context of the national standard. Each site collects over 8000 samples annually. All of the data collected, and other information, are available on the world wide web at the address given at the end of this article.

Each site is provided with a 26 component calibration standard supplied by the National Physical Laboratory. A rigorous QA/QC procedure is applied to the network giving a carefully calibrated and reliable dataset.

The UK Standard for 1,3-Butadiene

Figure 9 illustrates the background to the standard which has been set at 1 ppb as a running annual mean (Figure 10).

Ambient Data

Figure 11 illustrates data for 6 year periods at one of the roadside sites, this one located in London. The 1998 data are distinct because the plot only includes data up to the end of May 1998. There is a consistent pattern over the day for the different years. This plot effectively shows the annual (hourly) mean for each hour of the day. The morning and afternoon peaks show the impact of rush hour traffic movements and the dip between effect of atmospheric mixing/ dispersion reducing concentrations. This illustrates a typical diurnal pattern for a pollutant originating from motor vehicles.

Figure 12 shows daily mean data over several years at the Birmingham site. Clear seasonal behaviour is evident with lower summer-time and higher winter-time concentrations. This pattern will be driven by seasonal differences in meteorology (mixing in the surface boundary layer) and different rates of chemical destruction of 1,3-butadiene in the atmosphere due to reaction with OH free radicals.

Figure 13 summarises monthly mean data and the maximum hourly values recorded at Birmingham during 1995, 1996 and 1997. It can be seen that maximum hourly values at this (typical) site can be up to 7 ppbv.

Figure 14 illustrates the behaviour of the rolling annual mean at 3 sites. This plot summarises over 40,000 hourly measurements collected at each of the 3 sites over the period. It is evident that the running mean is well below the 1 ppb level at which the standard is set. the London site is the highest as this is a roadside site and closer to emissions than is the case for the two other sites which are in urban background locations.

Figure 15 illustrates data for the site in Middlesbrough, which again is located at an urban background location, but which can be influenced by industrial emissions of butadiene. The running mean is well below the 1 ppb standard. it is interesting to note the large perturbation to the value in mid 1995.

Figure 16 shows a more detailed analysis of the period and shows hourly data, (along with the daily mean) for the period of the incident. It is evident that the ambient values climbed to very high levels compared to the normal concentrations recorded at this site. The maximum value exceeding 70 ppb. This was clearly a non motor vehicle source and was eventually tracked down to an emission following tank cleaning operations on a bulk hydrocarbon transporter at a nearby handling facility. These events are recorded relatively infrequently, but they perhaps indicate the need to be aware of impacts of such emissions on populations close to, or down wind to such sources.

Because emissions, of 1,3-butadiene are dominated by motor vehicle sources, a good understanding of the likely future concentrations of it in the atmosphere may be obtained from forecasts based upon traffic growth and impact of emission control legislation. Figure 17 shows the trend in emissions of 1,3-butadiene over the period 1970-2025. This is based upon emission factors for the types of vehicles currently on the road at average speeds for urban and motorway roads. The emission factors are scaled down according to emission standards currently being set in European legislation. Reductions due to planned changes in fuel quality are included. The latest national traffic forecast has been utilised, it assumes a 20% level of diesel car sales remains the same and 5% of catalysts fail each year, with repair after the vehicle is 3 years old.

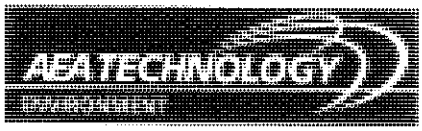
The plot shows the impact of legislation on the national total emissions. It is to be expected that as emissions reduce the ambient concentrations data will decline further from the levels reported here.

WWW address:

http://www.aeat.co.uk/netcen/airqual

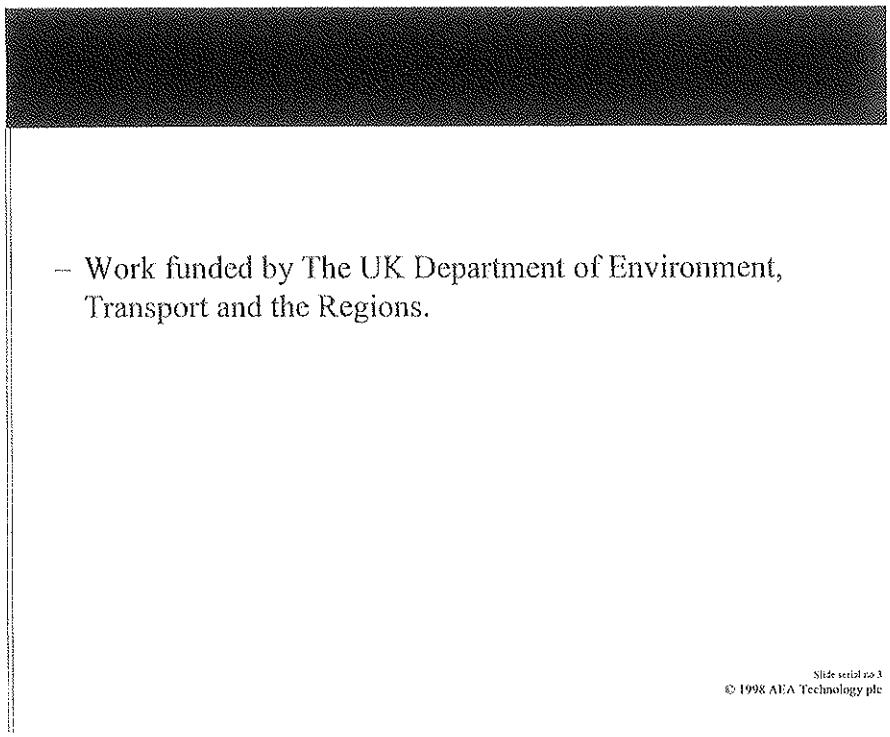
Ambient concentrations of 1,3 butadiene in the UK.

Geoff Dollard



- Background to the measurement programme
- Butadiene emissions in the UK
- Brief review of network operations
- The UK standard for butadiene
- Some examples of the data - trends and spatial distribution

Slide serial no 2


– Work funded by The UK Department of Environment, Transport and the Regions.
Slide serial no 3
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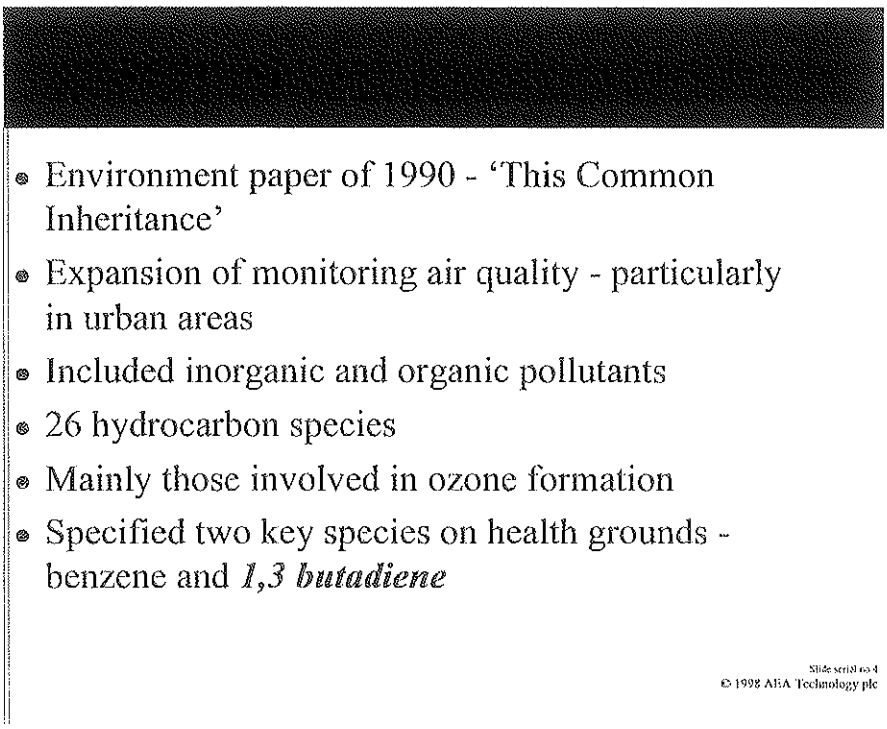
• Environment paper of 1990 - 'This Common Inheritance'
• Expansion of monitoring air quality - particularly in urban areas
• Included inorganic and organic pollutants
• 26 hydrocarbon species
• Mainly those involved in ozone formation
• Specified two key species on health grounds - benzene and *1,3 butadiene*
Slide serial no 4
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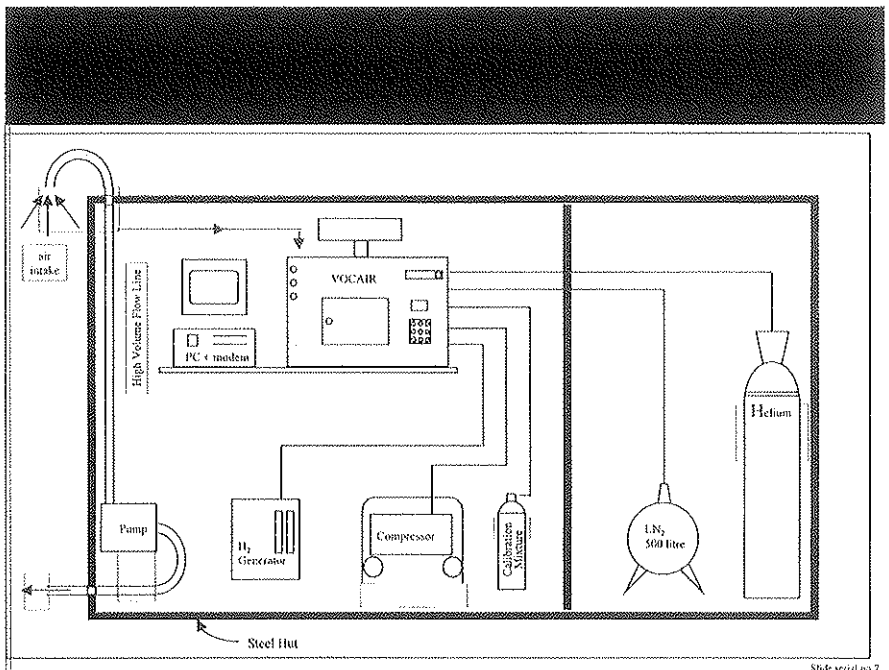
- Dominated by motor vehicle exhaust emissions - *generated during combustion*
- Localised industrial emissions where it is handled as a bulk chemical

Slide serial no 5

Source	Emission	Percentage
Petrol Vehicles	6 390	67
Diesel Vehicles	1 030	11
Feedstock	610	6
Manufacture	630	7
Gas leakage	400	4
Landfill	510	5
TOTAL	9 570	100

Slide serial no 6



Slide serial no 7

- Continuous gas chromatography
- Hourly values reported
- Data disseminated 1 hour after collection
- Rigorous QA/QC regime applied to sites and data
- Giving a carefully-calibrated and reliable dataset extending back several years

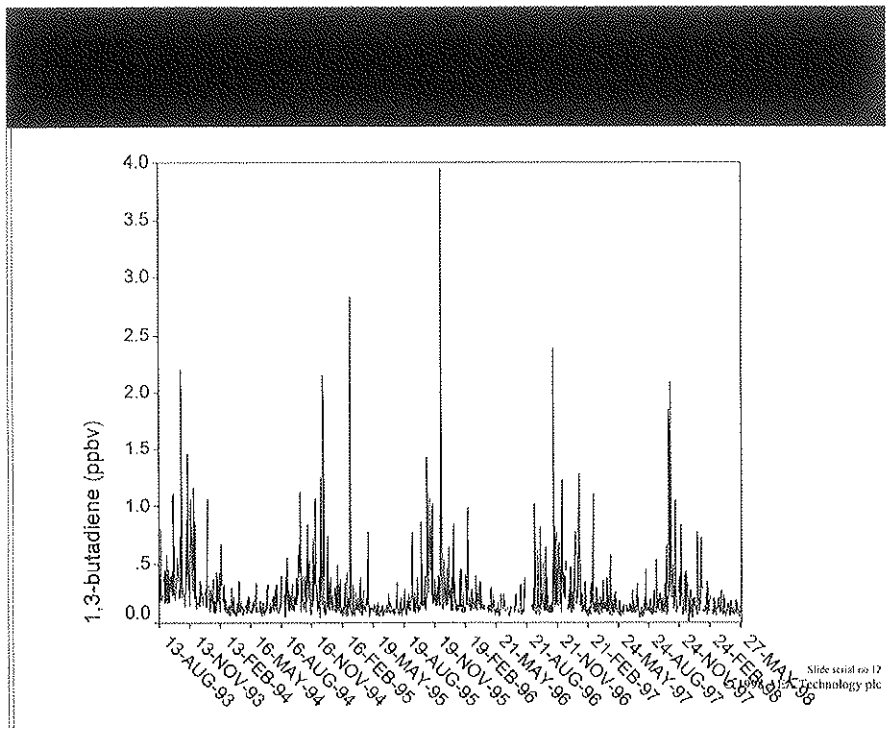
Slide serial no 8

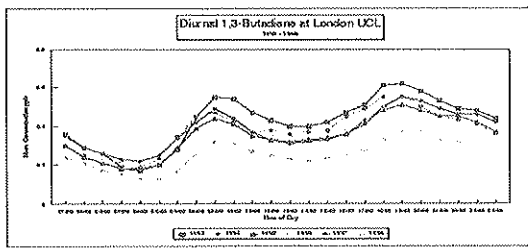
- Recommended by an expert review group (EPAQS) in 1994
- Group composed of medical and air pollution experts
- Butadiene considered a genotoxic carcinogen and in theory it is not possible to determine an absolutely safe level for human exposure
- Based opon the best information available the group recommended the standard
- Standard adopted within the National Air Quality Strategy in 1997

Slide serial no 9

- A concentration in air of 1 part per billion, (*1 part of butadiene in 1 000 000 000 parts of air*).
- Expressed as an annual running mean.

Slide serial no 10





Slide serial no 11
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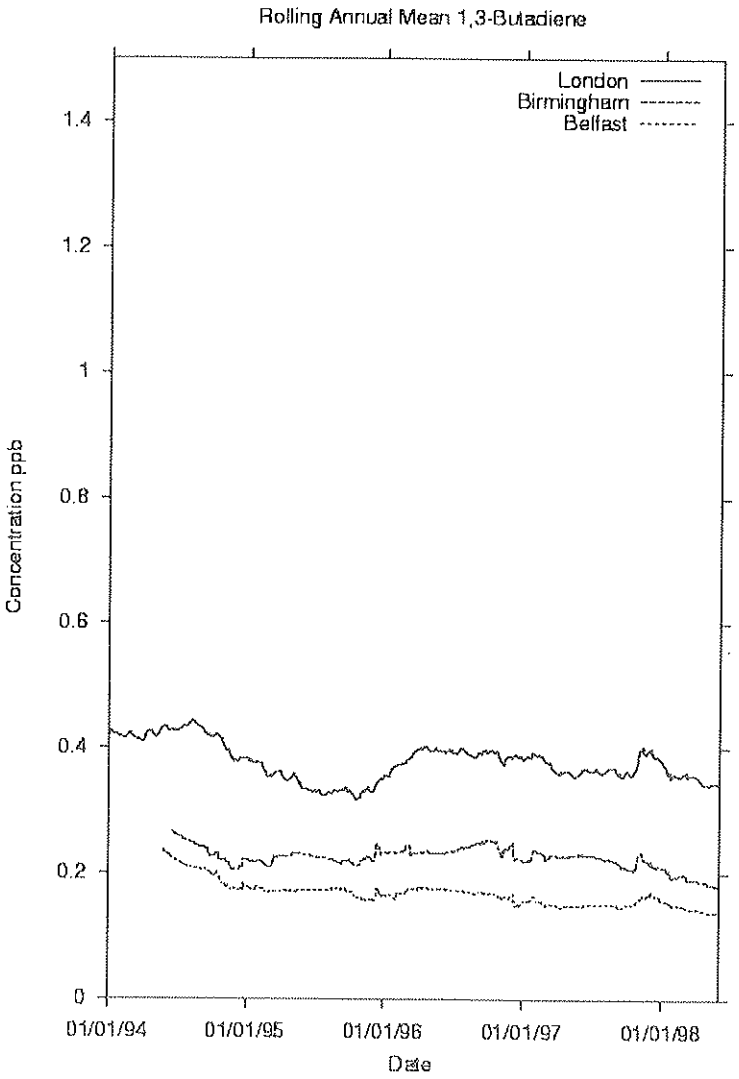
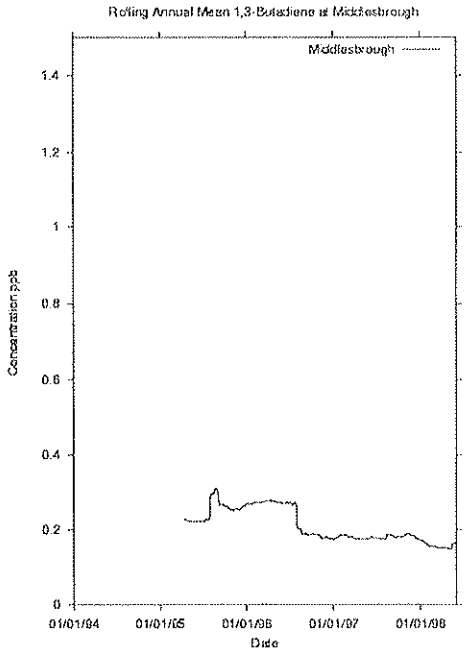
Month	Mean ppb	Hourly Max ppb	Month	Mean ppb	Hourly Max ppb	Month	Mean ppb	Hourly Max ppb
Jan-95	0.22	3.39	Jan-96	0.23	3.17	Jan-97	0.38	4.29
Feb-95	0.16	4.37	Feb-96	0.29	2.62	Feb-97	0.14	1.61
Mar-95	0.31	6.04	Mar-96	0.17	1.05	Mar-97	0.23	4.42
Apr-95	0.14	1.42	Apr-96	0.15	1.27	Apr-97	0.16	2.12
May-95	0.17	2.94	May-96	0.12	1.14	May-97	0.16	1.98
Jun-95	0.1	1.48	Jun-96	0.12	0.85	Jun-97	0.11	0.61
Jul-95	0.12	1.85	Jul-96	0.12	0.91	Jul-97	0.13	1.08
Aug-95	0.12	1.12	Aug-96	0.16	1.8	Aug-97	0.13	1.59
Sep-95	0.22	2.82	Sep-96	0.25	3.35	Sep-97	0.15	1.67
Oct-95	0.33	6.12	Oct-96	0.21	3	Oct-97	0.41	5.33
Nov-95	0.37	4.4	Nov-96	0.43	5.63	Nov-97	0.3	4.25
Dec-95	0.52	7.09	Dec-96	0.31	7.45			

Slide serial no 13





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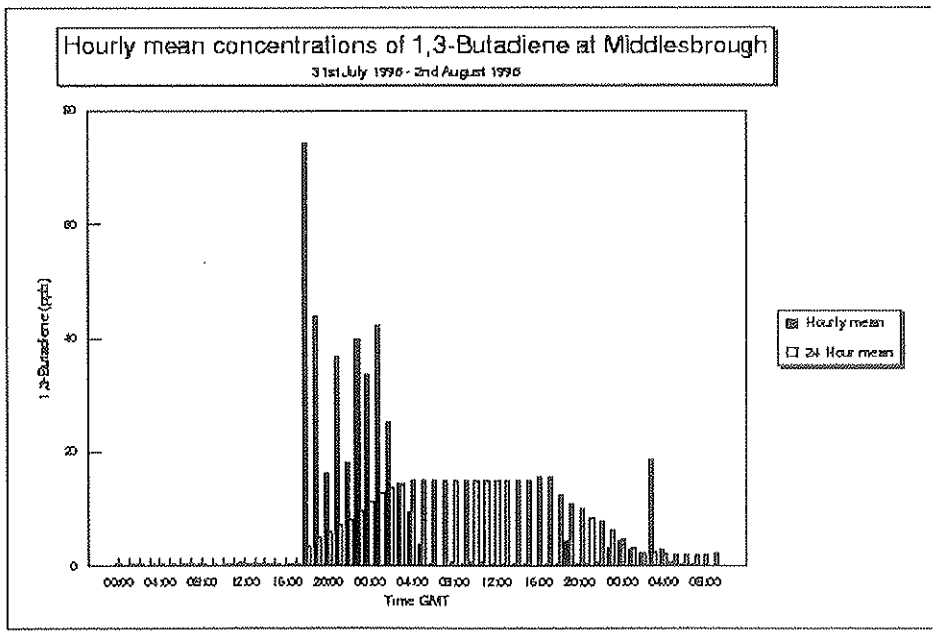
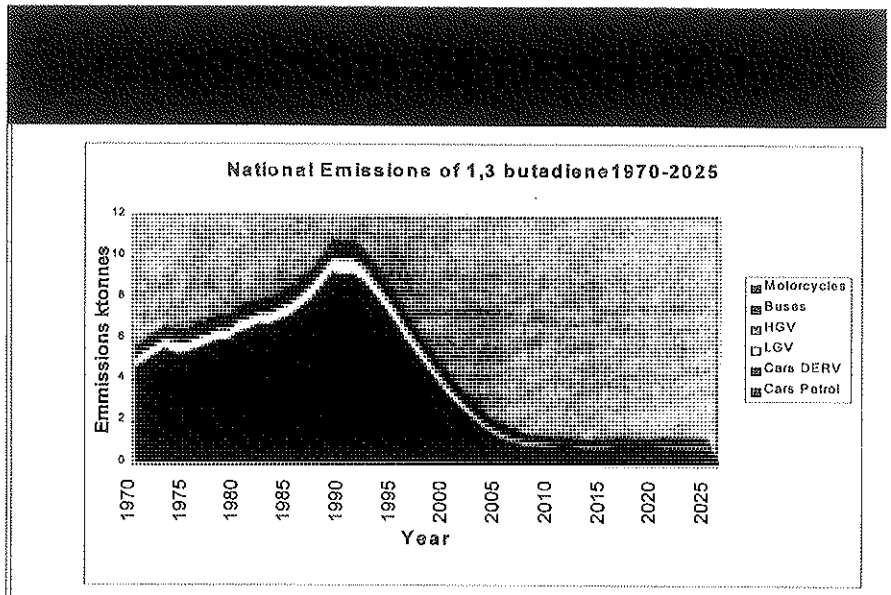




Slide script no 17

Metabolism of 1,3-Butadiene in Mouse, Rat and Human

J.G. Filser, GSF-Institut für Toxikologie, Neuherberg, Germany

The gaseous olefin 1,3-butadiene (BD) is primarily used in the production of synthetic rubbers. In long-term inhalation experiments, it was a strong carcinogen in mice but only a weak one in rats. In order to understand this difference and the relevance of these findings with respect to human exposure, metabolism of BD was investigated in several laboratories.

In mice and rats, BD is metabolized extensively. Its catabolism ends in the formation of CO_2. A greater number of metabolic intermediates has been detected in exhaled air, blood and urine. The first step of BD metabolism is catalyzed by cytochrome P450 dependent monooxygenases resulting in 1,2-epoxy-3-butene (BDO) which is found in exhaled air and in blood. Another oxidative pathway has been discussed which leads via 3-butenal to two mercapturic acids excreted in urine. BDO is in part exhaled unchanged and is further biotransformed either oxidatively or catalyzed by epoxidehydrolase (EH) or glutathione S-transferase (GST). BDO can be oxidized to 1,2:3,4-diepoxybutane (BDO2) measured in blood and urine. Both BDO and BDO2 can be hydrolysed by EH yielding 3-butene-1,2-diol and 3,4-epoxy-1,2-butanediol (BDO-diol), respectively. This epoxide, which can be formed by oxidation of 3-butene-1,2-diol, too, has been proven only in vitro. However, the urinary metabolites 1,3-dihydroxyacetone, presumably generated via EH mediated erythritol formation, and N-acetyl-S-(1-hydroxymethyl-2,3-dihydroxypropyl)-L-cysteine corroborate its intermediary production in vivo. Further adducts to L-cysteine which are excreted in urine probably result from GST catalyzed glutathione conjugations with BDO and with 3-butene-1,2-diol. Interestingly, exposure to high BD concentrations of 2000 ppm and more over several hours led to an almost complete depletion of cytosolic gluthatione in liver and other organs of mice, but not of rats. Under such conditions, the BDO burden in mice increased as a clear result from the loss of the GST dependent elimination.

The epoxides BDO, BDO2, and BDO-diol have been demonstrated to alkylate macromoleculs like DNA and HB and were mutagenic in several test systems. Both BDO and BDO-diol possess one BDO2, however, two sites which can react with nucleophilic groups. In contrast to the monofunctional epoxides BDO and BDO-diol, the bifunctional BDO2 is expected to act as cross-linking agent. DNA-DNA and DNA-protein cross-links, which were found in livers and lungs of BD exposed mice were ascribed to metabolically produced BDO2. Along with these chemical properties correspond the findings that BDO2 was by far more mutagenic than BDO and BDO-diol. BDO2 was positive in carcinogenicity studies with mice and rats following administration subcutaneously or onto skin. Skin application of BDO to mice yielded equivocal results, only.

Species specificity concerning the burden with BD and its epoxide metabolites was investigated in vivo and in vitro yielding significant differences. Mice metabolized BD several times faster than rats, if compared after normalization for body weight. The concentrations of the metabolically formed BDO in blood were up to 11 times higher in mice than in rats. In mice exposed to 62.5 ppm BD, concentrations of BDO and BDO2 in blood were very similar. In rats exposed to the same BD concentration however, BDO2 concentrations in blood were between 2.6 and 11 times smaller than the corresponding BDO values. Consequently, following exposure to 62.5 ppm BD, BDO2 concentrations in blood of rats were between 15 and 85 times smaller than in mice. Furthermore, BDO2 blood concentrations increased in mice from 0.20 nmol/ml at a BD exposure concentration of 62.5 ppm to 2.5 nmol/ml at 1250 ppm BD. In contrast, in rats exposed to 8000 ppm BD, BDO2 blood and tissue concentrations were practically identical with those measured at 62.5 ppm as has recently been demonstrated. Investigations in vitro on the kinetics of BD, BDO and BDO2 were carried out using lung and liver cell fractions of mice, rats, and humans. The activation step of BD to BDO was fastest in lung and liver microsomes of mice followed by those of humans and rats. Corresponding results were obtained regarding the oxidation of BDO to BDO2 in liver microsomes. The metabolic elimination of BDO and BDO2 catalyzed by EH was slowest in liver microsomes of mice and fastest in those of humans. In contrast, GST activity towards BDO and BDO2 was highest in lung and liver cytosol of mice and lowest in corresponding fractions of humans. Concerning the rodent species, these results agree with the findings in vivo that in BD exposed mice BDO and BDO2 burdens were higher than in equally exposed rats.

In order to get information on the human burden with BD and its metabolically produced epoxides, several physiological toxicokinetic models have been developed. The most advanced ones enable to simulate concentrations of BD, BDO and BDO2 in tissues of mice, rats and humans resulting from various exposure conditions. For comparable conditions of BD exposure, these models predict distinctly lower burdens with both epoxides for humans than for the rodent species. Also, adducts of BDO to the N-terminal valine of haemoglobin, which can be used to estimate the BDO burden of blood resulting from BD exposure indicate for humans by far lower BDO burdens: Their haemoglobin binding indices - defined as pmol adducts / (g globin x ppm x h) - have been determined to be 0.3-0.5 in mice, 0.09-0.3 in rats and 0.0005-0.004 in humans. Only limited data are reported on adducts of the intermediate BDO-diol on the N-terminal valine of haemoglobin. No findings are published on BDO2 in humans. Due to these gaps of knowledge a rational, on mechanistic data based estimate of the carcinogenic human risk (the dose dependent probability of getting cancer) from BD is not yet possible.

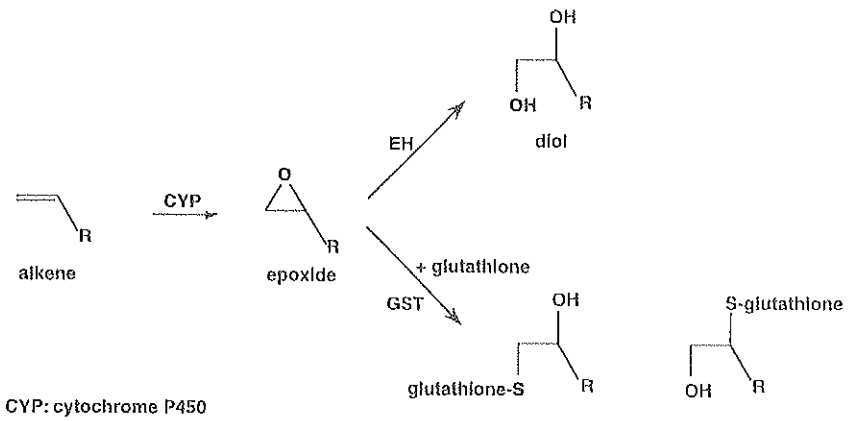
To find a reasonable basis for deriving threshold exposure limits, a comparison of the unavoidable body burden by the endogenously produced BD derivative 2-methyl-1,3-butadiene (isoprene) with the BD body burden resulting from exogenous, atmospheric exposure to BD might be helpful. Isoprene was carcinogenic in mice, but not in rats. In mice, its carcinogenic potency was about 10 times weaker than that of BD. Isoprene is formed naturally in large quantities in humans leading to a mean concentration in blood of about 40 nmol/l. Using a physiological toxicokinetic model, a ten times lower average BD concentration in blood of 4 nmol/l, calculated for a life span of 80 years is computed to result from a workplace exposure (8h/d; 5d/w; 40 y) to around 2 ppm BD. At such exposure conditions, the carcinogenic risk of BD might be similar to that of endogenous isoprene and could be negligibly low.

References: IARC Monograph 60 (1994); Toxicology 113 (1996); IARC Monographs Vol. 72 (in press); Thornton-Manning et al. (Toxicol Sci 41, 1998).

Metabolism of 1,3-Butadiene in Mouse, Rat and Human

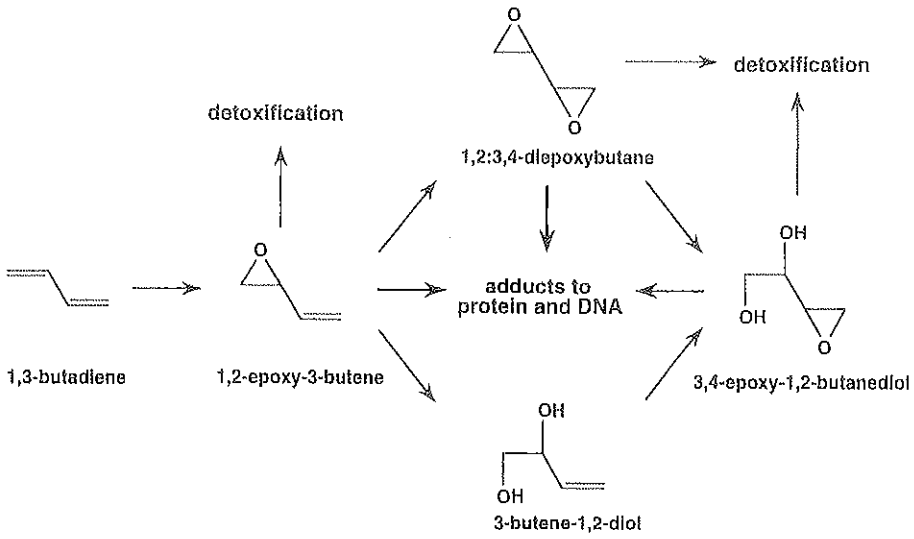
J.G. Filser
GSF-Institute of Toxicology,
Neuherberg, Germany

Biotransformation of alkenes:
Formation and metabolic elimination of epoxides



CYP: cytochrome P450
EH: epoxide hydrolase
GST: glutathione S-transferase

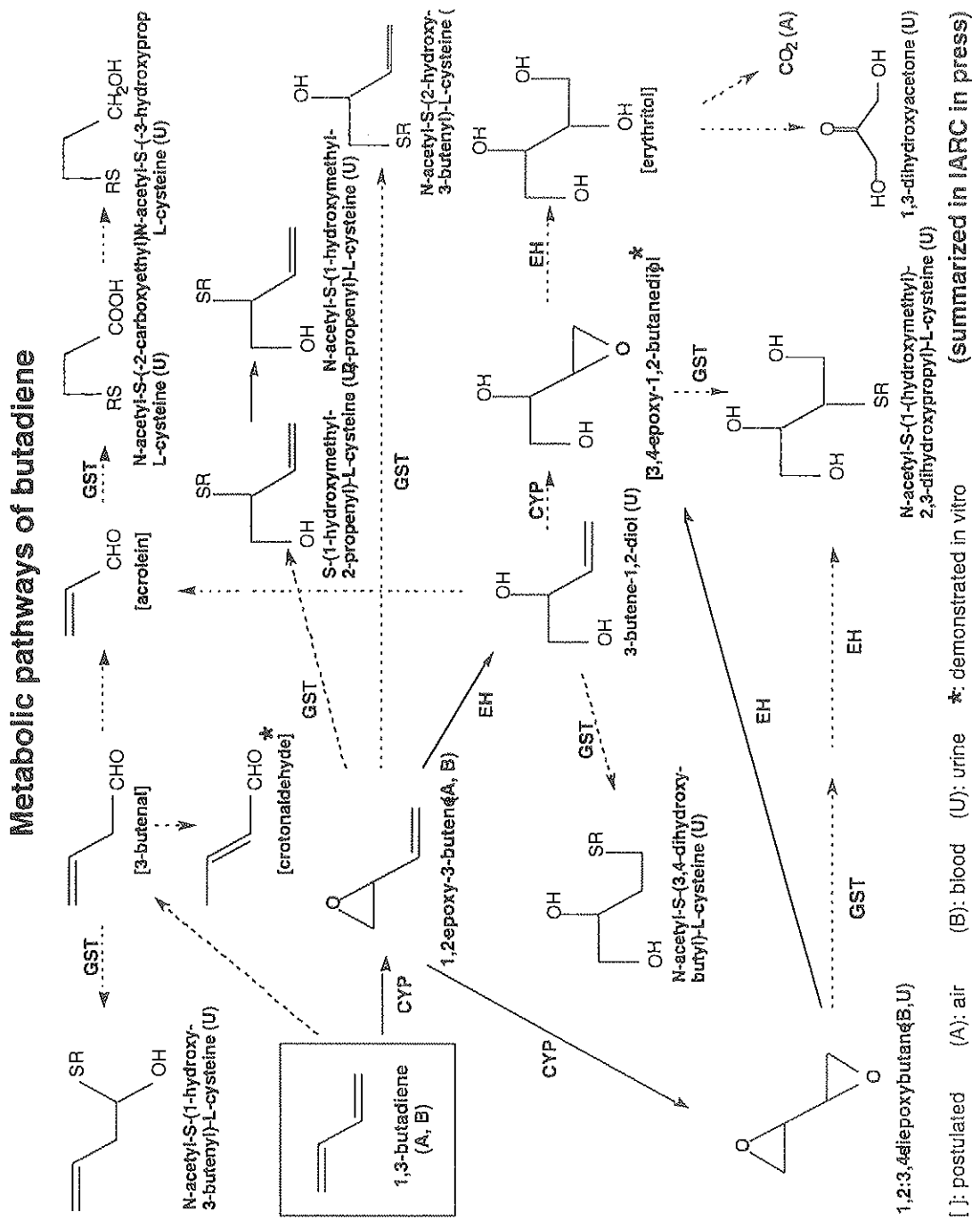
Formation of reactive metabolites of butadiene



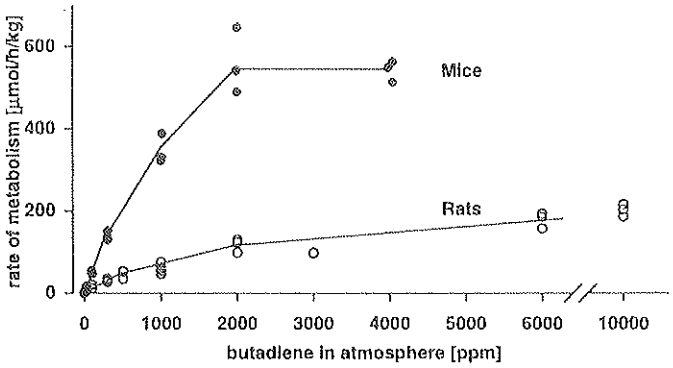
Carcinogenicity studies with butadiene in mice and rats

Species	Lowest effective concentration [ppm]	Highest tested concentration [ppm]	Carcinogenic potency
Mice	6.25	1250	high
Rats	1000	8000	low

(from Huff et al. 1985, Melnick et al. 1990, Owen et al. 1987)


Metabolic pathways of butadiene
1,3-butadiene (A, B)
CYP
GST
EH
[3-butenal]
[acrolein]
[crotonaldehyde]
[erythritol]
[3,4-epoxy-1,2-butanediol]
[1,3-dihydroxyacetone]
CO2 (A)
[ ]: postulated
(A): air
(B): blood
(U): urine
*: demonstrated in vitro
(summarized in IARC in press)

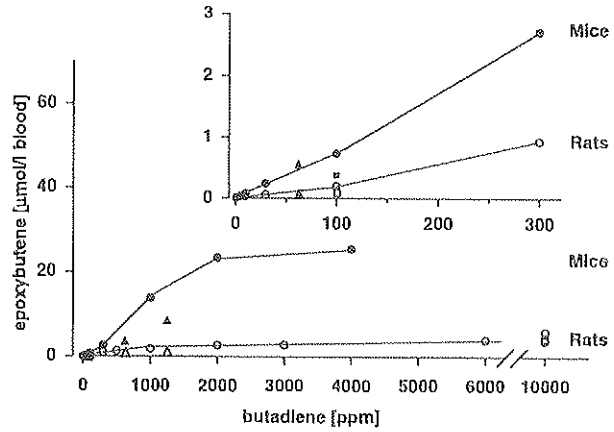
Rates of metabolism of butadiene at steady state related to the exposure concentration in atmosphere



◆ male B6C3F1 mice (n= 5)
○ male Sprague-Dawley rats (n= 2)

(Melschner et al. 1998)

Epoxybutene in blood of mice and rats exposed to constant concentrations of butadiene in air for (3 - 6 h)



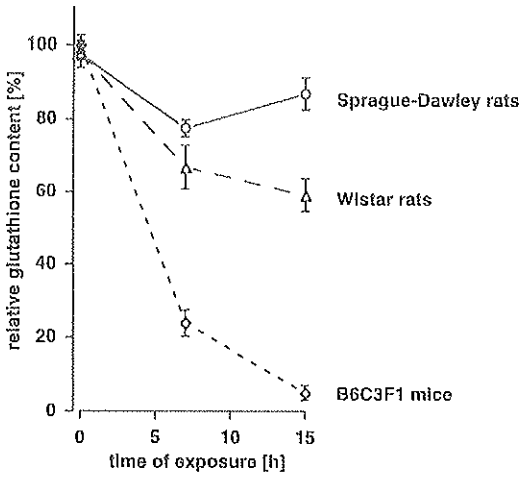
Filled symbols: Mice

▪ Bechthold et al. 1995
▲ Himmelstein et al. 1994
◆ Thornton-Manning et al. 1995a,b; 1997
● Melschner et al. 1998

Hollow symbols: Rats

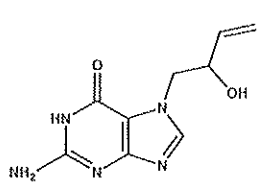
□ Bechthold et al. 1995
△ Himmelstein et al. 1994
◇ Thornton-Manning et al. 1995a,b; 1997
○ Melschner et al. 1998

Depletion of glutathione in livers of rats
and mice exposed to 2500 ppm butadiene

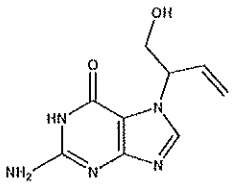


(from Krolling et al. 1988)

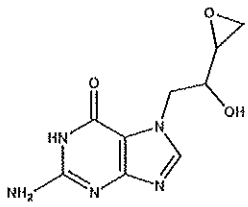
Adducts of the epoxide metabolites of butadiene
to N-7 of the DNA base guanine



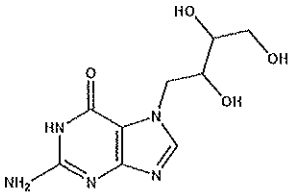
N-7-(2'-hydroxy-3'-buten-1'-yl)guanine



N-7-(1'-hydroxy-3'-buten-2'-yl)guanine



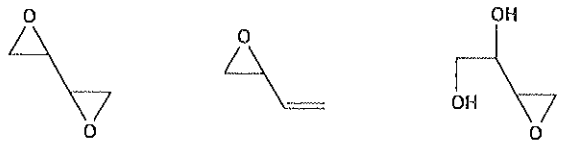
N-7-(2'-hydroxy-3',4'-epoxybut-1'-yl)guanine



N-7-(2',3',4'-trihydroxybut-1'-yl)guanine

(from Tretyakova et al. 1996, 1997)

Mutagenic potency of the epoxides deriving from butadiene



1,2:3,4 diepoxybutane (BDO2) > 1,2-epoxy-3-butene (BDO) ≥ 3,4-epoxy-1,2-butanediol (BDO-diol)

(summerized in IARC in press)

Metabolism of butadiene (BD), epoxybutene (BDO) and diepoxybutane (BDO2) in liver cell fractions of mouse, rat and human

		Comparison of V_{max}/K_m
Toxification	Cytochrome P450	
	BD → BDO	mouse > rat ≈ human
	BDO → BDO2	mouse > rat ≥ human
Detoxification	Epoxide hydrolase	
	substrate BDO	mouse < rat < human
	substrate BDO2	mouse << rat < human
	Glutathione S-transferase	
	substrate BDO	mouse ≈ rat > human
	substrate BDO2	mouse > rat > human

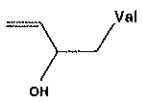
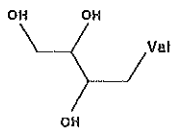
(from Boogaard and Bond 1996, Boogaard et al. 1996, Csanády et al. 1992, Filser et al. 1992, Kreuzer et al. 1991, Seaton et al. 1995)

Epoxybutene (BDO) and diepoxybutane (BDO2) in blood of rodents following exposures (4 or 6 h) to butadiene in air

Butadiene in air [ppm]	Epoxybutene in blood [µmol/l]		Ratio mice to rats	Reference
	Mice	Rats		
62.5	0.28	0.04	7	Thornton-Manning et al. 1995a, b; 1997

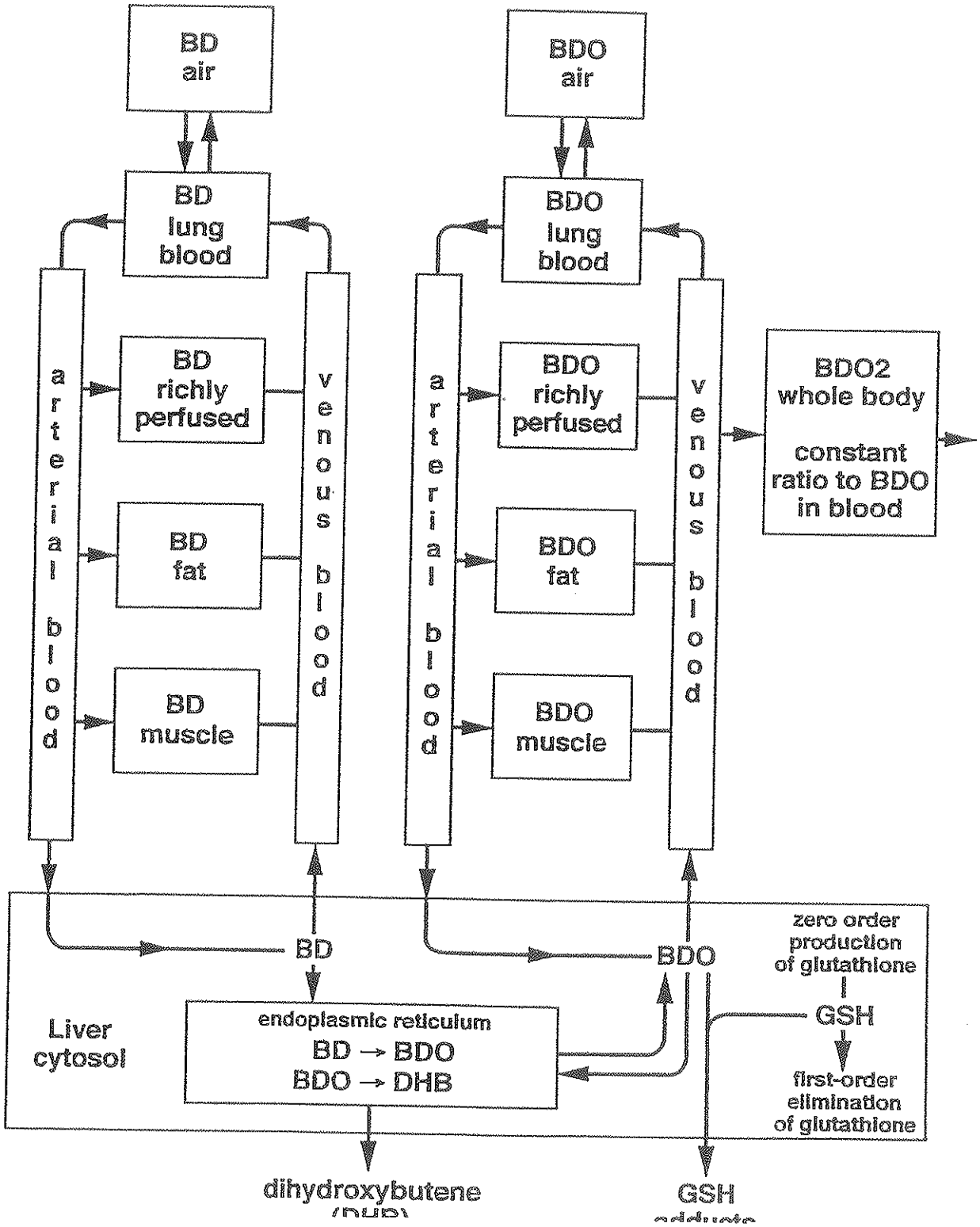
Butadiene in air [ppm]	Diepoxybutane in blood [µmol/l]		Ratio mice to rats	Reference
	Mice	Rats		
62.5	0.27	0.01	27	Thornton-Manning et al. 1995a, b; 1997

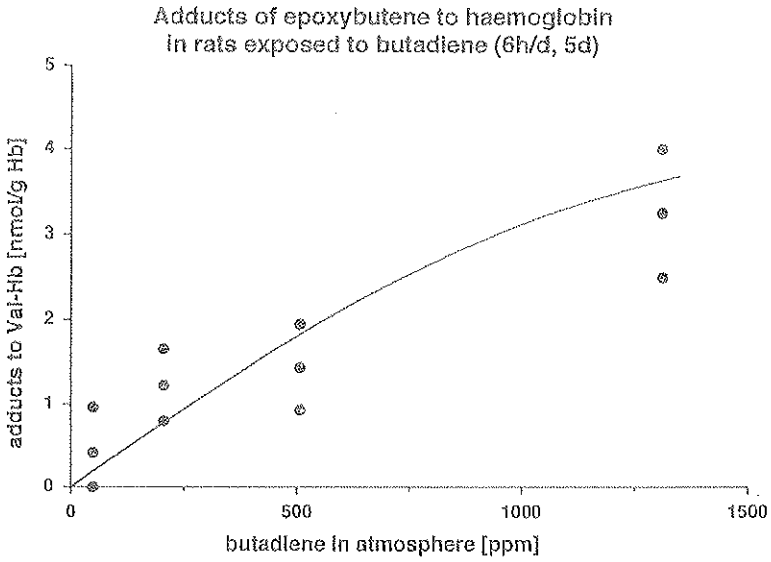
Haemoglobin binding indices (HBI) for N-terminal valine resulting from exposure to low concentrations of butadiene (BD)

Haemoglobin-adduct	Species	HBI*	HBI - ratio species/mouse
 *N*-(2-hydroxy-3-butenyl)-Val (from BDO)	Mouse	0.3 - 0.5	1
	Rat	0.09 - 0.3	0.2 - 1
	Human	0.0005 - 0.004	0.001 - 0.01
 *N*-(2,3,4-trihydroxybutyl)-Val (presumably from BDO-diol)	Rat	0.5 (50 ppm BD)	
	Human	0.02; 0.03 (only 2 measurements)	

*: calculated as pmol adducts per g globin per ppm · h
(from Osterman-Golkar et al. 1991, 1993, 1996; Pérez et al. 1997; Recio et al. 1992)

Physiological toxicokinetic model for butadiene (BD) and its metabolites epoxybutene (BDO) and diepoxybutane (BDO2)





symbols: measured (Albrecht et al. 1993) line: predicted by a toxicokinetic model

Gaps of knowledge concerning metabolism of butadiene

- Is it sufficient to explain the much higher carcinogenic potency of butadiene in mice as compared to rats by the different body burdens with epoxybutene (BDO) and diepoxybutane (BDO2) solely?
- How relevant is the formation of the butadiene metabolite epoxybutanediol (BDO-diol)?
- How high is the burden with the three epoxides in humans exposed to butadiene?

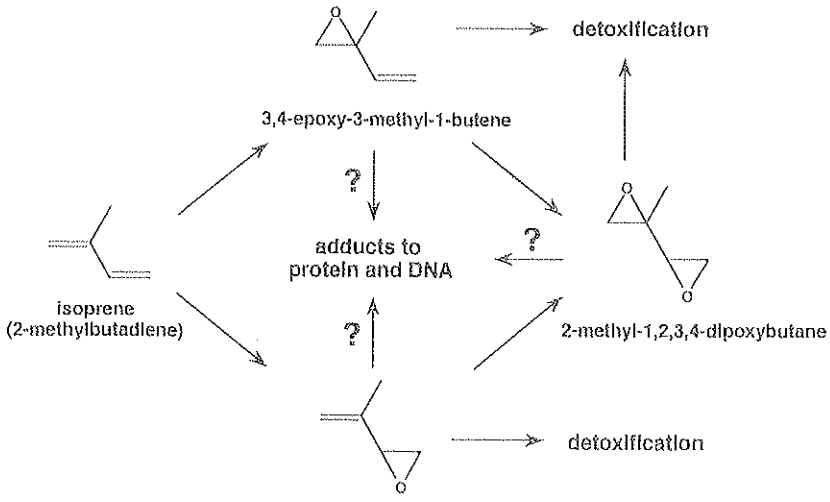
Summary

- Carcinogenic potency of butadiene is strong in mice but weak in rats.
- Carcinogenic hazard of butadiene is considered to result from epoxides formed as metabolic intermediates.
- Mutagenic potency of the epoxides decreases in the order: diepoxybutane >> epoxybutene ≥ epoxybutanediol.
- At equal conditions of exposure to butadiene the epoxides in blood are:

diepoxybutane:	mouse	>>	rat;	human: no data
epoxybutene:	mouse	>	rat;	human: no data
epoxybutanediol:			no data	

- At equal conditions of exposure to butadiene binding of epoxybutene to haemoglobin is highest in mouse, smaller in rat, much smaller in human.

Formation of reactive metabolites of isoprene



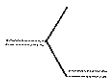
(according to Wistuba et al. 1994)

Carcinogenicity studies with isoprene in mice and rats

Species	Lowest effective concentration [ppm]	Highest tested concentration [ppm]	Carcinogenic potency
Mice	70	7000	high
Rats		7000	negative

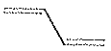
(from Melnick et al. 1994, NTP 1994, Placke et al. 1996)

Comparison between endogenous isoprene in humans and exogenous 1,3-butadiene



Isoprene

mean endogenous concentration in blood 40 [nmol/l] [a]



1,3-butadiene

mean concentration in blood (calculated for a lifespan of 80 y) 4 [nmol/l] [b]

corresponding concentration in air of workplace (8h/d; 5d/w; 40y) 2 [ppm] [b]

[a]: Filser et al. 1996, Möllers et al. 1995

[b]: Based on Bolt et al. 1984

Conclusions based on mechanistic considerations

- There are gaps of knowledge of the butadiene metabolism in humans.
- Therefore, a rational estimate of a carcinogenic risk is not possible.
- The risk might be negligibly low at exposure conditions in the range of current threshold limit values.

1,3 Butadiene: Evidence for Genotoxicity

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1,3 butadiene is a synthetic organic chemical with large worldwide distribution. It is present in outdoor and indoor air. The potential for butadiene exposure in the workplace is great, where it often coexists with styrene and other chemicals.

Although 1,3 butadiene is clearly carcinogenic in mice and rats, the sensitivities of the two species are strikingly dissimilar. Mice are exceedingly sensitive, with tumors being observed at lifetime exposure concentrations as low as six parts per million (ppm). By contrast, rats are relatively insensitive, with malignant tumors appearing at exposure concentrations up to three orders of magnitude higher.

These species differences in butadiene sensitivity are attributable, at least in part, to variations in metabolism between mice and rats. Mice show a much greater capacity for butadiene oxidation than do rats, with a decidedly higher ratio of activation to detoxification. Butadiene's oxidation products are the mono- and diepoxide and the monoepoxide diol. Mice are much more efficient than are rats in forming the diepoxide. Butadiene metabolism in humans is under active investigation. Indications are that, in many respects, humans resemble rats.

Assessment of human cancer risk from 1,3 butadiene is, therefore, complex. Added to the usual uncertainty in extrapolating rodent carcinogenicity results to humans is a decision as to which rodent species constitutes the best model for extrapolation.

More direct evidence of butadiene's cancer potential in humans comes from epidemiological studies. A recent large analysis of deaths among styrene butadiene rubber workers revealed a clear excess of leukemia deaths that were related to probable lifetime butadiene exposure concentrations. However, interpretation of this finding is complicated by the results of another recent study of butadiene monomer production workers where the leukemia effect was not seen. There was instead a nonsignificant increase in lymphoma deaths in the monomer workers that was not related to lifetime exposures. This worker group should have experienced the "purest" butadiene exposures. These findings in the latter group raises the question of a coexposure to some other agent(s) producing the leukemias in the styrene butadiene rubber workers.

The confidence with which rodent carcinogenicity data can be extrapolated to humans depends on how the agent in question produces cancer. There are mechanisms unique to the animals which are not generalizable to humans. Examples are some hormonal and viral causes of cancer. Other mechanisms, however, are general and may be extrapolated to humans. The best example is genotoxicity. A caveat is that the responsible genotoxic metabolites generated in the rodents must also be generated in humans.

1,3 butadiene has the hallmarks of a genotoxic carcinogen in animals in that it induces cancers of many histological types in both sexes of two rodent species. Metabolites of 1,3 butadiene can also be shown directly to be genotoxic agents. (1) They are electrophilic substances that react with the nucleophilic centers in proteins and DNA. (2) They induce gene and/or chromosome level mutations in model systems. (3) They probably also induce gene and/or chromosome level mutations in humans.

Numerous studies have shown that 1,3 butadiene (metabolites) form covalent binding products (adducts) with proteins. In fact, hemoglobin adducts of various metabolites are now being used to monitor humans for evidence of butadiene exposures. Similarly, *in vivo* butadiene metabolites have been shown to react with the nucleobases in DNA, forming a wide variety of DNA adducts. These too are being developed as human biomarkers of butadiene exposure.

Prokaryotic and eukaryotic microorganism test systems have been used to study the mutagenicity of 1,3 butadiene and its metabolites. Positive responses have been seen in all systems that allowed for metabolism of the parent compound to its several oxidative products. The same has been seen for insect test systems and in studies of mammalian cells (including human) in culture.

In vivo studies in mice and rats have also demonstrated the induction of gene mutations in both species. Interestingly, the mutagenic potency of 1,3 butadiene administered by inhalation was five times greater in mice than in rats, paralleling the magnitude of the greater metabolic capacity for butadiene in the mouse. However, this difference in genotoxic effect between mice and rats, although in the right direction, is far less than the relative sensitivities of these two species to carcinogenicity, as noted above.

The three oxidative products of 1,3 butadiene, i.e. the diepoxide, the monoepoxide and the monoepoxide diol, have mutagenic potencies in that same order. By far the most potent mutagen is the diepoxide, which is 40-100 times more potent in this regard than is the monoepoxide. The monoepoxide is more potent than is the monoepoxide diol. Furthermore, the diepoxide is more prone to cause deletion types of gene mutations than is the monoepoxide. Apparently, all metabolites induce base substitution gene mutations that have a preference for A:T base pairs.

Examinations of tumors from mice treated with 1,3 butadiene have demonstrated mutations in oncogenes and in tumor suppressor genes in the malignancies. The supposition is that the metabolites of 1,3 butadiene that induced the tumors did so by a genotoxic mechanism.

Studies of chromosomal level mutations and of genotoxic effects in germinal cells in animals have indicated similar positive findings. These are discussed separately in this symposium.

Gene and chromosome mutations have also been studied in workers occupationally exposed to 1,3 butadiene. Findings have been mixed, with some studies clearly indicating these genotoxic effects and others not finding them. Also seen in humans are indications that some genetically inherited metabolic capacities modulate sensitivities to some of the genotoxic effects of the metabolites of 1,3 butadiene. These traits involve the glutathione-S-transferases (GST's) systems that detoxify butadiene's metabolites.

In an effort to define sensitivities with which the several biomarkers of human genotoxicity reflect actual 1,3 butadiene exposures, a large transitional epidemiological study of Czech workers is now in progress. Among the biomarkers being investigated are those of metabolism, of adduct formation, of somatic gene mutations, of chromosome changes, and of GST genotypes. This study, and the exhaustive efforts being expended on precise exposure measurements, will be presented in detail.

IARC currently classifies 1,3 butadiene as a known animal and probable human carcinogen (Category 2A). This classification will continually be reviewed. Further assessment of butadiene's human risk potential may depend, in part, on whether or not this agent is convincingly shown to produce or not produce genotoxic effects in humans at realistic exposure concentrations.

BRUSSELS.ABS
June 18, 1998

1,3 BUTADIENE

CONCLUSIONS REGARDING ANIMAL CARCINOGENICITY STUDIES

- 1,3 Butadiene induces tumors in both sexes of two rodent species (mice and rats)
-
- Tumors of mutliple types at multiple organ sites are induced in the two rodent species
- Remarkable species difference in cancer susceptibility
  - Mice > > > > > Rats
- In susceptible B6F3F1 mice - tumors induced at lowest exposure dose tested
  - 6.25 ppm x 6 hours/day x 5 days/week x 104 weeks for a total of 650 ppm weeks
- Stop exposure studies show that concentration more important than duration of exposure
- Retroviral activation does not account for all of butadiene lymphoma production in mice

1,3 BUTADIENE

MECHANISM OF ANIMAL CARCINOGENICITY

- Some mechanisms of carcinogenicity are unique to animals and may not be generalizable to humans

  e.g. Some hormonal mechanisms

- Some mechanisms of carcinogenicity are general and may be extrapolated to humans

  e.g. Genotoxicity

(A caveat is that genotoxic metabolites must be generated in the species to which extrapolation is made.)

1,3 BUTADIENE

CHARACTERISTICS OF GENOTOXIC CARCINOGENS

- Induce cancers in both sexes of multiple species (Animal Cancer Bioassays)
- Induce multiple types of cancer (Animal Cancer Bioassays)
- Agents can be shown to be genotoxic
  - Agents are (or are metabolized to) electrophilic substances that react with nucleophilic centers in proteins and DNA
  - Induce gene and/or chromosome level mutations in model systems
    - Prokaryotic organism
    - *In vitro* cell systems
    - *In vivo* animal systems
  - Induce gene and/or chromosome level mutations in humans

1,3 BUTADIENE

ELECTROPHILICITY OF METABOLITES

Reactions with Proteins

- Radiolabeled 1,3 butadiene (inhalation) results in covalent binding of radioactivity to liver nucleoproteins (mice > rats) (1986)
- Butadiene diepoxide induces protein - DNA crosslinks in mice (1989)
- Butadiene monoepoxide is bound to N-terminal valine of hemoglobin in Wistar rats exposed to 1,3 butadiene by inhalation (1991)
- Butadiene monoepoxide is bound to N-terminal valine of hemoglobin in Sprague-Dawley rats and B6C3F1 mice exposed to 1,3 butadiene by inhalation (1994)
- Repeat demonstration of binding to N-terminal valine of mouse and rat hemoglobin. Results similar for C3HX 101 EL mice
- Hemoglobin adducts of 1,3 butadiene metabolites found in occupationally exposed humans (1993, 1994, 1996)

1,3 BUTADIENE

ELECTROPHILICITY OF METABOLITES

Reactions with DNA

- Butadiene diepoxide forms inter-strand crosslinks in DNA through bifunctional alkylation of N-7 guanines (1961)
- B6C3F1 mice and Wistar rats exposed to ^{14}C 1,3 butadiene by inhalation have covalently bound radioactivity in liver DNA
  (Mice = Rats) (1986)
- 1,3 butadiene metabolites form complex adducts in DNA
  - Several groups have characterized various nucleobase adducts formed in DNA by metabolites of 1,3 butadiene (1994, 1995, 1996)
  - Specific enantio- and regioisomeric butadiene monoepoxide adducts are formed (1997)
  - N-7 position of guanine is most reactive with butadiene monoepoxide, followed by N-3 and N-1 positions of adenine
- Sensitive ^{32}P post-labeling methods are being studied for human biomonitoring
- Urinary mono- and diepoxide N-7 guanine adducts are being studied for human biomonitoring

1,3 BUTADIENE
GENOTOXIC PROFILE
Prokaryotic and Eukaryotic Microorganism Test Systems

Agent	Test Organism	Conditions	Results
1,3 Butadiene	S. typhimurium TA 1530 & TA 1535	S-9 (Rat, Human)	+ (Multiple Tests)
1,3 Butadiene	Same	No S-9	- Usual (+ attributed to volatile metabolites)
Butadiene Monoepoxide	S. typhimurium TA 1530, TA 1535, TA 100	No S-9	+
"	E. coli	No S-9	+
"	Klebsiella pneumoniae	No S-9	+
Butadiene Diepoxide	S. typhimurium	No S-9	+ More
	E. coli	No S-9	+ Potent
	Bacillus megaterium	No S-9	+ than
	Pseudormonas pyocyanea	No S-9	+ 1,3 butadiene
	Klebsiella pneumoniae	No S-9	+ monoepoxide
Butadiene Diepoxide	Neurospora crassa	No S-9	+
	Schizosaccharomyces pombe	No S-9	+
	Saccharomyces cerevisae	No S-9	+

1,3 BUTADIENE

GEN-TOX PROFILE

Insect Test Systems

Agent	Test Organism	Result
1,3 Butadiene	Drosophila melanogaster	
	Sex linked recessive lethal mutations	-
	Spot Wing test somatic mutations	-
Butadiene Diepxide	Drosophila melanogaster mutations	
	Recessive lethal	+
	Visible	+
	Semi-lethal	+

1,3 BUTADIENE
GEN-TOX PROFILE
In Vitro Mammalian Cell Systems

Agent	Test Cells	Results
1,3 Butadiene	Mouse lymphoma	+ with S-9
1,3 Butadiene	Mouse lymphoma	- with S-9
Butadiene diepoxide	Human TK6 * (*hprt* and tk loci)	+ (40-100 x more potent than below)
Butadiene monoepoxide	Human TK6 * (*hprt* and tk loci)	+ (3.5x more potent than below)
Butadiene monoepoxide diol	Human TK6 * (*hprt* and tk loci)	+
Butadiene diepoxide	Human TK6 * (*hprt* locus)	+
Butadiene monoepoxide	Human TK6 * (*hprt* locus)	+

(* Mutations induced preferentially at A:T base pairs)

1,3 BUTADIENE

GEN-TOX PROFILE

IN VIVO MUTAGENICITY STUDIES IN RODENTS

Agent: 1,3 Butadiene by Inhalation

Mouse		Rat
◦ Melanocyte Mutations (Mouse Spot Test)		
Multiple loci	+	
◦ *hprt* (B6C3F1) *		◦ *hprt* * (Fischer 344)
– Splenic lymphocytes	+ (x2)	– Splenic lymphocytes +
– Thymic lymphocytes	+	– Thymic lymphocytes +
◦ *hprt* (102XC3H)		
– Splenic lymphocytes	+	
– Splenic lymphocytes	-	
◦ *hprt* (CD1)		
– Splenic lymphocytes	-	
◦ *lac Z* (MM)		
– Bone marrow	-	
– Lung	+	
– Liver	-	
◦ *lac I* (BB) **		
– Bone marrow	+	

* Mutagenic potency at *hprt:* Mouse/rats = 5

** Preferential mutations at A:T base pairs

1,3 BUTADIENE

GEN-TOX PROFILE

IN VIVO MUTGENICITY STUDIES IN RODENTS

Agent: Butadiene Monoepoxide

Mouse

- *hprt* (B6C3F1)
  - Splenic lymphocytes +
- *hprt* (102XC3H)
  - Splenic lymphocytes +/-
- *hprt* (B6C3F1)
  - Splenic lymphocytes +

Rat

- *hprt* (Lewis)
  - Splenic lymphocytes -
- *hprt* (Fischer 344)
  - Splenic lymphocytes +/-

1,3 BUTADIENE
GEN-TOX PROFILE
IN VIVO MUTAGENICITY STUDIES IN RODENTS
Agent: Butadiene Diepoxide

Mouse

- *hprt* (B6C3F1)
  - Splenic lymphocytes +

- *hprt* (B6C3F1)
  - Splenic lymphocytes +

- *hprt* (102XC3H)
  - Splenic lymphocytes -

- *hprt* (C57Bl)
  - Splenic lymphocytes -

Rat

- *hprt* (Fischer 344)
  - Splenic lymphocytes (inhalation) +

- *hprt* (Lewis)
  - Splenic lymphocytes -

1,3 BUTADIENE

GEN-TOX PROFILE

MUTATIONS IN ONCOGENES/TUMOR SUPPRESSOR GENES IN MOUSE TUMORS

- B6C3F1 - 2-year Bioassay

  K-ras + (Lung, liver, lymphoma)
  H-ras + (Liver)

- B6C3F1 - 2-year Bioassay

  H-ras + (Haderian Gland - also in controls)

- B6C3F1 - 2-year Bioassay

  p53 - allele loss)
  Rb - allele loss) Lung and mammary tumors
  Chr. 4 gene - allele loss)

1,3 BUTADIENE

GEN-TOX PROFILE

IN VITRO CYTOGENETIC STUDIES
(Mammalian Cell System)

- Chinese Hamster Ovary Cells
  - SCE (with S-9) +

- Human Lymhocytes
  - SCE (with and without S-9) + and -
    (S-9 not necessary)

1,3 BUTADIENE
GEN-TOX PROFILE

IN VIVO CYTOGENETIC STUDIES IN RODENTS

1,3 Butadiene by Inhalation

Mouse

- Chromosome aberrations
  - Lymphocytes +'s (multiple)
  - Bone marrow +'s (multiple)
- Micronuclei
  - Lymphocytes +'s (multiple)
  - Bone marrow * +'s (multiple)
- SCE
  - Lymphocytes +'s (multiple)
  - Bone marrow +'s (multiple)

Rat

- Chromosome aberrations -
- Micronuclei -
- SCE +/-

Metabolites (butadiene mono- and diepoxide) produce cytogenetic effects in both species

* Found in bone marrow of tumor-bearing animals in stop exposure bioassay

1,3 BUTADIENE

GEN-TOX PROFILE

IN VIVO GENOTOXICITY TO GERMINAL CELLS

1,3 Butadiene by Inhalation

Mouse

◦ Dominant lethals	+'s (multiple)
◦ Heritable translocations	+'s (x2)
◦ Sperm head abnormalities	+
◦ Spermatid micronuclei	+
◦ Comet Assay (Testicular Cells)	+

Rat

◦ Dominant lethal	-

Metabolites (butadiene mono- and diepoxide produce germ cell genotoxic effects in both species.

1,3 BUTADIENE

GEN-TOX PROFILE IN HUMANS

CYTOGENETIC ENDPOINTS

◦ Chromosome Aberrations - Production Workers	
– 1st Study	-
– Reevaluation by GST Genotype	+ ($GST^{-/-}$)
– 2nd Study	+
◦ SCE - Production Workers	
– 1st Study	-
– 2nd Study	+
–	
◦ Micronuclei - Production Workers	
– 1st Study	-
– 2nd Study	-
◦ Comet Assay	-
◦ Chromosome Aberrrations - SBR Workers	
– *In vivo*	-
– Challenge	+
◦ SCE - "Butadiene Exposed"	-

1,3 BUTADIENE

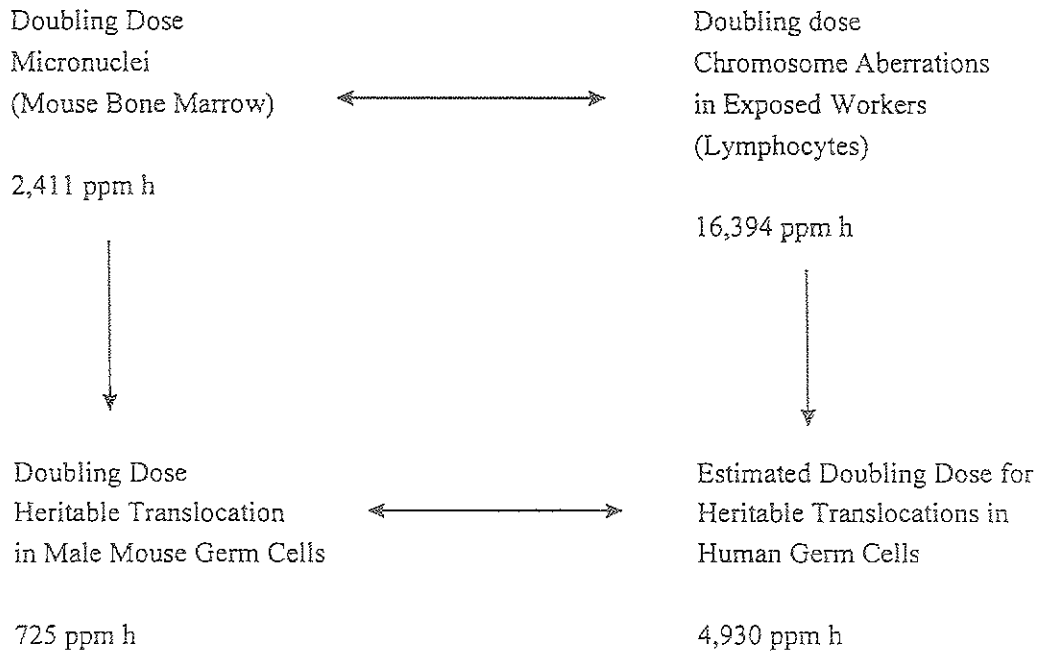
GEN-TOX PROFILE IN HUMANS

GENE MUTATION ENDPOINTS

◦ *hprt* T-cell (Autoradiography)	Monomer Production Facility	+
	Monomer Production Facility	+
	SBR Workers	+
◦ *hprt* T-cell (Cloning Assay)	Polybutadiene Rubber Workers	-
◦ *hprt* T-cell (Cloning Assay	Butadiene Production	-
◦ *ras* Oncoprotein	Butadiene (Styrene) Exposure	-

1,3 BUTADIENE
RISK ASSESSMENT FOR HUMAN HERITABLE EFFECTS

DOUBLING DOSE METHOD
(Pacchierotti et al., Mut. Res., 1998)



If sperm are sensitive over 6 weeks = 240 working hours; a worker exposed to 20 ppm 1,3 butadiene has a risk of having a child with a heritable translocation that is 2x background (background = 879/million live born)

Genotoxicity of 1,3-Butadiene. The effects on chromosome integrity and chromosome segregation

Francesca Pacchierotti
Section of Toxicology and Biomedical Sciences, ENEA, CR Casaccia, Rome, Italy

Clastogenicity is the capacity of a physical or chemical agent to produce chromosome breaks. Chromosome breaks can start processes leading to structural chromosome aberrations, such as deletions or translocations, with adverse health effects in somatic and germinal cells. The induction of chromosome aberrations can be demonstrated by cytogenetic analysis of metaphase chromosomes or cytogenetic analysis of micronuclei in interphase cells.

1,3-Butadiene has been shown to be a potent clastogenic chemical in mice exposed by inhalation. The frequency of micronuclei was significantly increased in peripheral blood erythrocytes of mice exposed for 6 h/day, 5 days/week for a total of 13 weeks to 6.25 ppm or higher butadiene concentrations (Jauhar et al., 1988; Tice et al., 1987). Even a short exposure of 23 h to a butadiene concentration of 10 ppm induced a significant elevation of the background frequency of micronuclei (Victorin et al., 1990). In view of the present permissible exposure levels that regulate occupational exposure to butadiene, these data show that butadiene is strongly clastogenic for mouse bone marrow erythropoietic cells.

In addition to bone marrow cells, other cell types in the inhalation-exposed mouse can be target of butadiene-induced chromosome breaks. The frequency of micronuclei is significantly increased in spleen lymphocytes after 5 days of exposure to 130 ppm (the lowest concentration tested; Stephanou et al., 1998) and in lung fibroblasts after 5 days of exposure to 500 ppm (the lowest concentration tested; Pacchierotti, unpublished data). In male mice post meiotic germ cells, DNA damage is induced after inhalation exposure to butadiene which causes the formation of breaks and rearrangements in paternal chrosomoses of the fertilized egg (Pacchierotti et al., 1998), leading to dominant lethal effects (Adler et al., 1998).

As a matter of fact, the clastogenic effects of butadiene are produced by its metabolites. The metabolism of butadiene produces different intermediates. Three of them are epoxides with a chemical structure making them highly reactive with DNA molecules: 1,2-epoxy-3-butene (BDO), 1,2:3,4-diepoxybutane (BDO_2) and 3,4-epoxy-1,2-butanediol (BDO-diol). Since these metabolites are produced with different rates in different species, it is of interest to know the clastogenic potency of each one of them. This is not straightforward because, for instance, testing the genotoxicity of BDO per se would require excluding, in the specific test system, its biotrasformation into the other metabolites. Nevertheless, both considerations on structure-activity relationships and experimental data on micronucleus induction in bone marrow (Adler et al., 1997), and spleen cells (Xiao and Tates, 1995) of treated mice, and SCE induction in cultured human

lymphocytes (Bernardini et al., 1996) suggest that the clastogenicity of the three metabolites can be ranked in the order BDO_2 > BDO > BDO-diol.

The type of clastogenic effects induced by butadiene is relevant for human health, at least as far as heritable damage is concerned. A linear dose-effect relationship has been described for the induction of heritable translocations in male mice exposed for one week to 500 or 1300 ppm butadiene (Adler et al., 1998). In man, heritable translocations are associated with reduced fertility, and, in some cases, with increased probability of miscarriages and skeletal and neurological defects.

Contrary to the observations collected in butadiene-exposed mice, micronucleus induction could not be demonstrated in bone marrow cells of rats exposed for two days to butadiene concentrations as high as 10000 ppm (Cunningham et al., 1986). As regards the effects produced in rats by treatment with butadiene metabolites, BDO_2 is approximately as effective as in the mouse for the induction of bone marrow (Lähdetie and Grawe, 1997) or spleen micronuclei (Xiao and Tates, 1995), while BDO and BDO-diol are clearly less effective than in mice or even not effective at all (Lähdetie and Grawe, 1997; Xiao and Tates, 1995). These observations are in line with a pharmacokinetic model postulating a lower rate of biotransformation of butadiene and BDO to BDO_2 in rats than in mice.

It is interesting to note that the yield of clastogenic effects can be conditioned also by species-specific variables different from the metabolic rates. For instance, the same frequency of micronuclei in post meiotic germ cells was induced by a 6 times lower dose of BDO in rats than in mice possibly because rat germ cells are more resistant to chemically-induced cell killing than mouse germ cells (Lähdetie et al., 1997).

In spite of the evidence showing that butadiene metabolites can be clastogenic for rat germ cells when directly injected, butadiene could not be demonstrated to induce dominant lethal effects in rats (Anderson et al., 1998), the highest ineffective concentration tested being 1250 ppm (the lowest effective concentration tested in mice was 65 ppm).

Two cytogenetic biomonitoring studies have been conducted on butadiene-exposed workers measuring the frequencies of micronuclei and chromosome aberrations in peripheral blood lymphocytes. Exposure levels measured by personal dosimeters were shown to average 1.6 - 1.8 ppm. The first study (Sorsa et al., 1994) did not show any significant difference between the exposed and the unexposed subjects; the second study (Tates et al., 1996) reported a frequency of 3.11 % chromosome aberrations in the exposed group to be statistically higher than the frequency of 2.03 % measured in the matched controls.

Conjugation with glutathione is a step in the detoxification pathway of BDO and BDO_2. In vitro experiments have demonstrated that lymphocytes of individuals with a null genotype for the glutathione S-transferase T1 (GSTT1) gene treated with BDO_2 respond with higher frequencies of SCEs, micronuclei and chromosome aberrations than lymphocytes of GSTT1-positive individuals (Vlachodimitropoulos et al., 1997). Also the

in vitro response to BDO seems to be modulated by the glutathione S-transferase genotype (Bernardini et al., 1998). A retrospective genotyping of exposed workers enroled in the first biomonitoring study cited above showed that the frequency of lymphocytes with chromosome aberrations was significantly higher in workers with a null *GSTT1* genotype than in GSTT1-positive workers (Sorsa et al., 1996).

In addition to structural chromosome aberrations, numerical chromosome changes (aneuploidies) are a genotoxic effect which may have health relevant consequences being implicated in carcinogenesis, miscarriages and genetic diseases. Aneuploidies are generated by errors of chromosome distribution in mitosis and meiosis and can be detected in interphase cells by counting fluorescent signals from chromosome-specific hybridization probes and micronuclei contanining whole chromosomes. BDO and BDO_2 have been shown to induce aneuploidies in human lymphocytes treated in vitro (Xi et al., 1998; Vlachodimitropoulos et al., 1997). The mechanism responsible for these effects has not yet been clarified.

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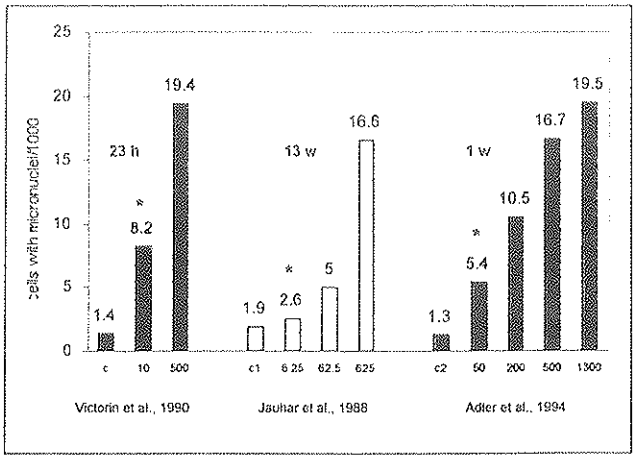
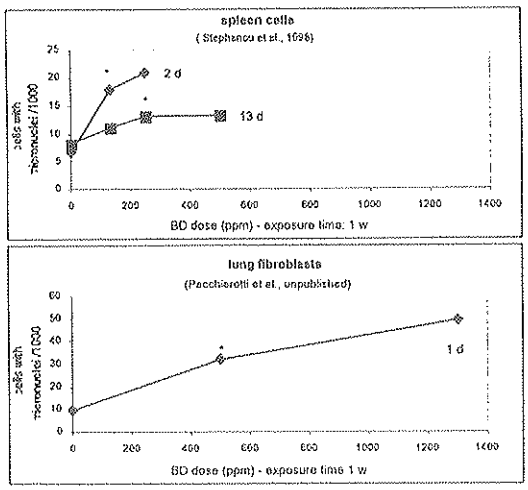
Induction of micronuclei in bone marrow cells



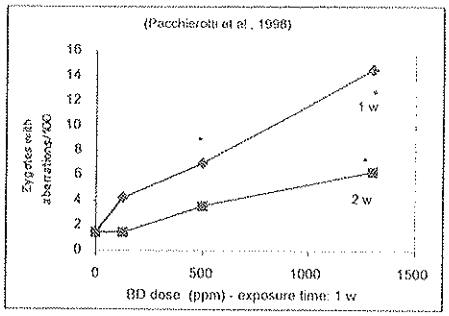
Genotoxicity of 1,3-Butadiene. Effects on chromosome integrity and chromosome segregation (Pacchierotti, 1998)

Induction of micronuclei in spleen and lung cells



Genotoxicity of 1,3-Butadiene. Effects on chromosome integrity and chromosome segregation (Pacchierotti, 1998)

Induction of chromosome damage in germ cells

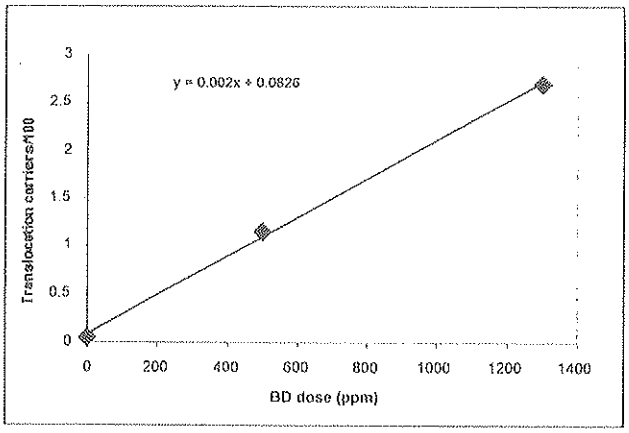


Induction of dominant lethal mutations
(Adler et al., 1994; Adler et al., 1998)

BD dose	1 w	2 w
130	n.s.	n.s.
500	$p < 0.05$	n.s.
1300	$p < 0.05$	$p < 0.05$

Genotoxicity of 1,3-Butadiene. Effects on chromosome integrity and chromosome segregation (Pacchierotti, 1998)

Induction of heritable chromosome translocations

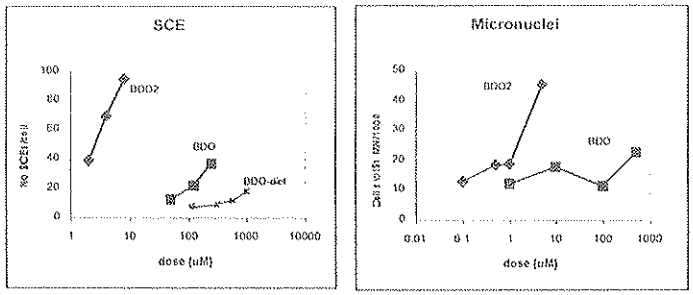


Adler et al., 1998

Genotoxicity of 1,3-Butadiene. effects on chromosome integrity and chromosome segregation (Pacchierotti, 1998)

Relative clastogenic effectiveness of BDO, BDO2 and BDO-diol.

Induction of SCEs and micronuclei in human lymphocytes in vitro



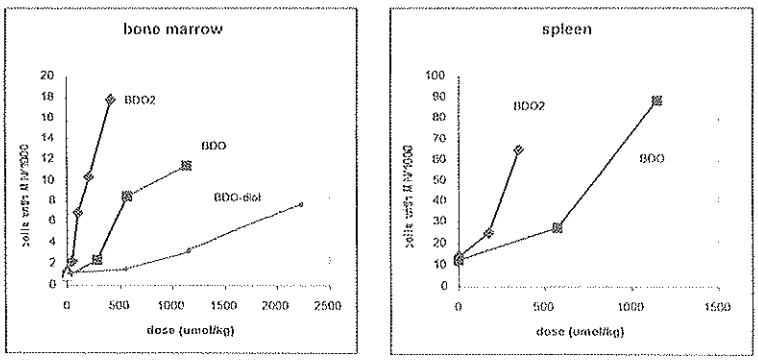
SCEs: Bernardini et al., 1996

Micronuclei: Xi et al., 1997

Genotoxicity of 1,3-Butadiene. Effects on chromosome integrity and chromosome segregation (Pacchierotti, 1998)

Relative clastogenic effectiveness of BDO, BDO2 and BDO-diol.

Induction of micronuclei in mouse somatic cells

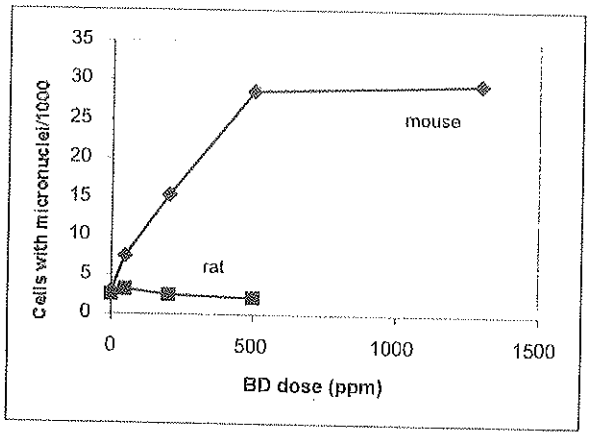


Bone marrow: Adler et al., 1997

Spleen: Xiao and Tates, 1995

Genotoxicity of 1,3-Butadiene. Effects on chromosome integrity and chromosome segregation (Pacchierotti, 1998)

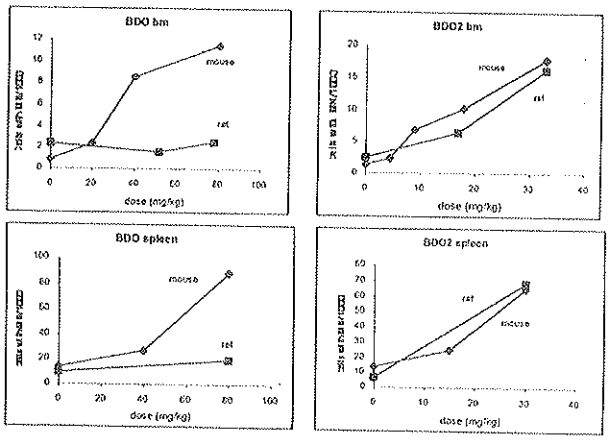
Induction of micronuclei in bone marrow cells. Comparison of mouse and rat responses



Autio et al., 1994

Genotoxicity of 1,3-Butadiene. Effects on chromosome integrity and chromosome segregation (Pacchierotti, 1998)

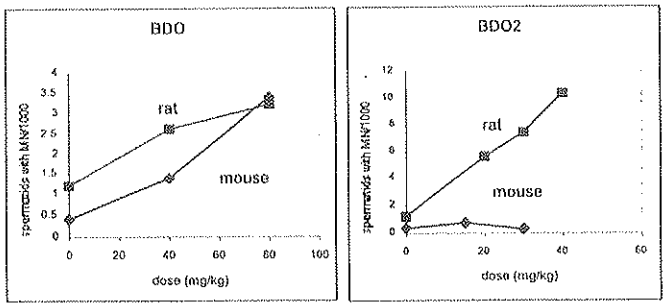
Somatic clastogenicity of BD metabolites. Comparison of mouse and rat responses



Mouse bone marrow: Adler et al., 1995; Adler et al., 1997 - Rat bone marrow: Lahdetie and Grawe, 1997 - Mouse and rat spleen: Xiao and Tates, 1995

Genotoxicity of 1,3-Butadiene. effects on chromosome integrity and chromosome segregation (Pacchierotti, 1998)

Germ cell clastogenicity of BD metabolites. Comparison of mouse and rat responses



Xiao and Tates, 1995

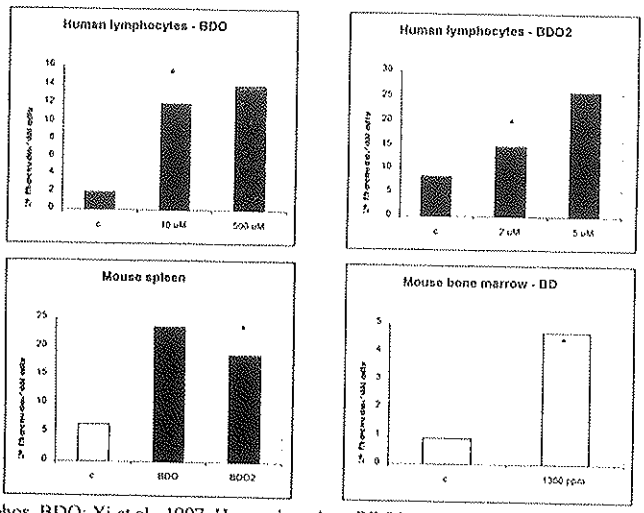
Genotoxicity of 1,3-Butadiene. effects on chromosome integrity and chromosome segregation (Pacchierotti, 1998)

Induction of dominant lethal mutations. Comparison of mouse and rat responses.

	Early deaths	Late deaths	Malformations	Ref.
Mouse				
12.5 ppm / 10 w	ns	significant increase over historical controls	significant increase over historical controls	Anderson et al., 1996
1250 ppm / 10 w	p<0.001	ns	ns	
12.5 ppm / 10 w	ns	ns	ns	Brinkworth et al., 1998
125 ppm / 10 w	p<0.01	ns	ns	
12.5 ppm / 4 w	ns	ns	ns	Anderson et al., 1998
62.5 ppm / 4 w	p<0.01	ns	ns	
130 ppm / 4 w	p<0.01	ns	ns	
Rat				
65 ppm / 10 w	ns	ns	ns	Anderson et al., 1998
400 ppm / 10 w	ns	ns	ns	
1250 ppm / 10 w	ns	ns	ns	

Genotoxicity of 1,3-Butadiene. Effects on chromosome integrity and chromosome segregation. (Pacchierotti, 1998)

Induction of chromosome loss by BD and its metabolites



Human lymphos, BDO: Xi et al., 1997. Human lymphos, BDO2: Vlachodimitropoulos et al., 1997

Mouse spleen and bone marrow: Xiao et al., 1996

Human cytogenetic biomonitoring studies of BD-exposed workers

	C (10)	E (17)	C (20)	E (10)	C (19)	E (19)
SCE	6	6.2	6	6.3		
MN	15.9	12	10.7	11	14.6	16.3
CA	2.1	2.5	2.1	2.9	2	3.1*

* $p < 0.01$

Sorsa et al., 1994; Tates et al., 1996

Summary 1/2

- In the mouse, butadiene is a potent clastogenic chemical
- Butadiene induces chromosome breakage in many different organs of inhalation-exposed mice: bone marrow, spleen, lung, testis
- The clastogenic effectiveness of butadiene metabolites can be ranked in the order BDO2 > BDO > BDO-diol
- The clastogenic effectiveness of butadiene metabolites may vary across organs and species as a function of cytotoxic processes

Summary 2/2

- In the mouse, butadiene induces health relevant effects, like heritable translocations
- In rats, butadiene is not clastogenic for bone marrow cells and does not induce dominant lethal mutations
- In humans, butadiene is possibly clastogenic for peripheral blood lymphocytes
- The clastogenic potency of BD and its metabolites can be modulated by glutathione S-transferase genotype
- Butadiene and its metabolites may also interfere with chromosome segregation

Epidemiology of 1,3-Butadiene

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Introduction

The epidemiology of 1,3-butadiene (hereafter, butadiene) concerns mainly its possible carcinogenicity. Additional potential chronic toxic effects include reproductive toxicity, on the basis of positive data in mice but no data in humans, and various disorders of the urinary, respiratory and neurological systems, based on uncontrolled observations (IARC, 1998). A single study reported minimal haematological changes among eight workers exposed to butadiene and styrene (Checkoway & Williams, 1982).

The carcinogenicity of butadiene in humans has been recently evaluated by a IARC Working Group (IARC, 1998). The conclusion was that there is limited evidence for the carcinogenicity of butadiene in humans. The overall classification of butadiene was in IARC Group 2A (probably carcinogenic to humans). This review is largely based on the document prepared for the recent IARC evaluation and focuses on the risk of lymphohaematopoietic neoplasms, since the results on the risk of other neoplasms lack consistency. The results of some of the studies have been reported in several publications: only the most recent reports are quoted here.

Data on the carcinogenicity of butadiene in humans derive essentially from studies conducted among workers employed in the production of the monomer and in the production of styrene-butadiene rubber (SBR). Additional information is available from community-based studies, in which exposure to butadiene has been estimated from employment in different industries.

1,3-Butadiene production

The mortality in a cohort of 2795 male workers who have been employed for at least six months between 1942 and 1993 in the manufacture of 1,3-butadiene in Texas,

USA has been reported (Divine & Hartman, 1996). Exposure assessment was based on job history and industrial hygiene sampling. A total of 1222 deaths were identified; the standardized mortality ratio (SMR) for all causes of death was 0.88 (95% confidence interval [CI], 0.83–0.93), that for lymphohaematopoietic neoplasms was 1.47 (95% CI, 1.06–1.98), and that for leukaemia was 1.13 (95% CI, 0.60–1.93). The SMRs for the lymphohaematopoietic neoplasms decreased with length of employment. Subcohort analyses were made for background, low and varied exposure groups based on industrial hygiene sampling. The background exposure group included persons in offices, transportation, utilities and warehouse. The low exposure group had spent some time in operating units and the varied exposure group includes those with greatest potential exposure in operating units, laboratories and maintenance. Workers in the varied exposure group had higher mortality from lymphohaematopoietic neoplasms than workers in the low exposure group (Table 1). Modelling was done using a qualitative cumulative exposure score as a time-dependent explanatory variable for all lymphohaematopoietic neoplasms, non-Hodgkin's lymphoma and leukaemia. None of these neoplasms were significantly associated with the cumulative exposure score and all risk estimates were close to unity.

This is one of the most informative studies on carcinogenicity of butadiene. In particular, strengths include the large size and the semi-quantitative assessment of exposure.

A cohort study included 364 men who worked in three 1,3-butadiene production units in the Kanawha Valley of West Virginia, USA (Ward *et al.*, 1996) . Departments included in the study were those where butadiene was a primary product and neither benzene nor ethylene oxide was present. A total of 185 deaths were observed; the SMR for all causes of death was 0.91 (95% CI 0.78-1.05). There was a non-significant increase in mortality from lymphohaematopoietic neoplasms (SMR, 1.75; 95% CI, 0.70–3.61). The increase was greater for lymphoreticulosarcoma (SMR, 5.77; 95% CI, 1.57–14.8, based on four deaths). The four deaths all had duration of employment of 2 years or more (SMR, 8.27; 95% CI 2.25-21.16). This study is of interest but suffers from limited size and lack of quantitative information on exposure.

Another cohort study was performed among 614 male employees who had worked for five years or more in jobs with potential exposure to 1,3-butadiene from 1948 to 1989

at the Shell Deer Park Manufacturing Complex in the United States (Cowles *et al.*, 1994). Industrial hygiene data from 1979 to 1992 showed that most butadiene exposures did not exceed 10 ppm as an 8-hour time-weighted average, and most were below 1 ppm, with an arithmetic mean of 3.5 ppm. For all causes of death, the SMR was 0.48 (95% CI, 0.31–0.72) and the all cancer SMR was 0.34 (95% CI, 0.09–0.87) by comparison to local (county) rates. There were no deaths from lymphohaematopoietic neoplasms (1.2 expected). The very low risk of overall and cancer mortality in this cohort raises questions on the completeness of the follow-up or other possible sources of bias (e.g., unusually strong healthy worker effect.

SBR production

In a case–control study nested within a cohort of 6678 US male rubber workers, 51 deaths from lymphohaematopoietic neoplasms and 14 deaths from lymphatic leukaemia were compared to those in a sample of the whole cohort (McMichael *et al.*, 1976). A 6.2-fold increase in risk for lymphohaematopoietic neoplasms (99.9% CI, 4.1–12.5) and a 3.9-fold increase for lymphatic leukaemia (99.9% CI, 2.6–8.0) were found in association with more than five years' work in manufacturing units producing mainly SBR during 1940–60. However, there was no attempt in this study to assess exposure to specific substances; thus, the relevance of the reported findings to the carcinogenicity of butadiene is unclear. This study suffers from the lack of any quantitative data on exposure to either butadiene or styrene.

Bond *et al.* (1992) reported a mortality study on workers engaged in the development and manufacture of styrene-based products, including styrene-butadiene latex production. The person-years of follow-up during 1970-1986 for workers in this production were 11 754. The SMR for all causes of death was 0.85 (95% CI 0.68-1.06), that for all neoplasms was 0.59 (95% CI 0.31-1.01). There was one death from lymphohaematopoietic neoplasms (2.2 expected). This study provides only limited information on butadiene exposure; in addition, the low overall cancer mortality is unusual.

Delzell *et al.* (1996) and Sathiakumar *et al.* (1998) evaluated the mortality experience of 15 649 men employed for at least 1 year between 1943 and 1991 at eight SBR plants in the United States and Canada. About 75% of the subjects were exposed to

butadiene and 83% were exposed to styrene. A total of 3976 deaths were observed (SMR, 0.87; 95% CI, 0.85–0.90). Fifteen deaths from non-Hodgkin's lymphoma were observed (SMR, 1.37; 95% CI, 0.77–2.26); there was no association with duration of employment. There were 48 observed leukaemia deaths in the overall cohort (SMR, 1.31; 95% CI, 0.97–1.74). The excess was concentrated among ever hourly subjects with 10 or more years of employment and 20 or more years since hire (28 deaths; SMR, 2.24; 95% CI, 1.49–3.23) and among subjects in polymerization (15 deaths; SMR, 2.51; 95% CI, 1.40–4.14), maintenance labour (13 deaths; SMR, 2.65; 95% CI, 1.41–4.53) and laboratories (10 deaths; SMR, 4.31; 95% CI, 2.07–7.93), i.e. three areas with potential for relatively high exposure to butadiene or styrene monomers.

Macaluso *et al.* (1996) reported an additional analysis of leukaemia mortality among 16 610 subjects employed at six of these eight North American SBR plants; 14 295 workers were included in the cohort analysis while another 2350 non-SBR workers were not. There were 58 leukaemia deaths, seven of which were reported as contributory cause of death. Retrospective, quantitative estimates of exposure to 1,3-butadiene, styrene and benzene were developed and the estimation procedure entailed identifying work areas within each manufacturing process, historical changes in exposure potential and specific tasks involving exposure, and using mathematical models to calculate job- and time period-specific average exposures. After reciprocal adjustment, the relative risks increased with cumulative butadiene exposure (p-value of test for linear trend = 0.03), while the increase in risk was less clear for styrene exposure (p-value of test for linear trend = 0.09) (Table 2).

This is the most informative study on butadiene carcinogenicity. Strengths include the large size and the detailed assessment of exposure to butadiene, styrene and benzene, in particular in the case-control analysis.

Community-based studies

Siemiatycki (1991) conducted a community-based study in Montreal in which male cases of 20 types of cancer, including non-Hodgkin's lymphoma but excluding leukaemia, were interviewed with respect to their occupational history. A group of chemists and industrial hygienists reviewed each job and assessed the exposure to about 300 agents and mixtures, including SBR. A total of 3730 cases of cancer and

533 controls were interviewed. In most analyses, each cancer site was compared with cases of other types of cancer. Four percent of study subjects, mainly construction painters, motor vehicle mechanics and shoemakers, had exposure to SBR. There was no increased risk for non-Hodgkin's lymphoma (odds ratios for any exposure: 0.9, 90% confidence interval 0.5-1.7, and for 'substantial' exposure: 1.5, 90% confidence interval 0.4-5.1). This study suffers from lack of exact knowledge on exposure circumstance, assessment of exposure to SBR rather than to butadiene and possible presence of co-exposures among subjects classified as exposed to SBR.

Conclusions

The most informative cohort study of workers exposed to butadiene showed a clear increased risk of leukaemia with a dose-response with butadiene that was not explained by other exposures (Macaluso *et al.*, 1996; Sathiakumar *et al.*, 1998). These results receive a limited support by the only other large cohort study, conducted among butadiene production workers (Divine and Hartman, 1996). The results for other lymphohaematopoietic neoplasms are inconsistent among the studies: one should keep in mind however the limitations of the additional studies, their limited statistical power and the possible misclassification between leukaemia and other lymphatic neoplasms, in particular in studies based on death certificates. One general problem of occupational epidemiological studies with limited information of exposure, as it is the case in most of the studies of butadiene exposed workers, is non-differential misclassification of exposure, that in most instance has the net effect of obscuring existing associations (i.e., a false negative result is more likely than a false positive result). In conclusion, the presence of a causal association between butadiene exposure and occurrence of cancer (leukaemia in particular) in humans is plausible: this conclusion is supported by a single large and very carefully conducted study and is compatible with the results of an additional valid, although less informative, study. The inconsistency in the results of additional studies should be seen in the light of their important limitations. The fact that only one - although the best - study clearly points towards a causal association between butadiene exposure and leukaemia in humans represents a major drawback of the available data on the epidemiology of butadiene.

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Table 1. Standardized mortality ratios and 95% confidence intervals of lympho-haematopoietic neoplasms by exposure to butadiene and duration of employment (Divine & Hartman, 1996)

Duration of employment	*Butadiene exposure*		
	Background	*Low*	*Varied*
<10 years	2.38 (0.77-5.56)	1.00 (0.40-2.06)	1.75 (1.07-2.71)
10+ years	0.56 (0.01-3.09)	0.99 (0.27-2.54)	1.66 (0.83-2.97)
Total	1.56 (0.57-3.39)	1.00 (0.50-1.79)	1.72 (1.17-2.44)

Table 2. Relative risks and 95% confidence intervals of leukaemia by cumulative butadiene and styrene exposure (Macaluso *et al.*, 1996)

Butadiene exposure (ppm-years)	*Styrene exposure (ppm-years)*			
	0.1-9	*10-39*	*40+*	*Overall*
0.1-19	1.0 (Ref.)	1.7 (0.5-6.0)	7.0 (2.2-22)	1.0 (Ref.)
20-79	3.5 (1.1-11)	2.3 (0.7-7.3)	2.7 (0.7-11)	1.5 (0.7-3.2)
80+	5.1 (1.3-21)	4.9 (1.6-15)	5.7 (2.0-16)	1.7 (0.8-3.9)
Overall	1.0 (Ref.)	0.9 (0.4-2.0)	1.6 (0.6-3.7)	

Ref. = reference category

Epidemiology of 1,3-butadiene

Paolo Boffetta
International Agency for Research
on Cancer, Lyon

Potential health effects of butadiene

- Various disorders
  - Uncontrolled reports only
- Reproductive toxicity
  - No data in humans
- Cancer
  - Leukaemia and lymphoma
  - Other neoplasms

IARC Monograph evaluation

- Limited evidence of carcinogenicity in humans
- Sufficient evidence of carcinogenicity in animals
- Other relevant data not sufficient to modify the evaluation
- Overall evaluation: group 2A (probable human carcinogen)

Volume 71, 1998

Epidemiological studies on cancer

- Butadiene production
- Styrene-butadiene rubber (SBR) production
- Community-based studies

Butadiene production
Texas study 1

- 2795 workers emp. 1942-1993
- Semi-quantitative exposure assessment
- 1222 deaths, overall SMR 0.88

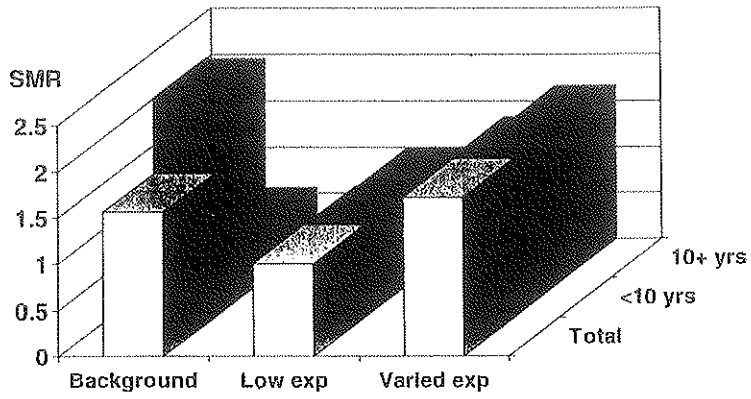
- *Large size; good exposure information*

Divine and Hartman, 1996

Butadiene production
Texas study 2

- SMR LHP neopl. 1.47 (CI 1.06-1.98)
- SMR leukaemia 1.13 (CI 0.60-1.93)
- Negative trend with duration
- Higher LHP mortality in highest exposure group, but no trend with estimated cumulative exposure

SMR of LHP by duration of employment and estimated exposure



Divine and Hartman, 1996

Butadiene production NIOSH study

- 364 workers
- 185 deaths, overall SMR 0.91
- SMR LHP 1.75 (CI 0.70-3.61), mainly LRS

- *Small size; limited exposure information*

Ward et al., 1996

Butadiene production
Shell study

- 614 workers, empl. 1948-1989
- overall SMR 0.48, all cancer SMR 0.34
- no LHP deaths (1.2 exp)

- *Small size; low mortality*

Cowles et al., 1994

SBR production
Nested case-control study

- cohort of 6678 US rubber workers
- employment in SBR:
  - LHP: RR 6.2 (CI 4.1-12.5)
  - CLL: RR 3.9 (CI 2.6-8.0)

- *Lack of quantitative data on exposure*

McMichael et al., 1976

SBR production
Cohort of latex workers

- approx. 700 U.S. workers
- SMR all causes 0.85, all cancer 0.59
- 1 LHP death (2.2 exp.)

- *Small size; low mortality*

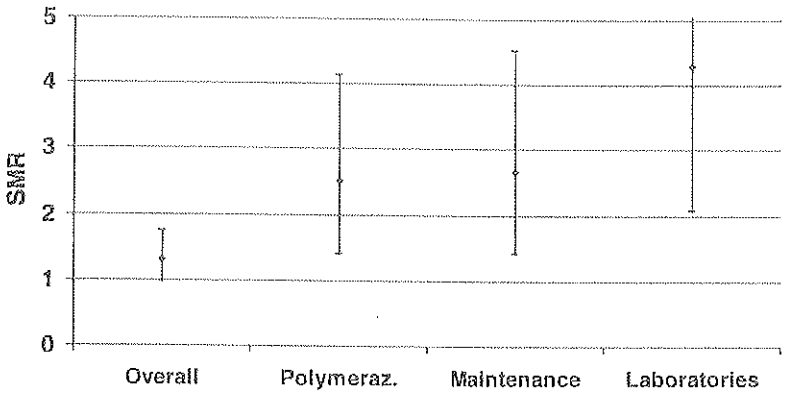
Bond et al., 1992

SBR production
Multicentric US-Canada study 1

- 15,649 workers empl. 1943-1991, 8 plants; nested case-control analysis
- 75% exp. butadiene; 83% exp. styrene
- detailed retrospective assessment of exposure to butadiene, styrene and benzene (job- and period specific exposure estimates)
- SMR all causes 0.87

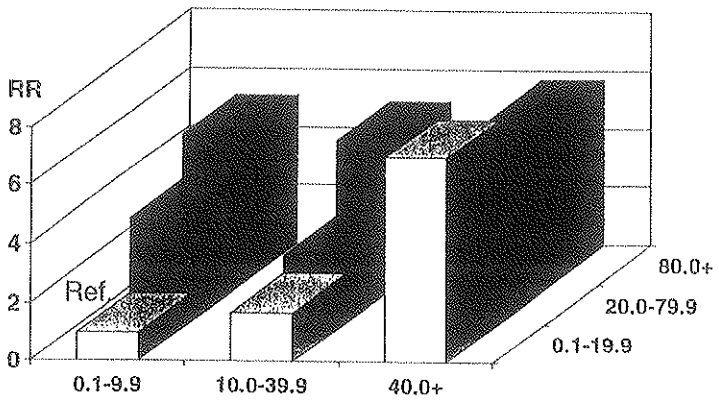
Sathiakumar et al., 1998; Macaluso et al., 1996

SMR of leukaemia by department of employment

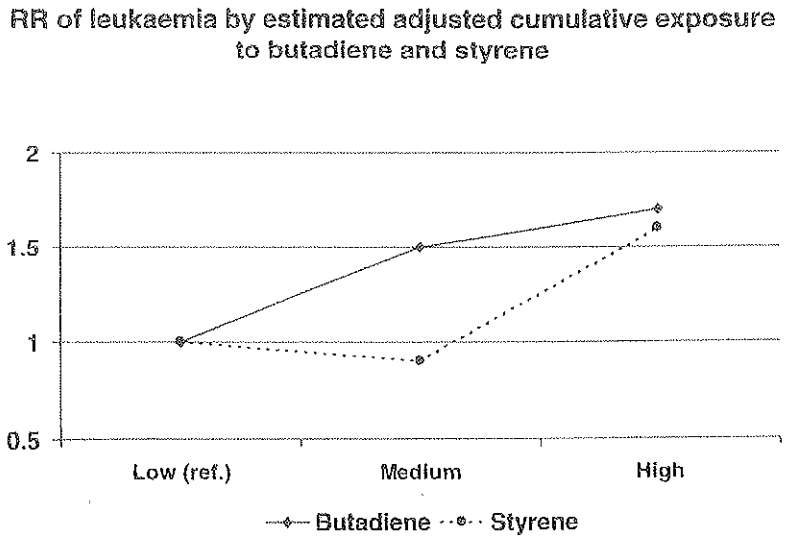


Delzell et al., 1996

RR of leukaemia by estimated combined cumulative exposure to butadiene and styrene



Macaluso et al., 1996



Macaluso et al., 1996

Montreal community-based study

- 20 types of cancer, population controls
- detailed occupational questionnaire, assessment of exposure to 300 agents
- 4% exposed to SBR
- RR NHL: any exposure 0.9 (CI 0.5-1.7)
- substantial exposure 1.5 (CI 0.4-5.1)
- *Limitation in exposure assessment; possible co-exposures*

Siemiatycki, 1991

Conclusions 1

- Association between butadiene exposure and leukaemia risk in the most informative study
- Consistent with only other good study
- Inconsistencies of other studies
  - low statistical power
  - misclassification (underestimation of risk)

Conclusions 2

- Carcinogenicity of butadiene is plausible
- Quantitative risk estimates are weak

ISSUES TO CONSIDER FOR REGULATING RISKS FROM 1,3 BUTADIENE

Seymour Garte, Department of Environmental Medicine, New York University Medical Center, N.Y. N.Y. USA

An important issue to consider in the regulation of butadiene is whether or not it can be stated with assurance that the agent is a known human carcinogen. The designation of a chemical compound mixture or industrial process as a known human carcinogen is a serious matter, which implies a series of regulatory measures in order to protect human health. For any chemical there is always the possibility of debate over quantitative issues such as dose extrapolation models, thresholds or no effect levels, mechanisms, and issues related to quantitative risk assessment. However the qualitative assessment of a carcinogen as a known, probable or possible human carcinogen is usually the starting point for both regulatory action and further discussion on the science and policy issues of regulation.

A number of scientific principles have been accepted by most agencies including the International Agency for Research on Cancer (IARC) that carry out carcinogen risk assessment of individual agents, and these principles are incorporated into the decision making process. These include: 1 The demonstration of carcinogenic activity in a non-human species is not sufficient evidence to prove carcinogenicity in man. This principle follows from a large volume of data showing that certain agents may produce tumors in rodents by mechanisms that cannot or do not function in people. Furthermore it is well known that extrapolation between species of the effects of chemicals is often difficult and sometimes impossible. 2. The demonstration of a carcinogenic effect in humans by epidemiological evidence is sufficient to define an agent as a known human carcinogen (IARC Class 1) agent, even if any other information on the chemical is lacking. This principle, which may seem to be obvious, is based on the rigorous development of cancer epidemiology as a scientific discipline capable of making and testing predictions, and allowing for definitive conclusions to be drawn from properly designed, well conducted, epidemiological studies. 3. In the absence of definitive epidemiological evidence, an agent may still be classified as a known human carcinogen if the appropriate mechanistic data is available to allow extrapolation from results in animals, or to otherwise indicate a strong logical reason to believe that *based on biological mechanisms*, the agent is carcinogenic in humans. This is the most difficult and challenging of the three principles governing classification, but it is critical, because without it, classification into group 1 would rely solely on epidemiology. Despite the value of epidemiological research, the many well known limitations of the epidemiological approach to the identification of carcinogenic compounds would make assessment of many agents impossible, if only epidemiologiacl data were to be considered.

The US EPA and the Scientific Expert Group on Ocupational Exposure Limits of DG V of the European Commission both classified butadiene as a known human carcinogen, whereas a recent IARC re-examination of the carcinogenicity of butadiene left it as a probable human carcinogen. One of the clear conclusions from the outcome of the IARC Monograph meeting was the urgent need for research to clarify the various unresolved issues regarding the epidemiological and mechanistic evidence on butadiene carcinogenicity. The areas of research that must be addressed in the near future include 1. Epidemiology - New studies are needed to take into account the

possibility of confounding exposures, and to expand the results obtained from the rubber industry into other sources of exposure. 2. Mechanistic - More precise data on the comparative metabolic pathways between species including humans, and a clearer understanding of the role of the mono and diepoxide intermediates in human cells. Also a better understanding of the identity if the active carcinogenic metabolite in animal models is needed.

Considering the uncertainties reflected in the IARC decision making process concerning butadiene, and the inconsistency in the classification of butadiene emerging from different scientific committees, regulatory agencies might justifiably sense a high degree of ambiguity regarding any consensus about the carcinogenicity of butadiene from the research community. The best remedy for this situation is a rapid, intense and highly focused research program to resolve the issues one way or the other, and to allow for a true consensus to be reached that can be used to guide the critical regulatory decisions that must be taken on this important industrial and environmental chemical.

QUESTION: CAN THE UNCERTAINITES REGARDING HUMAN CARCINOGENICITY OF BUTADIENE BE RESOLVED IN A TIMELY AND DEFINITIVE MANNER BY FURTHER RESEARCH?

ANSWER: YES

QUESTION: CAN THE SAME BE SAID ABOUT ALL UNCERTAINTIES RELATED TO CHEMICAL CARCINOGENICITY?

ANSWER: NO

HUMAN BIOMARKER ISSUES

1. METABOLITES AS MARKERS OF EXPOSURE

2. ADDUCTS AS MARKERS OF EFFECTIVE DOSE

3. MUTATIONAL EFFECTS AS MARKERS OF EFFECT

MECHANISTIC ISSUES

1. CLARIFICATION OF THE FORMATION OF THE DIEPOXIDE METABOLITE IN HUMANS

2. RESOLUTION OF GENOTOXICITY IN HUMAN CELLS, INCLUDING HRPT MUTATIONS

3. UNDERSTANDING OF THE BASIS FOR THE RAT-MOUSE DIFFERENCES, AND APPLICATION OF THE RELEVANT MECHANISMS TO HUMANS.

4. TISSUE SPECIFICITY IN METABOLISM AND DOSIMETRY

5. ROLE OF GENDER DIFFERENCES IN METABOLISM.

BUTADIENE SPECIES COMPARISONS

	MOUSE	RAT	MAN
CARCINOGENESIS	+	-	?
METABOLISM	+	-	+/-
GENOTOXICITY	+	-	+/-
CLASTOGENICITY	+	-	+/-

INTERSPECIES EXTRAPOLATION

- THERE IS ALWAYS UNCERTAINTY IN EXTRAPOLATION FROM DATA IN ANIMALS TO MAN

- THE DEGREE OF UNCERTAINTY IS INVERSELY RELATED TO THE DEGREE OF CONSISTENCY BETWEEN DIFFERENT ANIMAL SPECIES

EPIDEMIOLOGY ISSUES

1. CONFOUNDING EXPOSURES

2. ONLY ONE STRONG POSITIVE STUDY FROM ONLY ONE TYPE OF EXPOSURE (RUBBER INDUSTRY)

3. SPECIFIC CANCER TYPE WITHIN THE CATEGORY OF LYMPHOHEMATOPOIETIC CANCER

IARC MONOGRAPH MEETING ON BUTADIENE

1. Sufficient Evidence for Human Carcinogenicity based on Epidemiology -

Epidemiology subgroup - 3 yes, 2 no
Working Group - 12 yes, 19 no

2. Animal Carcinogenicity

Working Group - yes

3. Considering Animal and Mechanistic Data, Butadiene is a Known (Class 1) Human Carcinogen

Working Group - Vote 1 15 yes, 14 no
Vote 2 13 yes, 15 no

Final Conclusion: Butadiene remains Class 2A (probable), with early re-review to be considered.

ECETOC (EUROPEAN CENTRE FOR ECOTOXICOLOGY AND TOXICOLOGY OF CHEMICALS)

TASK FORCE ON BUTADIENE 1997

OEL OF 1 PPM TWA WOULD BE PROTECTIVE

STEL OF 10 PPM, 15 MIN

EUROPEAN COMMISSION DG V
OCCUPATIONAL HEALTH AND HYGIENE

SCIENTIFIC EXPERT GROUP ON
OCUPATIONAL EXPOSURE LIMITS (SCOEL)

ONGOING EVALUATION OF BUTADIENE

FROM A DRAFT DOCUMENT (NOT FINAL)
"1 TO 7 EXTRA LEUKEMIA DEATHS IN ADDITION TO 5 EXPECTED, FROM EXPOSURE TO 1 PPM BUTADIENE FOR 40 YEARS BETWEEN AGES 20 AND 65 IN 1000 MALE WORKERS"

NO OEL (OCCUPATIONAL EXPOSURE LIMIT) FOR BUTADIENE AS A KNOWN HUMAN CARCINOGEN

NO STEL (SHORT TERM EXPOSURE LIMIT)

EPA ASSESSMENT (FEB 1998)

KNOWN HUMAN CARCINOGEN

- EPIDEMIOLOGY (EXCESS LEUKEMIA)
- ANIMAL CARCINOGENICITY
- GENOTOXIC METABOLITE FORMED IN HUMANS

QUANTITATIVE RISK ASSESSMENT

0.1 PPB = 1 IN 10^6 EXCESS RISK.

RECENT RISK ASSESSMENTS

- US EPA - KNOWN HUMAN CARCINOGEN
- EC DG V- KNOWN HUMAN CARCINOGEN, NO OEL
- ECETOC - NO HAZARD BELOW 1 PPM TWA EXPOSURE
- IARC - PROBABLE HUMAN CARCINOGEN

POSTER SESSION

Posters presented current research findings regarding BD and featured recently completed research funded by the EC and HEI. Among the topics included were development of biomarkers of exposure, dose, and effect; assessments of genetic damage in animal models; assessment of exposure and genetic damage in occupationally exposed humans; and risk assessment applications. The following section presents abstracts from these posters.

BIOMARKER RESPONSES IN BUTADIENE EXPOSED CZECH WORKERS: A TRANSITIONAL EPIDEMIOLOGICAL STUDY

R. J. ALBERTINI[1] and R. J. Šrám[2]. [1]University of Vermont Genetic Toxicology Laboratory, Burlington, VT, USA, and [2]Laboratory of Genetic Ecotoxicology, Prague, Czech Republic

The current transitional epidemiological study of 1,3 butadiene workers in the Czech Republic will measure responses of eleven different biomarkers, chosen to span the continuum from worker exposure to potential genotoxic effect. Six of these biomarkers asses "exposure", four "effect" and one "individual susceptibility." Although it is advantageous to measure multiple biological responses in a single transitional study, a complication is that each has a different expression time, i.e. the time that must elapse between worker exposure and biomarker manifestation. As the purpose of a transitional epidemiological study is to asses the different biomarkers in terms of their sensitivity for detecting an external exposure (the independent variable), it is important that an appropriate reference external exposure measurement be available for each. The current study achieves this by making multiple individual worker personal exposure measurements over a period of 60-90 days prior to the collection of biological samples. Thus, biomarkers such as urinary metabolites, with short expression times (hours), and somatic mutations, with long expression times (weeks to months) and all between, will have appropriate reference external exposure measurements. In addition, area ambient measurements and peak exposure determinations are being made to develop a composite picture of butadiene dose and dose-metric for each worker in the study. This exposure assessment scheme relative to the various biomarkers will be presented.

Somatic and germ cell effects in rats and mice after treatment with 1,3-butadiene and its metabolites, 1,2-epoxybutene and 1,2,3,4-diepoxybutane

D. Anderson[1], M.M. Dobrzynska[1*], L.I. Jackson[1], T.W.-Yu[1], A.J. Edwards[1], J.A. Hughes[1] and M.H. Brinkworth[2]

[1]BIBRA International, Woodmansterne Road, Carshalton, Surrey SM5 4DS; [2]University of Münster, Institute of Reproductive Medicine of the University Domagkstrasse 11, D-48129 Münster, Germany; *Visiting fellow from National Institute of Hygiene, Warsaw, Poland

Abstract

1,3-butadiene is produced in large quantities for use in the manufacture of synthetic rubber. It is also an environmental pollutant. Exposure to butadiene has been shown to produce tumours in rats and mice and an increased risk of leukaemia in humans. Differences in responses have been reported in rats and mice possibly due to metabolic capabilities. The present studies determined somatic and germ cell effects in rats and mice after treatment with 1,3-butadiene and its metabolites 1,2-epoxybutene and 1,2,3,4-diepoxybutane using different endpoints for genotoxic effects. These included DNA strand breakage as measured in the single cell gel electrophoresis (COMET) assay in bone marrow and testicular cells, and micronuclei in bone marrow using both the acridine orange and Giemsa staining methods. Unscheduled DNA synthesis (UDS) was also measured in the testes of mice. CD-1 mice were exposed by inhalation for 6 hr/day for 5 days per week for 4 weeks and CD-1 mice and Sprague-Dawley rats to the metabolites after intraperitoneal injection. 1,3-butadiene did not affect liver, bone marrow and haploid testicular cells in mice as measured in the Comet assay at doses of 0, 12.5, 65 and 130 ppm. After treatment with 1,2-epoxybutene at doses of 0, 40, 80 and 120 mg/kg body weight, in the Comet assay there was a response in the testes in mice but not in rats, and there was little or no effect in the bone marrow in mice, but there was in rats. With 1,2,3,4-diepoxybutane in the Comet assay in mice at doses of 0, 15 and 30 mg/kg body weight, there was a response in bone marrow cells, but not in testicular cells, and in rats at doses of 0, 12.5, 25 and 50 mg/kg body weight, there was also a response only in bone marrow cells. There was an increase in micronuclei in both rats and mice with both metabolites, but clastogenicity was stronger with 1,2,3,4-diepoxybutane, occurring at lower doses than with 1,2-epoxybutene. In the UDS assay in the testes of mice, there was an

increase in response with 1,2,3,4-diepoxybutane treatment, but not with 1,2-epoxybutene. CD-1 mice exposed for 6 hr/per day, 5 days per week for 4 weeks to 0, 12.5, 65 and 130 ppm were compared to Sprague- Dawley rats also exposed by inhalation to 1,3-butadiene for 6 hr/day for 5 days per week for 10 weeks to 0, 65, 400 and 1250 ppm and examined for dominant lethal and associated effects to confirm an earlier finding in mice after a similar 10 weeks' exposure of mice (Anderson *et al.* (1996), *Toxicology* **113**, 120-127). There were positive effects in mice but no dominant lethal effects in rats. These present studies would confirm a species difference of CD-1 mice and Sprague-Dawley rats, where mice were sensitive at lower doses than rats.

word\anderson\butad-2\jld

Biomarkers for the Quantitation of 1,3-Butadiene Induced-DNA Damage in B6C3F1 Mice and F344 Rats.

* I. A. Blair[1], S. J. Kambouris[1], T. Oe[1], A. K. Chaudhary[2], R. R. Selzer[3], A. A. Elfarra[3], and L. Recio[4].

Center for Cancer Pharmacology[1], Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104, Taylor Technology, Inc.[2], Princeton, NJ, University of Wisconsin[3], Madison, WI, and Chemical Industry Institute for Toxicology[4], Research Triangle Park, NC.

Metabolism of 1,3-butadiene (BD) by CYP2E results in the formation of 1,2-epoxybutene (BDO) and 1,2,3,4-diepoxybutane (BDO_2). Both BDO and BDO_2 cause covalent modifications to DNA and they are both are genotoxic and carcinogenic. However, BD is much more carcinogenic to mice when compared with rats. Three DNA-adducts have been identified in BD-treated animals, N^7- (1-hydroxy-3-buten-2-yl) guanine (1HB2G), N^7- (2-hydroxy-3-buten-1-yl) guanine (2HB1G), and N^7- (2,3,4-trihydroxybutyl)guanine (BDO_2-G). These DNA-adducts are potential biomarkers of BD exposure and may provide insight into the species difference observed in the carcinogenicity of BD. This could ultimately make it possible to use these DNA-adducts as molecular dosimeters to assess the potential carcinogenic consequences of human BD exposure. BDO_2-G has been detected in liver and quantitated in urine of BD-exposed F344 rats and B6C3F1 mice using liquid chromatography/atmospheric pressure ionization/selected reaction monitoring mass spectrometry (LC/API/SRM/MS). 2HB1G and 1HB2G were also quantitated in the livers, kidneys and urine of the same animals using this methodology. The assays were linear with good reproducibility. The relative standard deviation ranged from 1.8% to 6.2% for the quality control samples of urinary BDO_2-G and 1.3 % to 5.6 % of 1HB2G and 2HB1G in tissue. For the urinary assay, the lower limit of detection for BDO_2-G was 200 pg/mL. For 1HB2G and 2HB1G in tissue the lower limit of detection was 6.25 pg on column. Livers from BD-exposed rats were shown to have 2 BDO_2-G adducts per 10^7 purine bases. BD-exposed rats were shown to have 30 and 3 adducts per 10^7 purine bases of 1HB2G and 2HB1G, respectively, in their liver after 5-days of exposure. Results from the livers of mice exposed to BD were quite different. The ratio of 1HB2G to 2HB1G was approximately 1:0.5 in the mouse liver compared with approximately 1:0.1 in the rat liver. In addition, there were significantly increased amonnts of both DNA-adducts in the mouse liver. These *in vivo* results were somewhat surprising since equal amounts of 1HB2G and 2HB1G were found in BDO-exposed calf thymus DNA and in the DNA of BDO-exposed TK6 cells. This rules out the possibility that regioselective incorporation of BDO into the DNA could have occurred. Therefore, the observed regioselectivity of the DNA-adducts found *in vivo* is thought to result from more efficient repair of 2HB1G present in DNA when compared with 1HB2G. It is noteworthy that increased susceptibility to the carcinogenic effects of BD was associated with an increase in BDO-derived DNA-adducts in the liver. Supported by HEI 94-4-3.

Interspecies Extrapolation of Butadiene Metabolism and Dosimetry: Relevance to Risk Assessment. James Bond, Pieter Boogaard, Gyorgy Csanády, Matthew Himmelstein, Michele Medinsky, Mark Seaton, and Lisa Sweeney. Chemical Industry Institute of Toxicology, Research Triangle Park, NC

1,3-Butadiene is a volatile gas that has high-volume usage in the synthesis of polybutadiene, styrene-butadiene, and other polymers. Due to its volatile nature, uptake of butadiene occurs almost exclusively by inhalation and absorption through the respiratory system. Sources of exposure include production, transport, and end-use processes in industrial settings or environmental exposures through automotive fuel, fossil fuel combustion, and cigarette smoke. Chronic inhalation studies established that butadiene is carcinogenic in B6C3F1 mice and Sprague-Dawley rats and that mice are considerably more sensitive than rats. The mechanisms of butadiene-induced carcinogenicity are not entirely understood but are thought to involve covalent interactions of the butadiene epoxide metabolites, epoxybutene and diepoxybutane, with DNA. The objective of the studies described here was to use quantitative in vitro-in vivo predictions of butadiene metabolism and dosimetry in rats and mice to extrapolate to humans where only *in vitro* measurements can be made. *In vitro* studies conducted in liver and lung tissues of rats, mice, and humans identified the formation of two reactive epoxide metabolites of butadiene, epoxybutene and diepoxybutane. The ratio of activation to detoxification for both of these metabolites was significantly higher (up to 10-fold) in mouse tissues compared with rat or human tissues. Experiments were also conducted in which rats and mice were exposed to a range of inhaled concentrations of butadiene (62.5 to 8000 ppm). While steady-state levels of butadiene in blood were similar between species, concentrations of epoxybutene and diepoxybutane were significantly higher in the blood and tissues of mice compared with rats. For example, blood concentrations of epoxybutene were up to 10-fold higher and diepoxybutane concentrations up to 100-fold higher in mice compared with rats. To predict concentrations of these metabolites in the blood and tissues of humans exposed to butadiene, a physiologically-based dosimetry model was developed that incorporated measured organ weights, blood flows, ventilation rates, and *in vitro* metabolic parameters for all three species. Model predictions for the concentrations of butadiene epoxides formed during and after butadiene exposure were similar to values measured *in vivo* for rats and mice, indicating the usefulness of the parameters derived *in vitro* for predicting *in vivo* behavior. When the dosimetry model was extended to humans by incorporating human physiology parameters and human metabolic constants, model predictions for concentrations of diepoxybutane were 100-fold lower than those predicted for mice and 5-fold lower than levels predicted for rats. Biochemical and molecular studies suggest that diepoxybutane may be responsible for the carcinogenic effects of butadiene. These simulations suggest that humans may be less sensitive to the carcinogenic effects of butadiene exposure compared with mice and thus at lower risk. (Research supported in part by a grant from the Chemical Manufacturers Association and the National Institute of Environmental Health Sciences.)

Determination of the dose dependent body burden of 1,2-epoxy-3-butene in male Sprague-Dawley rats and B6C3F1 mice exposed to 1,3-butadiene

V.P. Meischner, Gy.A. Csanády, J.G. Filser, GSF-Institut für Toxikologie, Neuherberg, Germany

1,3-Butadiene (BD) is used in the production of synthetic rubbers. BD is a strong carcinogen in mice but only a weak one in rats. In the first metabolic step, BD is oxidized via 1,2-epoxy-3-butene (BDO) to various further metabolic intermediates. A small share of BDO is exhaled directly. The aim of this work was to compare the dose dependent body burden of BDO in male Sprague-Dawley rats and B6C3F1 mice exposed to various BD exposure concentrations.

Rodents were exposed for 8 h in closed all-glass exposure chambers (6.4 l) to constant concentrations of BD. The exposure concentrations were between 1 and 6000 ppm (mice) and 1 and 10000 ppm (rats), respectively. BD concentrations were kept constant within a range of ± 5 % by injecting BD periodically into the air of the chambers. Gas samples were withdrawn from the exposure chamber and were analysed for BD and BDO using a gas chromatograph (Shimadzu GC-8A) equipped with a stainless steel column (2.5 m packed with Tenax TA) and a flame ionisation detector. Additionally, BDO was quantified simultaneously by a second gas chromatographic method using a Hewlett Packard GC (5890) with a Poraplot U column (25 m x 0.32 mm) coupled to a mass selective detector (HP-MSD 5972).

In both species, metabolism of BD showed saturation kinetics. The metabolic intermediate BDO was detected even at the lowest BD exposure concentration. BDO concentrations increased with duration of exposure and approached plateaus after about 2 h. These represent direct measures of the BDO body burden. In rats, BDO plateau levels ranged from 1 ppb at 1 ppm BD up to 1.3 ppm at BD concentrations above 3000 ppm. In mice, BDO plateau values were about twice as high as in rats at BD concentrations below 10 ppm. However, at BD exposures above 10 ppm this ratio increased and led to 13 times higher BDO levels in mice at 6000 ppm BU.

These results will help to explain the difference between mice and rats in the carcinogenic potency of BD.

CARCINOGENICITY OF BUTADIENE DIEPOXIDE IN B6C3F$_1$ MICE AND SPRAGUE DAWLEY RATS.

R.F. Henderson, J. M. Benson, S. A. Belinsky, E. B. Barr and F. F. Hahn. Inhalation Toxicology Laboratories, Lovelace Respiratory Research Institute, Albuquerque, NM

Reports in the literature suggest that one reason for the greater sensitivity of mice to the carcinogenicity of 1,3-butadiene is that exposed mice have much more butadiene diepoxide in their blood and tissues than do exposed rats. The purpose of this study is to determine if butadiene diepoxide (BDO_2) is as tumorigenic in rats as in mice if exposed to equivalent amounts of the agent. Female B6C3F$_1$ mice and Sprague Dawley rats, 10-11 weeks old, 56/group, were exposed by inhalation to 0, 2.5 or 5 ppm BDO_2, 6hr/day, 5 days/wk, for 6 weeks. Preliminary dosimetry studies in rodents exposed for 6 hr to 12 ppm BDO_2 indicated that blood levels would be expected to be approximately 100 and 200 pmol/g at the two exposure concentrations in the rat and twice those levels in the mouse. The mice at the high exposure level showed signs of labored breathing and 4 mice died during the last week of exposure, but these symptoms disappeared after exposures ended. Rats showed no clinical signs of toxicity during exposure but 13 rats died within 3 months of the exposure. In both species the only significant lesions were in the nose, concentrated around the main airflow pathway. Necrosis, inflammation and squamous metaplasia of the nasal mucosa, as well as atrophy of the turbinates were all present at the end of exposure to 5 ppm. Within 6 m, necrosis and inflammation subsided, but squamous metaplasia remained in the mice. In rats dying after exposure, squamous metaplasia was seen in areas of earlier inflammation and extended beyond those areas with time. The metaplasia was severe enough to cause restriction and occlusion of the nasopharyngeal duct. Later, keratinizing squamous cell carcinomas developed from the metaplastic foci in rats but not mice. These finding indicate that the metabolite of BD, BDO2, is carcinogenic in the respiratory tract of rats. Research supported by HEI, FIA No. DE-FI04-95AL86988.

Hemoglobin-Adducts as Biomarkers for Butadiene Exposure

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The reaction product of a metabolite of 1,3-butadiene, butadiene-monoepoxide, with the N-terminal valine of globin in hemoglobin was selected as a biomarker for human butadiene exposures. The method proposed by Törnqvist et al. (1986), and applied bei Osterman-Golkar et al. (1996), which uses a modified Edman degradation, has been adapted. The procedure previously developed in our laboratory to demonstrate the species differences in the bioavailability of this reactive and mutagenic metabolite in rats and mice (Albrecht et al. 1993), was not sensitive enough to be used with human blood samples. With additional purification steps, modification of the temperature program and evaluating only one diastereomer, the detection limit for GC/MS analysis of 2-hydroxy-3-butenylvaline could eventually be lowered to 0.2 pmol/g globin. Blood samples from two different occupational exposure situations were analysed and preliminary results are communicated.

From an EU-sponsered collaboration with V. Scram, Prague (Cipa CT93-0228) we obtained blood samples from workers and controls of the Kralupy plant (Czech Republic). External butadiene exposure levels measured by ambient monitoring were below 0.5 ppm. Personal samplers showed a range of 0.01-19 ppm (cf. Tates et al. 1996). The range of hemoglobin adducts is <0.2 - 1 and <0.2 - 0.9 pmol/g globin in 11 exposed and 13 controls, respectively. The mean value of the exposed (0.57 ± 0.33) is significantly higher than that of the controls (0.26 ± 0.24) at the $p < 0.05$ level with this incomplete set of data.

As part of an EU-coordination project (BMH-CT96-1640) 11 blood samples from workers of a factory in Ravenna (Italy) have so far been measured. External exposure levels in this case were below the detection limit of 0.02 ppm butadiene. The adduct levels were below the detection limit in 8 cases, at the detection limit in 2 cases, and at 0.4 pmol/g globin in 1 case.

It is concluded that the detection limit for this particular hemoglobin adduct - under favourable analytical conditions - is sufficiently low to assess the exposures with mean occupational butadiene levels somewhat below 0.5 ppm and to detect differences between background and occupational exposures. Work is in progress to adapt the method proposed by Licea Perez et al. (1997) which analyses N-(2,3,4-trihydroxybutyl)valine formed by the reaction of epoxybutanediol or diepoxybutane with the N-terminal valine of hemoglobin as a biomarker. This adduct seems to be more abundant and is possibly more relevant with respect to risk considerations.

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EC/HEI Workshop
"Health Effects of 1,3-Butadiene: Informing the Regulatory Process"
Brussels 29-30 June 1998

Abstract of poster presentation

Induction of micronuclei in lung cells of butadiene-exposed mice

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The induction of chromosome damage has been evaluated in lung cells of mice exposed to 1,3-butadiene by inhalation. Mice have been exposed for 5 days, 6 hours per day to concentrations of 500 or 1300 ppm at the inhalation facility of the GSF Institute of Saugetiergenetik, Neuherberg. Twentyfour hours after the end of exposure, the animals have been sacrificed and a suspension of lung cells has been prepared by mechanic/enzymatic treatment (1). The percentage of viable cells was consistently around 80 %. Aliquots of lung cells have been seeded on square coverslips and cultured for 72 hours in Williams medium E, the last 48 hours in the presence of cytochalasin B. At the end of this period, cells attached to the coverslips have been fixed and stained with Giemsa or propidium iodide and immunofluorescent antikinetochore antibodies for the quantification and characterization of micronuclei in binucleate cells. The frequency of cells with micronuclei was significantly higher ($p < 0.001$) in butadiene-exposed than in sham-exposed mice. A linear dose-effect relationship could be fitted to the data. Frequencies of kinetochore-positive and kinetochore-negative micronuclei were both significantly increased above the control level, suggesting that micronuclei originated during the first in vitro cell division were the consequences of both chromosome structural damage and disturbance of chromosome segregation.

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Partially supported by CEC contract EV5V-CT94-0543 within the Environment and Climate Programme of the European Union.

DNA adducts of the epoxy metabolites of 1,3-butadiene

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INTRODUCTION

1,3-Butadiene (BD) is carcinogenic to rodents. It is metabolized to three epoxides which are genotoxic. Butadiene monoepoxide (BMO), diepoxybutane (DEB) and diolepoxybutane (BDE) are capable to form DNA adducts. Aliphatic epoxides bind mainly at the N7 position of guanine. Butadiene derived epoxides exist as stereoisomers and stereoisomers may possess different genotoxic properties.

MATERIALS AND METHODS

Deoxyguanosine and 3' or 5' dGMP and DNA was exposed to BMO, DEB and BDE. Guanine N7 adducts were purified by HPLC and characterized by NMR and MS. Synthesized markers were used in the analyses of guanine N7 alkylation products *in vitro* and *in vivo*. Analysis were performed by ^{32}P-postlabeling and HPLC equipped with UV and radioactivity detectors. Mice were exposed to 1,3-butadiene through inhalation (5d, 6h/d).

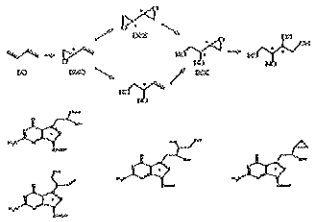

Fig 1. The BD metabolism and the corresponding guanine N7 adducts.

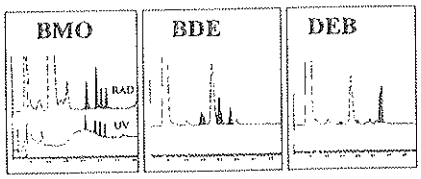

Fig 2. The adducts of BD metabolites analyzed by HPLC equipped with radioactivity detector.

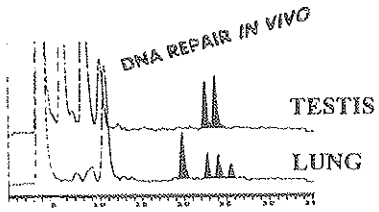

Fig 3. BMO derived N7 guanine adducts in mouse lung and testis after inhalation exposure to BD.

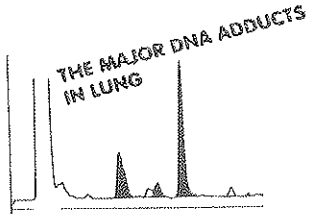

Fig 4. BDE derived N7 guanine adducts in mouse lung after inhalation exposure to BD.

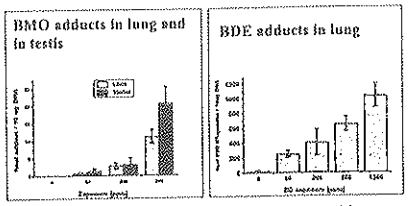

Fig 5. BMO and BDE derived guanine adducts are formed in a dose response manner *in vivo*.

CONCLUSIONS

- BMO, BDE and DEB produced specific guanine N7 alkylation products *in vitro*
- DEB adducts were not hydrolyzed to trihydroxy adducts in DNA -they depurinate
- BMO and BDE adducts were formed in a dose-response manner *in vivo*
- two BMO adducts out of four were not repaired in mouse testises
- BDE derived N7 guanine adducts are the most prevalent adducts in mouse lung

ACKNOWLEDGEMENTS

Financial support for the study was obtained from the project EV5V-CT94-0543 of the DG XII of European Communities coordinated by Francesca Pacchierotti.

ANALYSIS OF MUTAGENICITY AND DETERMINATION OF MUTATIONAL SPECTRUM IN RODENT AND HUMAN CELLS TO ASSESS THE ROLES OF EPOXYBUTENE AND DIEPOXYBUTANE IN MEDIATING THE IN VIVO GENOTOXICITY OF 1,3-BUTADIENE.

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1,3-Butadiene (BD) is carcinogenic and mutagenic in mice. The goal of this research was to determine the mutagenicity and the mutational spectrum of epoxybutene (EB) and diepoxybutane (DEB) in human and rodent cells in vitro and in vivo to assess their roles in the mutagenicity and mutational spectrum of the parent compound BD. In human TK6 lymphoblastoid (TK6) cells, both EB and DEB were mutagenic and induced an increased frequency of A:T→T:A transversions (Fisher's exact test; $P<0.05$), DEB also induced an increased frequency of *hprt* deletions ($P=0.05$). In *lacI* transgenic Rat2 cells, EB was mutagenic inducing an increased frequency of mutation at A:T base pairs including an increased frequency of A:T→T:A transversions. DEB was not mutagenic at the *lacI* transgene in Rat2 cells but did increase the frequency of micronuclei. Inhalation exposure of B6C3F1 *lacI* transgenic mice and Fisher 344 *lacI* transgenic rats to EB (25.0 ppm; 6 h/day; 5 days/wk × 2 weeks) did not increase the *lacI* mutant frequency in bone marrow or spleen of mice or in the spleen of rats. An increased *lacI* frequency was observed in the bone marrow of rats exposed to EB. Inhalation exposure of B6C3F1 *lacI* transgenic mice and Fisher 344 *lacI* transgenic rats to DEB (4.0 ppm; 6 h/day; 5 days/wk × 2 weeks) did not increase the *lacI* mutant frequency in bone marrow or spleen. In human cells and in Rat2 cells EB induces an increased frequency of mutations at A:T base pairs. This demonstrates the induction of a consistent mutation across biological systems induced by EB and indicates that EB but not DEB is mediating the mutational spectrum at the *lacI* transgene in animals exposed to the parent compound BD. This hypothesis is strengthened by the lack of detectable mutagenicity by DEB at the *lacI* transgene in vitro and in vivo that is likely due to the poor recovery of large deletions using this mutagenicity assay. These data demonstrate that DNA adducts at A:T base pairs may be useful biomarkers for BD genetic effects. However, other DNA lesions that can account for BD-induced deletions also need to be considered as biomarkers for BD-induced genotoxicity.

CHROMOSOMAL ABERRATIONS, SISTER-CHROMATED EXCHANGES, CELLS WITH HIGH FREQUENCY OF SCE, MICRONUCLEI, AND COMET ASSAY PARAMETERS IN 1,3-BUTADIENE EXPOSED WORKERS

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The association of occupational exposure to 1,3-butadiene (BD) and induction of cytogenetic damage in peripheral lymphocytes was studied in 19 male workers from a monomer production unit and 19 control subjects from a heat production unit. The exposure to BD was measured by passive monitors 3MN, type 3520. The following biomarkers were used : chromosomal aberrations (CA), sister chromatid exchanges (SCE), cells with a high frequency of SCE (HFC), micronuclei, comet assay parameters like tail length (TL), and % of DNA in tail (%T), and genotyping GSTM1 and GSTT1. BD exposure to 0.53 mg/m^3 (range 0.024–23.0 mg/m^3) significantly increased a) the percentage of cells with chromosomal aberrations in exposed vs. control groups (3.11% vs. 2.03%, $P < 0.01$, b) the frequency of SCEs per cell (6.99 vs. 4.75, $P<0.01$), and c) the percentage of HFC (20.2% vs. 4.1%, $P<0.01$). BD exposure had no significant effects on production of micronuclei or modification of comet assay parameters. Smoking enhanced the effect of BD exposure with respect to induction of SCE and HFC. The data collected suggest that smoking increased the adverse effect of BD exposure.

These results are the first data revealing convincing evidence that chromosomal aberrations and SCE are induced in the peripheral lymphocytes of humans exposed at the generally low level (1μg/m^3) in ambient air in the BD-manufacturing industry.

Supported by the EC grant ERBCIPA-CT93-0228 and the grant from the Regional Institute of Hygiene of Central Bohemia.

QUANTITATION OF EPOXYBUTENE AND TRIHYDROXYBUTANE HEMOGLOBIN ADDUCTS WITH IN EXPOSED AND CONTROL MICE, RATS AND HUMANS.

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Butadiene (BD) is an important industrial chemical used largely in the manufacture of synthetic rubber and thermoplastic resins. It has been identified in cigarette smoke, automobile exhaust, and gasoline vapor. The objective of this research is to develop highly sensitive and specific assays for the detection and quantitation of hemoglobin adducts of epoxybutene, diepoxybutane and epoxybutanediol. We have successfully developed an assay for N-(2-hydroxy-3-butenyl)valine (HBVal) and N-(2,3,4-trihydroxybutyl)valine (THBVal) in hemoglobin. HBVal and THBVal were measured in control and exposed mice and rats (1000 ppm BD, 13 weeks, 6 h/d, 5 d/w). The same adducts were also monitored in smoker and non-smoker humans. The adducts measured were the two diastereomers (named isomer I and II) of HBVal and the three diastereomers of THBVal, named isomer I, II and III. The study utilized the modified Edman degradation method of Tornqvist et al (1986) plus Centricon-30 ultrafiltration and washing on C_{18} columns, followed by GC-MS quantitation (following acetylation for THBVal). For the HBVal assay an authentic internal standard globin alkylated with [$^2H_6$]-EB was used, and for the THBVal a synthesized internal standard THB[$^{13}C_5$]Val was used post-Edman degradation. The amounts of HBVal measured in exposed mice and rats (in pmol/g globin) were 9,600 ± 1,800 and 6,970 ± 1,670 (n=4) for female and 7,020 ± 1,110 and 5,430 ± 700 (n=5) for male mice for isomer I and II, respectively. The corresponding values for rats were 4,910 ± 490 and 3,630 ± 440 (n=6) for female and 3,150 ± 1270 and 2,340 ± 1080 (n=3) for male rats, respectively. The amounts of THBVal in male mice were 22,596 ± 4,616, 23,501 ± 3,901, and 36,339 ± 5,538 (n=3) and in females they were 15,394 ± 441, 16,592 ± 48, and 24,108 ± 551 (n=2) for isomer I, II and III, respectively. In male rats, the detected values were 2,833 ± 540, 2,928 ± 540, and 4,610 ± 819 (n=3) and in females the values were 6,223 ± 1,239, 6,364 ± 1,174, and 9,574 ± 2,070 for the same isomers. In control mice (male) the levels of THBVal were 9.7, 11.4, and 5.6 and in control rats (male) were 4.6, 6.9, and 3.4. HBVal was analyzed in one control human globin sample (male, lab worker, non-smoker) at 2.7 and 1.9. We measured the amounts of THBVal in 11 non-occupationally-exposed individuals (7 non-smokers and 4 smokers). The mean values for the three isomers of THBVal were 14.7 ± 6.4, 14.2 ± 7.5, 6.7 ± 2.7 pmol/g and 35.7 total for non-smokers and 15.6 ± 3.2, 17.0 ± 3.0 and 9.7 ± 2.7 pmol/g and 42.3 total for smokers. The difference between smokers and non-smokers was not significant. These data demonstrate that the methods developed in this project have adequate sensitivity to permit human biomonitoring for BD hemoglobin adducts. This method has been applied to a molecular epidemiology study of Chinese workers and was able to discriminate between exposed and non-exposed workers.

INDUCTION OF MICRONUCLEI AND POINT MUTATIONS IN LYMPHOCYTES AND MALE GERM CELLS OF RATS AND MICE EXPOSED TO BUTADIENE AND TWO OF ITS METABOLITES

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OBJECTIVES:

- to study the genotoxic potential of 1,3-butadiene (BD) and its metabolites 1,2-epoxybutene (EB; monofunctional) and 1,2,3,4,-diepoxybutane (DEB; bifunctional)
- to investigate whether differences exist in genetic sensitivity between rats and mice
- to contribute to the estimation of genetic risks for man following occupational exposure to BD

Methodology I:

Rodent species investigated:
Male rats: Lewis
Male mice: (102/E1 x C3H)F1, CD1 or C57Bl

Exposure routes:
BD: via inhalation
EB: via one or more intraperitoneal injections or via drinking water
DEB: via intraperitoneal injection or via drinking water

Methodology II:

Organs/cells studied:

- lymphocytes from spleen (=splenocytes)
- immature male germ cell stages such as spermatogonia and young/old spermatocytes

Methodology and results obtained are described in:
Tates A.D. et al., Mutation Res. 339 (1994) 249-258
Xiao Y. and Tates A.D., Environ. Mol. Mutagen., 23 (1994) 60-68
Xiao Y. et al., Mutation Res., 324 (1994) 49-57
Tates A.D. et al., Mutation Res. 297 (1993) 21-35

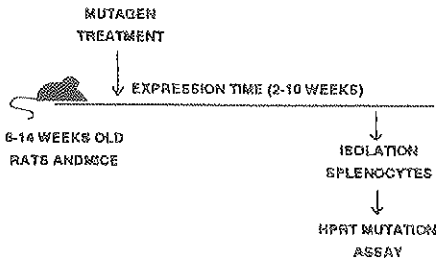
Types of genetic parameters studied:

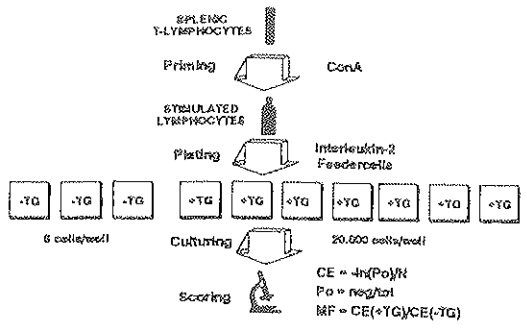
A) Indicators for induction of chromosomal damage
Somatic cells:
- Micronuclei in cytochalasine-B blocked binucleated splenocytes
- Analysis of centromeres in micronuclei of mouse and rat splenocytes to differentiate between the clastogenic potential of (=chromosome breakage) and aneugenic potential (=induction of numerical chromosomal changes) of the test chemical

Germ cells:
- Micronuclei in early spermatids derived from treated spermatogonia and spermatocytes

B) Indicator for induction of point mutations
HPRT mutations in splenocytes
(HPRT stands for the enzyme Hypoxanthine Phosphoribosyl Transferase)

IN VIVO MUTAGEN TREATMENT





CONCLUSIONS CONCERNING INDUCTION OF CHROMOSOMAL DAMAGE IN SPLENOCYTES:

- BD induced damage in splenocytes of mice (rats not studied)
- EB induced more damage in mice than in rats
- DEB induced equal amounts of damage in rats and mice
- DEB induced more damage than EB (on a molecular basis)
- Centromere staining indicated that BD, EB and DEB were predominantly clastogens in rats and mice, but also weak aneugens in mice

CONCLUSIONS CONCERNING INDUCTION OF CHROMOSOMAL DAMAGE IN IMMATURE MALE GERM CELLS:

- BD induced chromosomal damage in young spermatocytes of mice (rats were not studied)
- The monofunctional EB and the bifunctional DEB metabolites induced chromosomal damage in rats and mice
- DEB always induced more chromosomal damage than EB
- EB and DEB induced chromosomal damage appeared to be more prominent in rats than in mice

CONCLUSIONS CONCERNING INDUCTION OF HPRT MUTATIONS IN SPLENOCYTES:

- BD induced a dose dependent increase of mutations in mice (rats not studied)
- EB was mutagenic in mice but not in rats
- DEB was not mutagenic in our study with adult rats and mice. However, other investigators (Recio, Boorman and Watson) reported that DEB was mutagenic in pre-weanling or adolescent rats and mice. Thus age of the animals at the time of treatment plays a role in the realization of mutational damage.
- Although we observed no mutation induction in EB treated rats and DEB treated rats or mice, it needs to emphasized that there was substantial induction of chromosomal damage in splenocytes and immature germ cells under the same treatment conditions.

Methodology and results obtained are described in:

Tates, A.D. et al., Mutation Res.,309 (1994) 299-306
Xiao, Y. and Tates, A.D., Environ. Mol. Mutagen., 26 (1995) 97-108
Xiao, Y. et al., Mutation Res., 354 (1996) 49-57
Tates, A.D. et al., Mutation Res. 397 (1998) 21-36

IN VIVO MUTAGENESIS OF 1,3-BUTADIENE AND ITS METABOLITES IN THE ENDOGENOUS *HPRT* GENE OF MICE VERSUS RATS. Vernon E. Walker and Quanxin Meng, Wadsworth Center, New York State Department of Health, Albany, NY

1,3-Butadiene (BD), is an indirect alkylating agent with the potential to induce DNA damage, mutation, and cancer. To provide a better understanding of the mutagenic mechanisms involved in the species differences in BD-induced oncogenesis, studies were conducted in rodents to test the hypotheses that (a) the mutagenic potency of BD at the *Hprt* locus of T-lymphocytes can be used to indicate the species differences in BD carcinogenicity in exposed rodents and (b) comparison of the mutagenic potency and specificity of BD and its epoxy metabolites at Hprt of T-cells can be used to define the relative contribution of each intermediate to BD mutagenicity in each species. The first hypothesis was investigated by determining the effects of exposure duration and time elapsed after exposures on the frequencies of *Hprt* mutations (*Mf*s) in T-cells from thymus and spleen of female B6C3F1 mice and F344 rats (4—5 weeks old) exposed by inhalation to 0 or 1250 ppm for up to 2 weeks or to 0 or 625 ppm BD for up to 4 weeks (6 h/day, 5 days/week). The second hypothesis was examined by defining the effects of exposure concentration and time elapsed after exposures on the *Hprt* *Mf*s in splenic T-cells from mice and rats exposed to BD (0, 20, 62.5, or 625 ppm), 1,2-epoxybutene (BDO) (0, 2.5, or 25 ppm), or 1,2,3,4-diepoxybutane (BDO_2) (0, 2, or 4 ppm) for 4 weeks by inhalation (6 h/day, 5 days/week). *Hprt* *Mf*s were measured weekly or biweekly for up to 10 weeks post exposure, using the T-cell cloning assay. The mutagenic potency of BD in mice [represented by the difference in the areas under the mutant T-cell 'manifestation' curves (or the 'change in mutant frequencies over time') of treated versus control animals] was 4.4-fold greater than in rats following 2 weeks of exposure to 1250 ppm BD and 8.5-fold greater than in rats following 4 weeks of exposure to 625 ppm BD. These *Hprt* *Mf* data demonstrated for the first time that BD is mutagenic in the rat, albeit the mutagenic response was significantly less than that observed in similarly exposed mice. The relative contribution of BDO versus BDO_2 to BD mutagenicity was evaluated by exposing mice and rats to carefully chosen concentrations of BD, BDO, and BDO_2 (that is, 62.5, 2.5, and 4.0 ppm, respectively) and comparing the mutagenic potency of each compound when comparable blood levels of metabolites are achieved. The resulting *Mf* data indicate that at lower BD exposures ($\leq$ 62.5 ppm) BDO_2 is a major contributor to the mutagenicity of BD in mice, whereas the available metabolism and current Hprt mutation data combined suggest that other metabolites are likely to be responsible for mutations in BD-exposed rats and for the incremental mutagenic effects at higher exposures to BD in mice. The role of BDO_2 in the mutagenic action of BD in mice and rats was further evaluated by characterizing the mutational spectra of BD and BDO_2 in *Hprt* exon 3, and these mutational

spectra data correlated well with the findings in the mutagenic potency studies. Results of these mutagenicity experiments, along with data from collaborative studies of DNA adducts from the same animals, should provide a better understanding of the species variation in tumorigenic response to BD and improve the extrapolation of rodent data to the estimation of cancer risk in exposed people. *Supported in part by HEI Agreement No. 94-6.*

PANEL: BUTADIENE RISK ASSESSMENT IN THE REGULATORY FRAMEWORK

The following section presents abstracts from a panel discussion of current worldwide evaluative and regulatory activity concerning BD.

EC/HEI Joint Workshop on 1,3-Butadiene - Brussels 29/30 June 1998
Overview of current and developing regulations for 1,3-Butadiene
MG Penman - Exxon Biomedical Sciences Inc.

Up to the early 1980's 1,3-butadiene was generally regarded as a highly reactive gas that could, on exposure to oxygen, polymerise readily leading to the formation of potentially explosive peroxides. For this reason the major cause for concern, and regulation, was driven by its potential flammability and explosivity. Whilst this is still an immediate concern for those running facilities using butadiene, the cancer bioassays done in the late 1970's by the International Institute for Synthetic Rubber Producers (IISRP) and subsequently by the US National Toxicology Program, gave rise to concerns about the potential carcinogenicity of the material. At about the same time epidemiology studies of styrene butadiene rubber workers and butadiene monomer workers were initiated. Subsequently, almost all the regulatory activity on butadiene has been directed at this considerable body of data and weighing its relevance to man. In 1996 with the release of the IISRP sponsored epidemiological study by Delzell *et al* the debate was rekindled. Most recent standard setting processes have been based upon the epidemiological data using the available animal and mechanistic information as support. The rate of research from academia, governments and industry has been remarkable and continues as our understanding of the biological mechanism of action of this economically important molecule grows. Achieving a balance in this ever shifting sea of data has been a major challenge to regulator, community and industry alike. New research will undoubtedly impact upon our understanding and effect the future regulatory scene. In the following paragraphs there follows a very brief guide to the major types a regulatory steps already considered for butadiene and their current status.

Classification

A major amount of regulatory and scientific energy is expended in classifying substances into categories of inherent hazard. This classification process, is often driven by rules or definition of effects then drives subsequent regulatory action. For example, within the EU, butadiene's current classification as a Category 2 carcinogen, (Substances which should be regarded as if they are carcinogenic to man) automatically means that the provisions of the Carcinogens Directive apply, which include effective prohibition from consumer products and substitution wherever possible. Butadiene's current classification also evokes other EU Directives such as the Marketing and Use Directive, Young persons Directive, Pregnant workers Directive, and Seveso Directive. Other national regulations may also be evoked such as the UK's Control of Substances Hazardous to Health code on carcinogens. The EU's current classification of butadiene is currently being revisited following recommendations from the UK in their recent risk assessment. Within the USA the EPA's current classification of butadiene is as a "Probable human Carcinogen". The WHO's International Agency for Research on Cancer (IARC) have a major programme to review all the available relevant data on substances and classify them with respect to their potential human carcinogenicity. It is a highly respected process and although it has no regulatory force in itself, it is often adopted by regulatory bodies for subsequent regional or local action. Butadiene was assessed in 1992 and more recently in February 1998 when it was concluded that the classification 2A, "Probably carcinogenic to humans", was still appropriate.

Risk Assessment

Another major regulatory activity is risk assessment. Within the EU there is now a defined process (within the context of the Existing Substances Regulation) that undertakes to define the hazard of a substance, determining at what concentration health (and environmental) effects may occur and then comparing these to actual or modeled exposures to approximate the margin of safety for human and environmental populations. By examining a wide range of scenarios of potential exposure or release, it is possible to determine if further controls are required to protect man and the environment in particular situations or if more information is required to narrow uncertainties. Within the US the approach is more numerically and individually driven, in that they attempt to define a concentration at which for chronically exposed individuals there will be a set level of risk, usually one in a million. At the present time (June 1998) the EU (with the UK acting as the rapporteur), the US EPA and Health / Environment Canada are involved in risk assessments on butadiene which should complete their respective regulatory processes during 1998.

Occupational exposures

Within the USA, the Occupational Safety and Health Administration (OSHA) reviewed occupational exposure to butadiene in 1996 and published a new Permitted Exposure Limit (PEL) of 1ppm 8 hour time weighted average (TWA) with a short term exposure limit (STEL) of 5 ppm TWA 15 minutes. The non governmental US organisation the ACGIH publishes a list of Threshold Limit values for occupational exposures which are widely adopted by many countries as the *de facto* standard. Their current value is 2ppm 8 hour TWA. In the EU there are a number of standards in operation although the process for harmonisation is under way. Some countries use the ACGIH standards whilst others have national mechanisms. Butadiene is currently being reviewed by DG V's Scientific Committee on occupational exposure limits (SCOEL). ECETOC acted as contractor to SCOEL providing a criteria document and an initial proposal for an OEL. SCOEL are currently reviewing its position and a final decision is expected this year (1998).

Air Quality Standards (AQS)

Within the EU the UK is the only country which has developed a published recommendation for an AQS for butadiene with the aim of implementing a 1ppb as a running annual average. However, the EU Integrated Pollution Prevention and Control (IPPC) Directive effectively legislates for major point source releases within Member States. In the US, butadiene is on the Clean Air Acts 1990 list of 188 Hazardous Air pollutants (HAPS).

Future

There is considerable current regulatory activity on butadiene covering most aspects of its production and use. Running parallel to the time line of regulatory processes, and acting upon it, is the ongoing research which challenges our theories on the biological activity of butadiene, giving a scientific basis for risk assessment and decision making. Such major ongoing work as the HEI collaborative transitional epidemiological study, as well as work on process cofounders, will continue to offer continued improvement in risk characterisation and assessment.

THE EXISTING CHEMICALS REGULATION 793/93

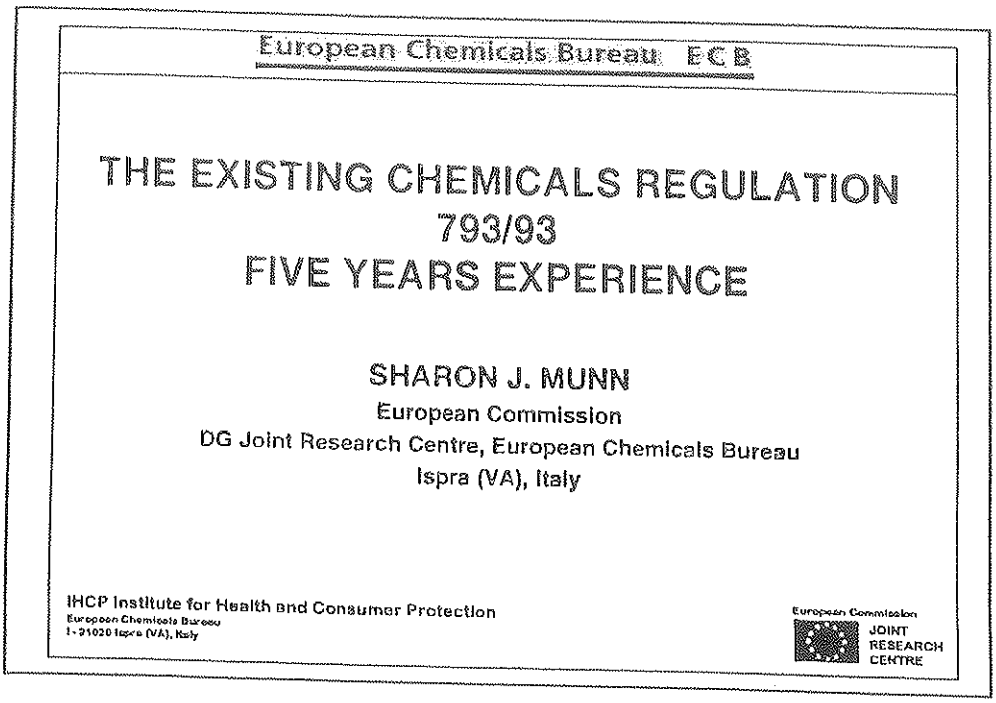
Five years experience

Sharon J Munn, European Chemicals Bureau, Ispra (VA), Italy

Council Regulation (EEC) 793/93 was adopted to provide a legal framework for the evaluation of the risks posed to the environment and to human health by existing chemical substances (i.e. those listed in EINECS). The regulation requires that the evaluations be carried out in four steps: a data collection step, a priority setting step, a risk assessment step, and a risk reduction step. All the data submitted by industry on EINECS substances produced or imported into the EU at over 1000 tonnes per producer/importer per year (the so-called High Production Volume Chemicals, HPVCs) have been consolidated to create the IUCLID database, thus completing the first two phases of the data collection procedure. 10,822 diskettes were submitted on 2471 substances.

Phase III, required the submission of a reduced data set for the so-called Low Production Volume Chemicals (LPVCs), with a deadline for industry of 4th June, 1998. The loading of these data is currently in progress at the European Chemicals Bureau. In the meantime, there has been intensive activity on the 110 HPVCs prioritised for risk assessment, involving submission of all original test reports from industry, preparation of risk assessment reports by Member State rapporteurs and discussion of the reports within the EU and OECD. Of the 40 draft risk assessment reports submitted for discussion, conclusions have been agreed for 19. For 14 of these substances, risk reduction measures are recommended; for 3 further testing is required; and for 2 substances no risks were identified on the basis of current data.

A Commission Recommendation concerning the results of the risk evaluation for 4 substances, including risk reduction strategies for three of them, is expected to be published by the end of 1998. For two substances voluntary agreements are foreseen. For one substance, restrictions on its marketing and use are recommended, through appropriate modification of 76/769/EEC.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THE EXISTING CHEMICALS REGULATION
793/93
FIVE YEARS EXPERIENCE
SHARON J. MUNN
European Commission
DG Joint Research Centre, European Chemicals Bureau
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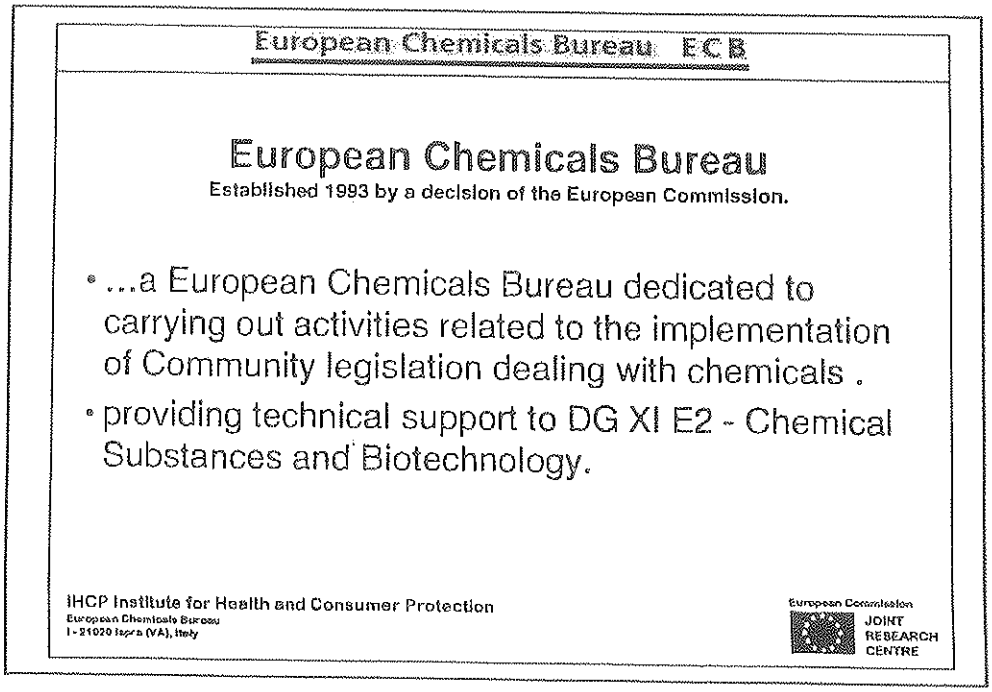
European Chemicals Bureau ECB
European Chemicals Bureau
Established 1993 by a decision of the European Commission.
• ...a European Chemicals Bureau dedicated to carrying out activities related to the implementation of Community legislation dealing with chemicals .
• providing technical support to DG XI E2 - Chemical Substances and Biotechnology.
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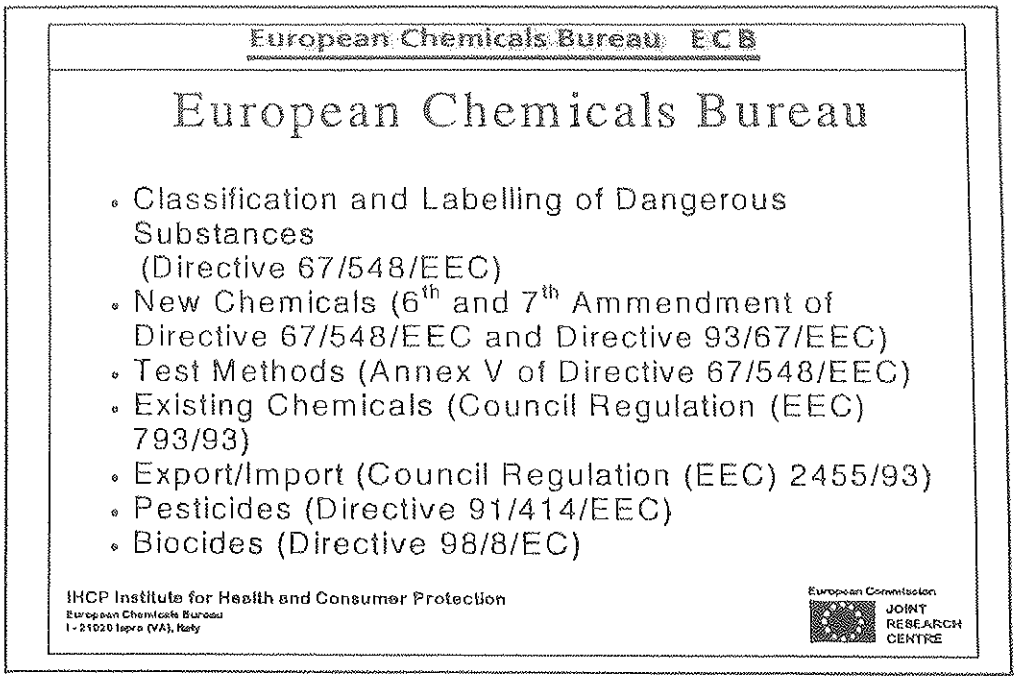
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European Chemicals Bureau
• Classification and Labelling of Dangerous Substances (Directive 67/548/EEC)
• New Chemicals (6th and 7th Ammendment of Directive 67/548/EEC and Directive 93/67/EEC)
• Test Methods (Annex V of Directive 67/548/EEC)
• Existing Chemicals (Council Regulation (EEC) 793/93)
• Export/Import (Council Regulation (EEC) 2455/93)
• Pesticides (Directive 91/414/EEC)
• Biocides (Directive 98/8/EC)
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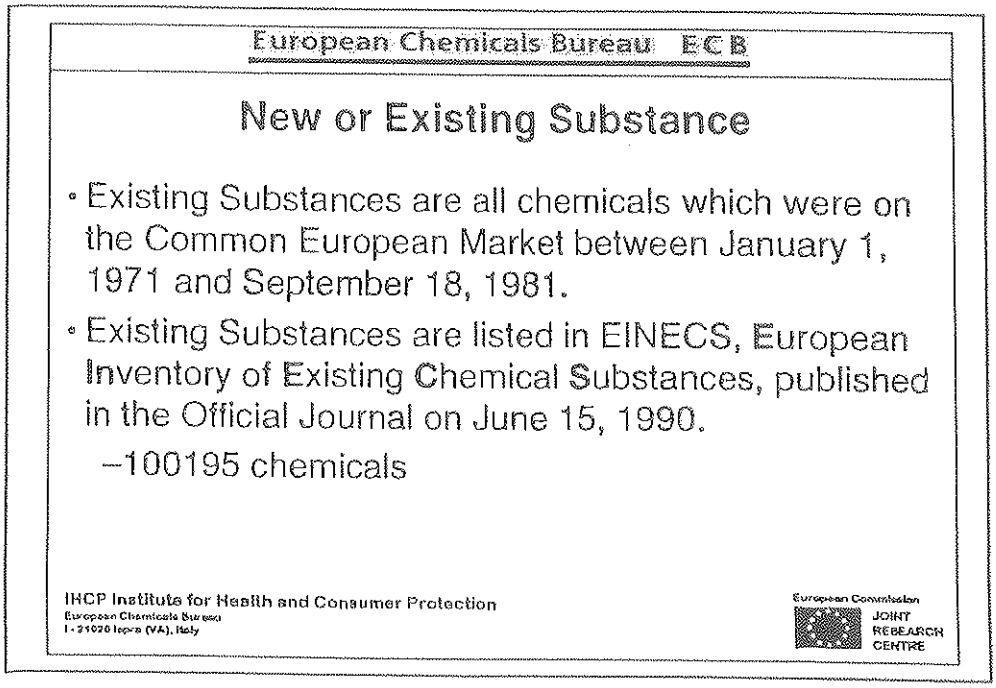
European Chemicals Bureau ECB
New or Existing Substance
• Existing Substances are all chemicals which were on the Common European Market between January 1, 1971 and September 18, 1981.
• Existing Substances are listed in EINECS, European Inventory of Existing Chemical Substances, published in the Official Journal on June 15, 1990.
–100195 chemicals
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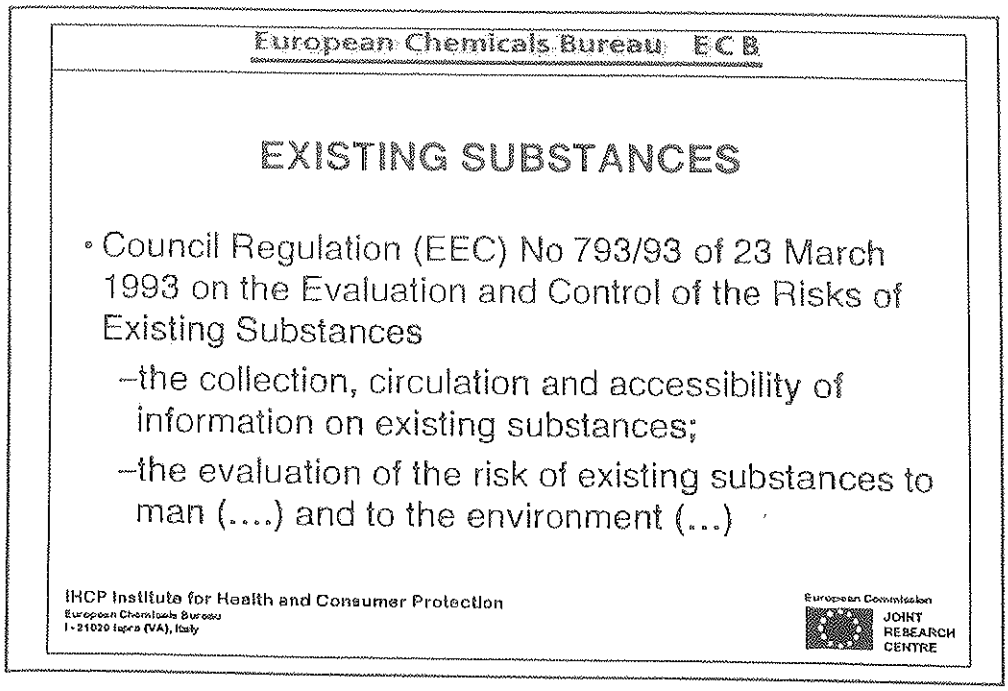
European Chemicals Bureau ECB
EXISTING SUBSTANCES
• Council Regulation (EEC) No 793/93 of 23 March 1993 on the Evaluation and Control of the Risks of Existing Substances
–the collection, circulation and accessibility of information on existing substances;
–the evaluation of the risk of existing substances to man (....) and to the environment (...)
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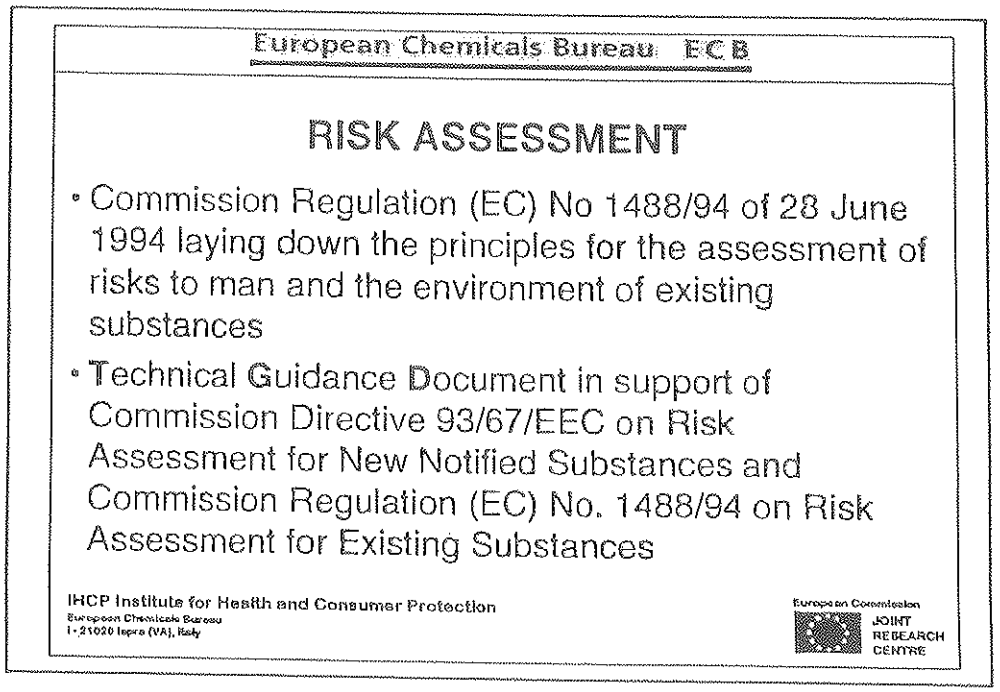
European Chemicals Bureau ECB
RISK ASSESSMENT
• Commission Regulation (EC) No 1488/94 of 28 June 1994 laying down the principles for the assessment of risks to man and the environment of existing substances
• Technical Guidance Document in support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No. 1488/94 on Risk Assessment for Existing Substances
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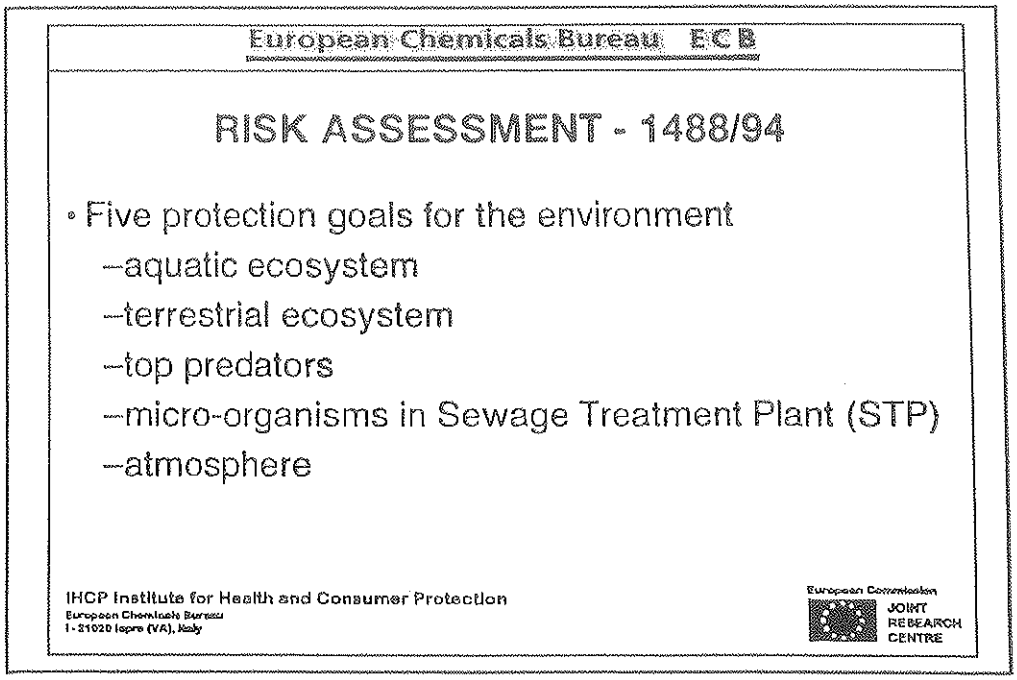
European Chemicals Bureau ECB
RISK ASSESSMENT - 1488/94
• Five protection goals for the environment
–aquatic ecosystem
–terrestrial ecosystem
–top predators
–micro-organisms in Sewage Treatment Plant (STP)
–atmosphere
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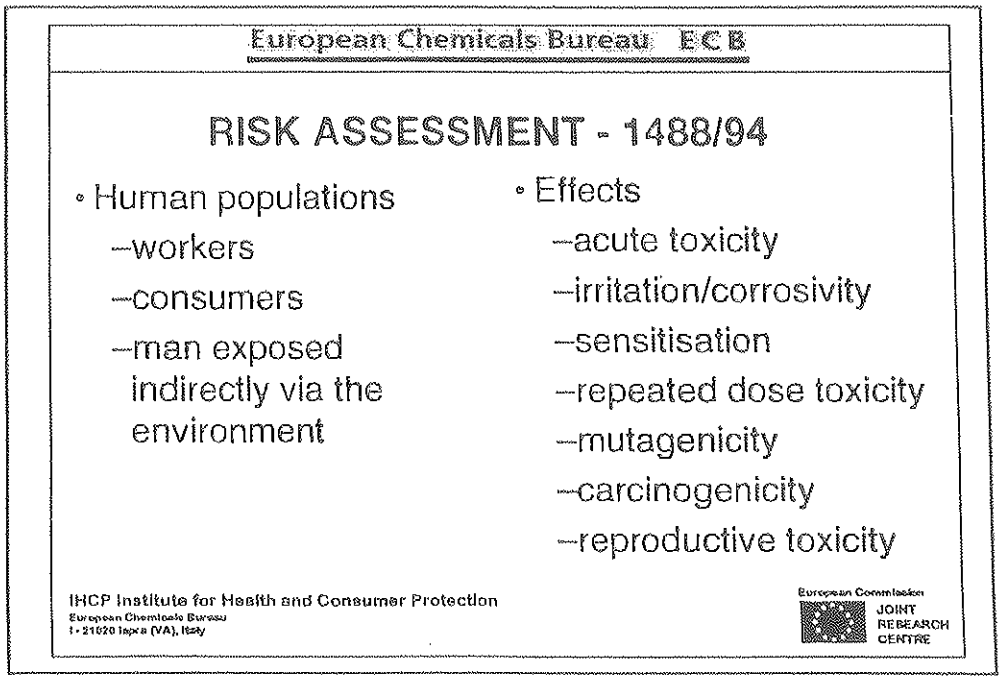
European Chemicals Bureau ECB
RISK ASSESSMENT - 1488/94
• Human populations
–workers
–consumers
–man exposed indirectly via the environment
• Effects
–acute toxicity
–irritation/corrosivity
–sensitisation
–repeated dose toxicity
–mutagenicity
–carcinogenicity
–reproductive toxicity
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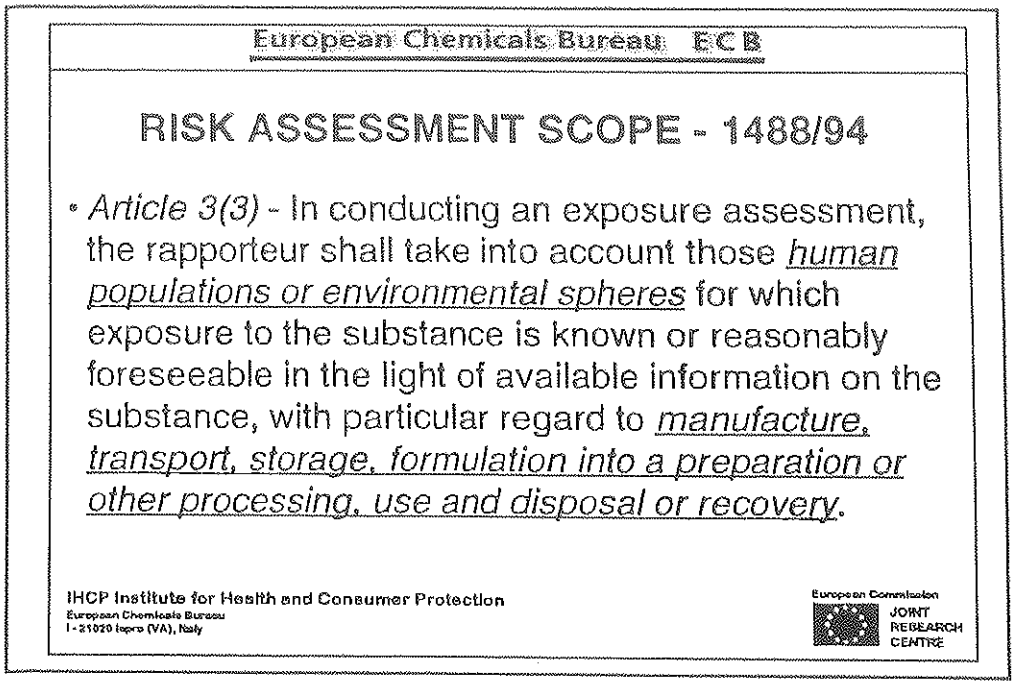
European Chemicals Bureau ECB
RISK ASSESSMENT SCOPE - 1488/94
• Article 3(3) - In conducting an exposure assessment, the rapporteur shall take into account those human populations or environmental spheres for which exposure to the substance is known or reasonably foreseeable in the light of available information on the substance, with particular regard to manufacture, transport, storage, formulation into a preparation or other processing, use and disposal or recovery.
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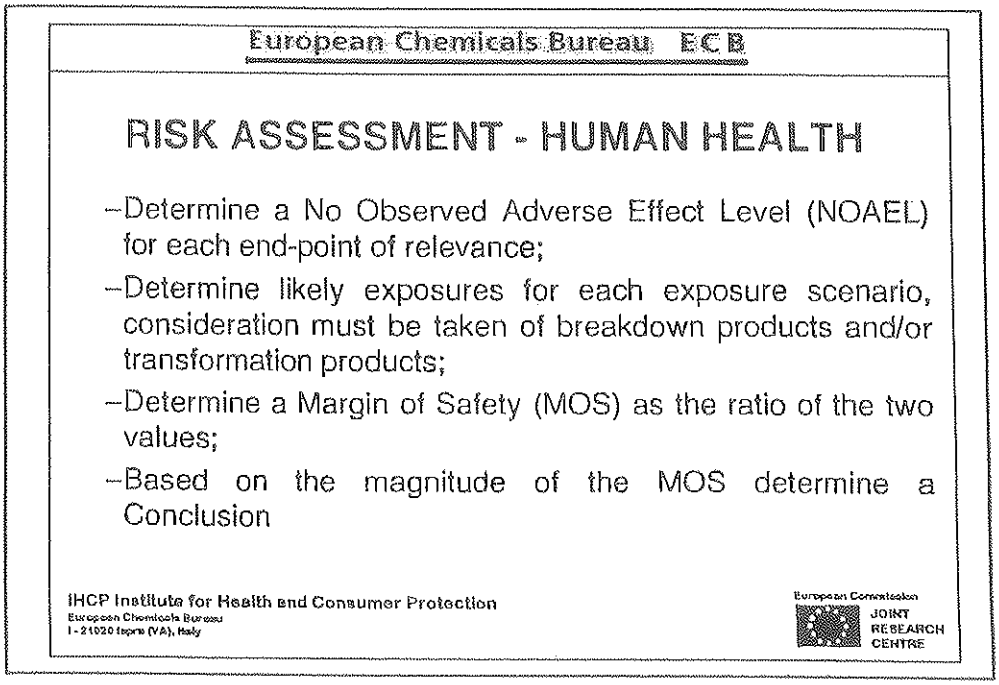
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RISK ASSESSMENT - HUMAN HEALTH
–Determine a No Observed Adverse Effect Level (NOAEL) for each end-point of relevance;
–Determine likely exposures for each exposure scenario, consideration must be taken of breakdown products and/or transformation products;
–Determine a Margin of Safety (MOS) as the ratio of the two values;
–Based on the magnitude of the MOS determine a Conclusion
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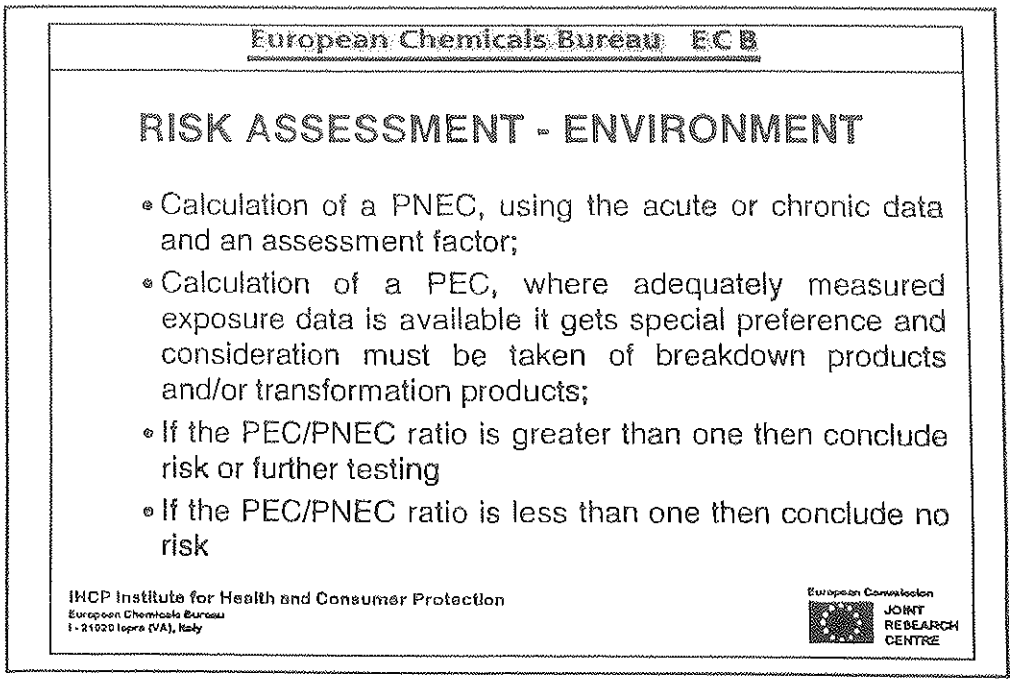
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RISK ASSESSMENT - ENVIRONMENT
• Calculation of a PNEC, using the acute or chronic data and an assessment factor;
• Calculation of a PEC, where adequately measured exposure data is available it gets special preference and consideration must be taken of breakdown products and/or transformation products;
• If the PEC/PNEC ratio is greater than one then conclude risk or further testing
• If the PEC/PNEC ratio is less than one then conclude no risk
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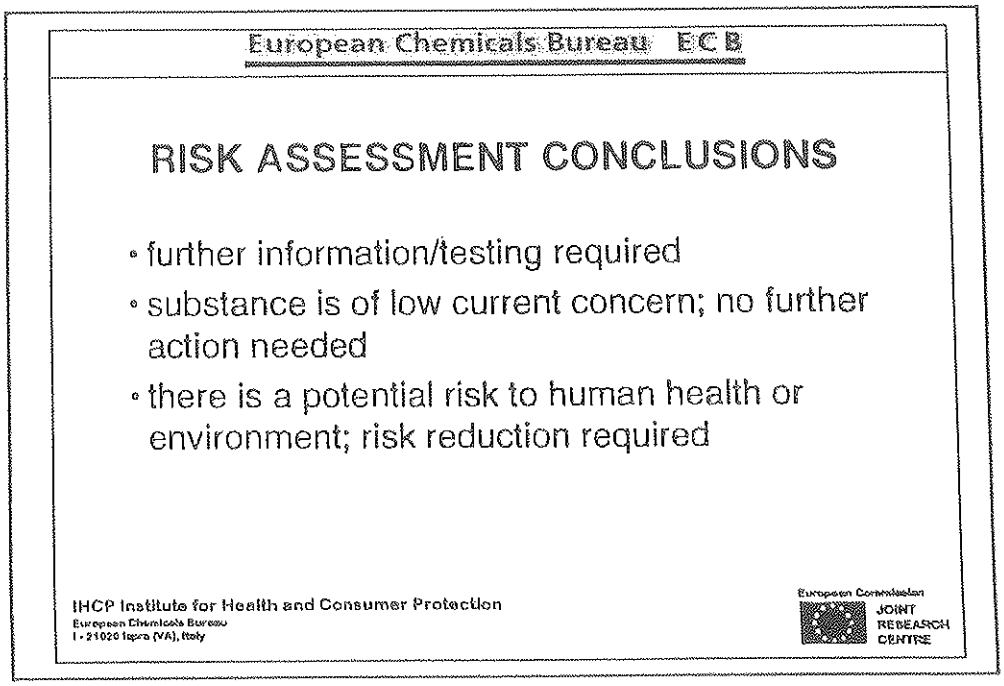
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RISK ASSESSMENT CONCLUSIONS
• further information/testing required
• substance is of low current concern; no further action needed
• there is a potential risk to human health or environment; risk reduction required
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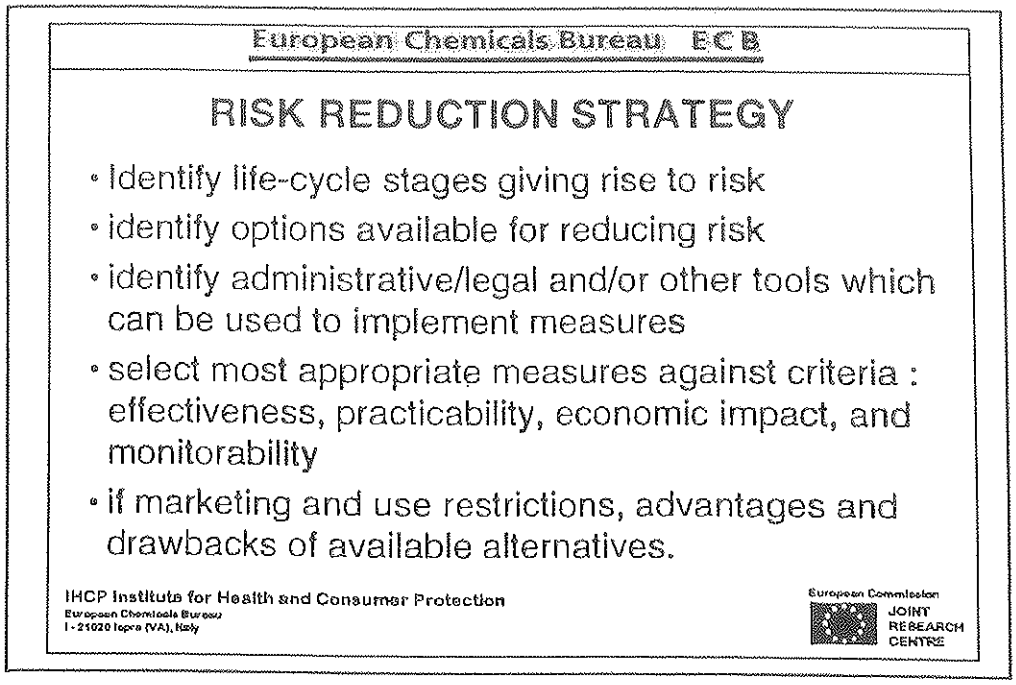
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RISK REDUCTION STRATEGY
• Identify life-cycle stages giving rise to risk
• identify options available for reducing risk
• identify administrative/legal and/or other tools which can be used to implement measures
• select most appropriate measures against criteria : effectiveness, practicability, economic impact, and monitorability
• if marketing and use restrictions, advantages and drawbacks of available alternatives.
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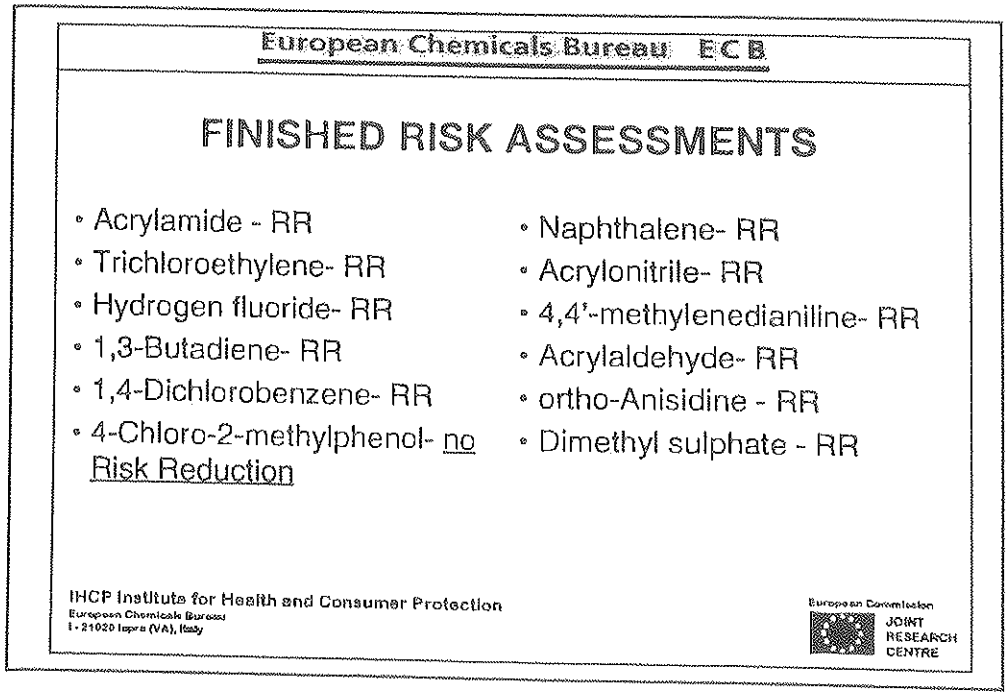
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FINISHED RISK ASSESSMENTS
• Acrylamide - RR
• Trichloroethylene- RR
• Hydrogen fluoride- RR
• 1,3-Butadiene- RR
• 1,4-Dichlorobenzene- RR
• 4-Chloro-2-methylphenol- no Risk Reduction
• Naphthalene- RR
• Acrylonitrile- RR
• 4,4'-methylenedianiline- RR
• Acrylaldehyde- RR
• ortho-Anisidine - RR
• Dimethyl sulphate - RR
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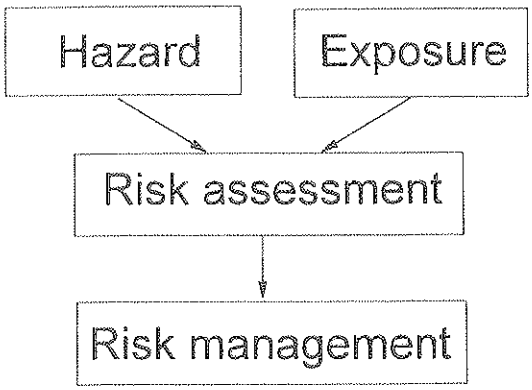
The UK ESR Risk Assessment of 1,3-butadiene

Isla M Brooke
Occupational Regulatory Toxicologist
HSE, UK

Risk assessment of 1,3-butadiene

What are we trying to achieve?



Workers
Consumers
Humans via the environment

Risk assessment of 1,3-butadiene

What are we concerned about?

- Hazard
  - cancer
  - heritable mutations?
  - fertility?
- Exposure
  - cumulative
  - peak?

Risk assessment of 1,3-butadiene

What are the uncertainties?

- Ultimate metabolite(s) of concern
- Underlying mechanism(s) of toxicity
- Dose-response relationships
- Extrapolation
  - mouse → human?
  - rat → human?

Risk assessment of 1,3-butadiene
The Battenburg Principle

Risk assessment of 1,3-butadiene

Risk assessment conclusions
- where are we currently?

- Uncertain but concerned
  - control exposures to the lowest reasonably practicable level

Health Effects of 1,3-Butadiene: Informing the Regulatory Process

Aparna M. Koppikar, M.D., Ph.D.

Brussels, June 29-30, 1998

EVALUATING AGENCIES IN NORTH AMERICA

- *Environmental Protection Agency (EPA)*
- *Occupational Safety and Health Administration (OSHA)*
- *National Institute for Occupational Safety & Health (NIOSH)*
- *National Institute of Environmental Health Sciences (NIEHS)*
- *California Air Resources Board (CARB)*
- *Canadian Environmental Protection Act (CEPA)*

HISTORY OF EPA EVALUATION

- *Office of Air Quality Planning & Standards (OAQPS): First request in 1984 to support decision making regarding possible designation of 1,3-butadiene as a "Hazardous Air Pollutant"*
- *Science Advisory Board Review: Public Meeting in February 1985*
- *Final product: September 1985 Classified as a "probable human carcinogen"*

EPA EVALUATION

- *Evaluation requested by EPA's Office of Mobil Sources*
- *The risk assessment will be used to support decision making regarding the Air Toxic Rules - Section 202(l)2 of the Clean Air Act Amendments*
- *The evaluation includes qualitative and quantitative assessments for cancer and certain non-cancer endpoints*

EPA EVALUATION CONTINUED

- *For the qualitative cancer endpoint, descriptive classification is used per 1996 Proposed Guidelines for Carcinogen Risk Assessment*
- *Classification of "known/likely" to be carcinogenic to humans is proposed based on totality of evidence derived from human & animal data, metabolism, genotoxicity, mutagenicity, toxicokinetics, & mode of action*

EPA EVALUATION CONTINUED

- *Lifetime risk of cancer is based on human data*
- *Quantitative risks to humans based on rat and mouse data are also presented*
- *ED_{01} and ED_{10} are presented*
- *Reproductive and developmental effects are qualitatively evaluated*
- *Non-cancer quantitative estimates are based on mouse data*

SCIENTIFIC ASSESSMENT & REVIEW PROCESS

- *Thorough literature review was conducted*
- *Scientific draft was prepared*
- *Internal review*
- *External review with 60-day public review and comment period*
- *Science Advisory Board (SAB) public meeting*
- *Finalization of the document will consider recommendations from the SAB & public comments*

OSHA EVALUATION

- *Conducted under the Occupational Safety & Health Act, 29 U. S. C. 651 et seq*
- *Standard is set for the permissible exposure limit (PEL) to an 8-hour time-weighted average (TWA)*
- *All standards must be reasonably necessary, economically & technologically feasible, cost effective, consistent with prior Agency action*
- *Classified as "potential occupational carcinogen." PEL is set at 1 ppm, a short-term exposure limit (15 min) is 5 ppm, and an "action level" is set at 0.5 ppm to an 8-hour TWA*

OSHA REVIEW PROCESS

- *OSHA initiated the rulemaking on 1,3-butadiene in 1986. After reviewing the available data & conducting risk assessment, a rule was proposed in 1990*
- *Public hearing was held in early 1991. Post-hearing submissions of briefs, arguments,& summations was extended to February 1992*
- *After the IARC evaluation in the Fall of 1992 rulemaking was delayed*

OSHA REVIEW PROCESS CONTINUED

- *Final rule was proposed in 1996 after a voluntary agreement between OSHA, union members, and industry representatives*
- *Public comment period was 45 days*
- *Based on comments the draft was revised*
- *Public hearings were held; the presiding judge closed the hearings and certified the proposed standards to the Assistant Secretary of Labor*

NIOSH EVALUATION

- *NIOSH is responsible for recommending health and safety standards for workers, employers, and health professionals*
- *NIOSH, in conjunction with OSHA, develops series of occupational health standards*
- *In 1991 evaluation using OSHA cancer policy, 1,3-butadiene was classifi[illegible] as "potential human health hazard" with respect to carcinogenicity*

NIOSH REVIEW PROCESS

- *NIOSH prepares three types of documents: policy, technical, & educational*
- *A team of experts is formed who prepare the documents*
- *The documents undergo an internal and external review*
- *Appropriate revisions are incorporated after each review*
- *Document is released*

NIEHS EVALUATION

- *NIEHS has no regulatory authority*
- *The annual (now biennial) Report on Carcinogens (RoC) is produced to provide information to the public regarding the environment and cancer*
- *The report includes a list of substances either known or reasonably anticipated to be carcinogenic to humans and to which significant numbers of individuals residing in the USA are exposed*
- *For the Ninth report (draft), 1,3-butadiene has been listed as a "known human carcinogen"*

NIEHS REVIEW PROCESS

- *Petition of chemicals for listing or delisting*
- *Information gathering/public comments/draft summary document preparation*
- *Review by NIEHS Report on Carcinogen (RoC) review group (RG1)*
- *Review by the NTP executive committee RoC review group (RG2)*
- *Review by the NTP Board of Scientific Counselors RoC subcommittee in public meeting*
- *Final public comments*
- *Review by the NTP executive committee*

Health Assessment of 1,3-Butadiene as a Priority Substance under the Canadian Environmental Protection Act

Dr. Kathryn Hughes, Health Canada

Under the Canadian Environmental Protect Act, Priority Substances Lists (PSLs) are compiled which include several substances considered to be of top priority for evaluation of potential health and environmental effects. As specified in the Act, the purpose of the health assessments is to determine if a substance is present in the environment in concentrations constituting "a danger in Canada to human life or health". The health assessment also provides guidance concerning the need for and priority for investigation of risk management options. Legislation requires that the assessments be completed for the 25 substances comprising the current PSL (PSL2), including butadiene, by the year 2000. The assessment for butadiene is scheduled to be completed this year.

In the draft PSL health assessment, effects associated with exposure to butadiene considered to be critical and for which exposure-response was quantified included both neoplastic and non-neoplastic endpoints. The evaluation of neoplastic effects was based on epidemiological studies in occupationally exposed populations, cancer bioassays in experimental animals as well as supporting *in vitro* and *in vivo* data on genotoxicity. Based on these data, it was concluded that butadiene is highly likely to be carcinogenic in humans and is also a somatic and germ cell mutagen.

Estimates of carcinogenic potency of butadiene were derived on the basis of the raw data for the University of Alabama epidemiological study, which were kindly provided by the study researchers. In the Priority Substances Program of Health Canada, potency is expressed as the concentration associated with a 5% increase in the incidence of or mortality due to cancer, and is referred to as the tumorigenic concentration (5%) or TC_{05}. Potency estimates were also derived on the basis of the animal bioassay data for comparison, taking into consideration available information on potential mechanisms of tumour induction. Although the numbers generated may require some further "fine-tuning", it appears that carcinogenic potency estimates based on epidemiological data are similar to the lower range of the valnes derived on the basis of studies in experimental animals.

With respect to non-neoplastic effects, the exposure-response relationship for the ovarian atrophy observed in the chronic study in mice was quantified by derivation of a benchmark concentration. This measure is expressed as the concentration associated with a 5% increased

incidence or BMC_{05}. We are also interacting further with staff at NIEHS concerning these lesions. Exposure-response relationships for other non-cancer effects are also being considered for derivation of benchmark concentrations.

With respect to risk characterization, in the Priority Substances Program, current exposure levels are compared to the TC_{05}s to calculate Exposure/Potency Indices, or EPIs. These EPIs are used to determine the need for and the priority for investigation of risk management options by comparison with values derived for other substances and to focus efforts in view of limited available resources. However, it should be noted that the results of the health assessment represent only one of the several factors considered in the risk management phase.

The peer review process for the Health Canada assessment on butadiene involves several stages. The initial draft of the background data review underwent internal review within the Environmental Health Directorate by senior staff with expertise in risk assessment, genotoxicity and reproductive and developmental toxicity. At the same time, the background sections of the supporting documentation were circulated to several individuals from industry and other government agencies primarily for comment on the adequacy of coverage of available data as well as the content of the data summaries. Based on comments received, the supporting documentation was revised, and these sections, accompanied by a draft of the hazard characterization and dose-response analyses, were forwarded for comment on technical content and interpretation to numerous individuals and agencies selected to cover areas critical to assessment of butadiene (including industry, government, academia and researchers).

The nature and format of the third stage of external peer review have yet to be determined and will be based on the comments received during the second stage of external review. In general, the comments received to date relate primarily to fine-tuning; there were no fundamental disagreements with the proposed Hazard Characterization and Dose-Response Analyses. The final review phase will involve public consultation which will likely involve publication of a shorter summary document on our web site as well as distribution of paper copies to interested parties. Once all the review stages are completed, which will hopefully be within the next couple of months, the final assessment report will be published.

WHAT IS HEI?

The Health Effects Institute (HEI) is a public–private partnership established in 1980 to provide decision makers, scientists, and the public with high-quality, impartial, and relevant scientific information that helps answer key questions about the health effects of emissions from motor vehicles and other sources in the environment. The intent of HEI has been to develop the facts concerning health effects carefully and credibly so that controversy about the facts themselves will be removed from the adversarial agenda and the debates over clean air can instead focus on national and international policy issues.

HEI is an unusual model of government-industry collaboration in support of research. The Institute has historically received half of its core funds from the U.S. Environmental Protection Agency and half from worldwide manufacturers of motor vehicles or engines marketed in the United States. HEI also receives funding from other organizations to supplement its funding for some of its projects. For example, the European Chemical Industry Council (CEFIC) and its U.S. counterpart, the Chemical Manufacturers Association, are contributing to funding of a transitional epidemiology study evaluating biomarkers of 1,3-butadiene, and the California Air Resources Board and the Engine Manufacturers Association are contributing to new diesel epidemiology studies. The Institute has developed consultation processes with its sponsors and others to help focus its research priorities. None of the contributors has control over the selection, conduct, or management of HEI studies, however, and HEI makes no recommendations on how to apply research to regulatory policy.

The Institute's antonomy is supported, even beyond the statements in its charter, by the integrity and commitment of both its scientific leadership and its Board of Directors. Subject to the approval of the Board of Directors, the work of the Institute is carried out by two external and independent committees for research and review, each consisting of distinguished scientists knowledgeable about the scientific issues inherent to investigating the health effects of air pollution. HEI's staff works with committee members in carrying out the work of the Institute.

HOW DOES HEI WORK?

Research Selection and Management

After seeking advice from HEI's sponsors and others interested in its work, the HEI Research Committee determines the research priorities of the Institute. When an area of inquiry has been defined, the Institute announces to the scientific community that applications are being solicited on specific topics by issuing requests for applications. Applications are reviewed first for scientific quality by an ad hoc panel of appropriate experts. They are then reviewed by the HEI Research Committee both for quality and relevance to the goals of the research program.

Studies recommended by the Research Committee undergo final approval by the Board of Directors, which also reviews the procedures, independence, and quality of the selection process.

During the course of each study, the Research Committee and scientific staff maintain close contact with HEI-funded investigators by means of progress reports, site visits, workshops, and the HEI Annual Conference. The ten-month progress report serves as the basis for contract renewal for multi-year projects. A site visit is conducted at many investigators' laboratories, not only to assess the conduct of the study, but also to provide an opportunity for discussion and exchange of ideas. At the annual conference, HEI investigators, Research Committee and Review Committee members, HEI staff, representatives of sponsor organizations, invited guests, and other participants meet to share information and develop new ties to strengthen the HEI community of scholars.

Independent Review Process

In order to fulfill its mission of providing timely, high-quality research results for decision makers, HEI has developed a rigorous review process to evaluate results of the research it funds. When a study is completed, the investigator is required to submit a comprehensive final report. The HEI Review Committee, which has no role in the review of applications or in the selection of projects, assesses the scientific quality of each completed study and evaluates its contribution to

unresolved scientific questions. The investigator's final report and the commentary of the Review Committee are published together by HEI. Additionally, all HEI investigators are urged to publish the results of their work in the peer-reviewed literature.

THE HEI RESEARCH PROGRAM

The HEI research program has addressed many important questions about the health effects of a variety of pollutants, including carbon monoxide, methanol, diesel exhaust particles, nitrogen oxides, and ozone. The current research program focuses on air toxics, ambient particles, and oxygenates added to gasoline. HEI has funded studies to understand the mechanisms of diseases, to develop better methods to assess health effects and determine exposure and dose, and to address issues common to many pollutants. The program has included theoretical, in vitro, animal, controlled human exposure, and epidemiologic studies. The choices of which pollutants to study or scientific questions to investigate have been based on many considerations, including evaluation of issues raised by sponsors and analysis of the regulatory needs and uncertainties about health effects of specific pollutants. HEI has, on some occasions, produced special reports to evaluate the state of existing science in areas related to policy and to determine research needs in new areas. HEI has also conducted special workshops on the health effects of specific pollutants such as 1,3-butadiene, the subject of this Communication.

To guide its research efforts, HEI consults regularly with its sponsors, scientists, and others to produce and regularly update a "Strategic Plan for the Health Effects of Air Pollution." This reflects HEI's belief that most pollutants from motor vehicles are not unique air pollutants but are also contributed by other sources, and that understanding the effects of exposure to each of several pollutants does not provide a complete picture of health effects of those pollutants together in the air. While emphasizing the broader relevance of its research program, HEI has maintained its efforts to study in a timely way pollutants that are specific to motor vehicles, such as ethers added to gasoline or particles in exhaust from diesel engines, and, recently, metals emitted from motor vehicles. HEI continually strives, through its strategic planning efforts, to project further into the future the possible new fuels and technologies for which health effects information will be needed to inform decisions.

As part of this effort, HEI seeks to develop a community of scientists and scholars who can generate new collaborations and fresh approaches to the problems of air pollution.

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