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Scientific Committee on Toxicity, Ecotoxicity and the Environment

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**SCIENTIFIC COMMITTEE ON TOXICITY, ECOTOXICITY AND
THE ENVIRONMENT (CSTEE)**

Opinion on the results of the Risk Assessment of:

BUTADIENE

CAS No.: 106-99-0

EINECS No.: 203-450-8

**REPORT VERSION (Human Health)
June 2001**

**Carried out in the framework of Council Regulation (EEC) 793/93 on
the evaluation and control of the risks of existing substances¹**

Opinion expressed at the 27th CSTEE plenary meeting

Brussels, 30 October 2001

¹ Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of those substances if they are produced or imported into the Community in volumes above 10 tonnes per year. The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94, which is supported by a technical guidance document.

Terms of reference

In the context of Regulation 793/93 (Existing Substances regulation), and on the basis of the examination of the Risk Assessment Report the CSTEE is invited to examine the following issues:

1. Does the CSTEE agree with the conclusions of the Risk Assessment Report?
2. If the CSTEE disagrees with such conclusions, the CSTEE is invited to elaborate on the reasons for this divergence of opinion

Introduction

According to the RAR, the EU production and consumption of 1,3-butadiene is 1,892 000 tonnes/year. Within the EU there are approximately 18 major companies using butadiene in the production of styrene-butadiene rubber (SBR), styrene-butadiene latex, acrylonitrile-butadiene-styrene thermoplastic resins (ABS) and other related products such as polybutadiene rubber. Butadiene is a gas, and is transported by road tanker, ship or pipeline. It is used in closed systems. The main exposure pathway to consider is by inhalation.

GENERAL COMMENTS

The report is of good quality, but the lack of a table of contents makes it difficult to read at present. As butadiene is a genotoxic carcinogen, conclusion iii) is reached for all exposure scenarios. However, the rationale behind conclusion iii)b for workers and iii)a for consumers and indirect exposure via the environment is not clear, as they are not qualified by any quantitative cancer risk estimates. According to the CSTEE, the exposure to butadiene should be kept as low as possible.

The CSTEE does not agree with the RAR that there is no need for further information or testing regarding the possibility of inhalatory sensitisation and toxicity for reproduction, conclusion i).

A recent update of the literature on butadiene can be found in the proceedings from a satellite meeting to Eurotox 2000: Evaluation of butadiene, isoprene and chloroprene health risks, Chemico-Biological Interactions Vol. 135-136 (2001).

SPECIFIC COMMENTS

Exposure assessment

Vehicle exhaust emissions and cigarette smoke are discussed in 3.1.1.1.9 and 3.1.1.1.10, respectively, and are included in table 3.6. In 3.1.1.1.13 it is mentioned that forest fires and biomass combustion is likely to be a significant source of butadiene, but as this source is not included in table 3.6, the modelled exposure to butadiene may have been underestimated ($PEC_{\text{local and regional}}$). In table 3.8 on measured levels in air, data from Canada (and possibly

other countries?) are missing. The Canadian measurements are briefly reviewed in the Priority Substances List Assessment Report on 1,3-butadiene, Environment Canada (2000). This report is included in the reference list.

Occupational exposure limits are cited on page 63 (table 4.1). These are mainly old data, and certainly outdated for some countries.

In table 4.15, page 77, 8-hour TWA exposure data for use in the risk characterisation are summarised as 1ppm for monomer production and 5 ppm for polymer production. However, these exposure levels are apparently not used in the Risk characterisation chapter (4.1.4.1.2), where lower exposures are used for comparisons.

Effects assessment

The structure of chapter 4, Human health, is somewhat confusing. According to page 62, 4.1 deals with human health (toxicity), but it starts with exposure assessment (page 62-86).

Butadiene is generally of low toxicity following repeated exposure in the rat, but is severely toxic to the mouse. In chronic studies, increased mortality occurs in both sexes of mice at 20 ppm and above, tumour development and ovarian atrophy occurs in females at 6.25 ppm and testicular atrophy occurs in male mice at 625 ppm for 9 months.

Toxicokinetics

The toxicokinetics of butadiene (4.1.3) is crucial in the risk assessment, as mice are much more sensitive than rats concerning the carcinogenic potency of butadiene, and there is limited information on the toxicokinetics in humans. Epoxybutene is formed in human liver (with considerable inter-individual variation), lung and bone marrow, but further metabolism of the monoepoxide to diepoxybutane has not been demonstrated as yet. Quantitatively higher concentrations of epoxybutene and diepoxybutane are formed in mice compared to rats. The CSTE agrees that the metabolism of butadiene in humans appears to be quantitatively more similar to that in the rat rather than the mouse, although it is not possible to attribute the differences in toxicity between the rat and the mouse solely to the quantitative differences in metabolism; nor to exclude the possibility that some humans could be similar to mice in their capacity for metabolism to active intermediates.

Sensitisation

According to the RAR, there are no data on the skin or respiratory potential of butadiene in animals, neither are there human data. In view of the fact that inhalation is the primary route of exposure and butadiene forms reactive epoxide metabolites, the possibility of inhalatory sensitisation should be investigated

Toxicity for reproduction

Results of long-term studies indicate that the ovaries and testes are target organs for butadiene toxicity in mice, with the testes also a target organ in rats. In one study, ovarian atrophy occurred in mice at a dose exposed to 6.25 ppm and above for 2 years. In addition, butadiene has an adverse effect on germ cells in mice. In view of these findings, a 2-generation reproduction study with butadiene is indicated.

Genotoxicity

In vitro and *in vivo* studies have indicated that butadiene is metabolised to reactive intermediates, primarily epoxides (1,2-epoxybutene-3 and 1,2;3,4-diepoxybutane) that can react with DNA and lead to somatic and germ cell mutations.

Butadiene is mutagenic in virtually all test systems both *in vitro* and *in vivo*.

In vitro

Butadiene was mutagenic in the Ames tests after metabolic activation. No *in vitro* cytogenetic study is available. A Mouse Lymphoma test was negative, but it is unclear whether the cells were adequately exposed. A second mouse lymphoma test was positive in the presence of S9 (small colonies, indicating a clastogenic activity).

Conflicting results have been found in three *in vitro* sister chromatid exchange assays (1 positive test in CHO cells after metabolic activation; 1 positive and 1 negative study in human lymphocytes, both studies with and without metabolic activation).

In vivo

In mice, butadiene did induce micronuclei in bone marrow, peripheral blood and germ cells. Chromosome aberrations were found in the bone marrow. Two hprt mutation assays and the mouse spot test were positive as well as various dominant lethal tests. Butadiene also induced heritable translocations (Adler *et al.*, 1998). Activated K-ras oncogenes have been detected in lymphomas and in liver and lung tumours induced in mice by 1,3-butadiene. Mutations in the p53 tumour-suppressor gene were seen in mouse lymphomas.

In rats, a well-documented micronucleus test was negative. Butadiene did not induce UDS in rats and was negative in the dominant lethal test in rats.

Butadiene induced DNA adducts and damage in both mice and rats *in vivo*, although the damage was significantly greater in mice than in rats. The mutation rate in the hprt gene was greater in mice than in rats (Meng *et al.*, 1999). DNA adducts have been found in mouse testis and lung after inhalation exposure to butadiene (Koivisto P. *et al.*, 1998). Metabolites of 1,3-butadiene induced *micronuclei* in rat spermatids (Lahdetie J. *et al.*, 1997)

Butadiene did not induce somatic mutations and recombination in *Drosophila*.

There are conflicting results on whether 1,3-butadiene increases hprt mutations in lymphocytes from butadiene-exposed humans (no increase was shown in 19 workers exposed on average to 1.8 ppm, while in a different population of workers exposed on average to 3.5 ppm a significant increase was detected). Sister chromatid exchanges, *micronuclei*,

chromosomal aberrations and DNA strand breaks were not significantly elevated above control levels in peripheral blood lymphocytes of workers. However, workers exposed to 1,3-butadiene who were deficient in GSTM1 were found to have higher levels of chromosome aberrations than those who were positive for the GSTM1 phenotype (Sram, 1998).

Analysis in occupationally exposed humans has shown the presence of adducts between haemoglobin and metabolites derived from 1,3-butadiene. The binding indices for 1,3-butadiene-exposed humans are more than one order of magnitude lower than those in exposed rats.

The CSTEE agrees with the Member State *rapporteur* that butadiene should be considered as genotoxic. Butadiene is classified as a Category 1 mutagen with the risk phrase R46: May cause heritable genetic damage in humans.

Carcinogenicity

Animal data

1,3-Butadiene is a potent multi-site carcinogen in the mouse, but has a weak carcinogenic potential in the rat.

In inhalation studies in mice, tumours were induced in multiple organs at all exposure levels (6.25 to 1250 ppm). The tumours induced included malignant lymphomas and haemangiosarcomas of the heart. Increases in the incidence of a number of other malignant and benign tumours were found.

In a lifetime inhalation study in rats, butadiene increased the incidence of tumour types that normally develop spontaneously in rats. The response was seen mainly at 8000 ppm.

These species differences in the susceptibility to tumour development have mainly been attributed to differences in the metabolism and toxicokinetics of butadiene, and the genotoxic potential of the metabolites.

Human Epidemiology

A number of epidemiological studies have been performed in workers of the butadiene manufacturing and styrene-butadiene rubber (SBR) industries. Some further information has been published since the production of the RAR. Although this additional information will not alter the conclusions drawn in the RAR, the information should be included into the final report.

Various studies showed an elevated risk of leukaemia or lymphomas, but either the exposure was to more than butadiene alone, no quantitative exposure information was given or the cohort was too small to have sufficient power.

The risk of leukaemia was elevated in SBR workers, and increased with cumulative exposure to butadiene (Delzell *et al*, 2001). Although there was an association between cumulative butadiene exposure and leukaemia, it was present only at relatively high butadiene exposures with peak exposures above 100 ppm (ECETOC, 2001). In a limited case-control study it was

suggested that leukaemia risk might be increased from butadiene exposures as low as 1 ppm (Matanoski *et al*, 1997). Confounding by styrene, the other major chemical used in the production of synthetic rubber, is unlikely since no excess of leukaemia has been found among workers with much higher exposures to styrene in the plastics industry. The role of dimethyl dithiocarbamate (DMDTC) in the development of leukaemias, however, is doubtful. DMDTC has been used in earlier industrial processes involving butadiene and has the potential to modify metabolic processes that are relevant to butadiene carcinogenesis (ECETOC, 2001). However, the CSTEE concludes that exposure to this compound is too low to affect metabolism and toxic effects of butadiene.

No excess of leukaemia was found in studies with butadiene manufacturing workers (Divine and Hartman, 2001; Tsai *et al*, 2001).

An increased risk for non-Hodgkins lymphoma was observed in two of three studies of butadiene manufacturers, but in the larger of these investigations the excess was restricted to short-term workers first employed before 1950 (Divine and Hartman, 2001). Furthermore, no similar excess was apparent in the large study of the synthetic rubber industry (Delzell *et al*, 2001). This suggests that butadiene may not have been responsible for the elevated incidence of lymphomas.

In 1999, IARC classified 1,3-butadiene as probably carcinogenic to humans (Group 2A). This evaluation should be included into the RAR. The CSTEE agrees with the Member State *rapporteur* that butadiene should be considered as carcinogenic. Butadiene is classified as a Category 1 carcinogen with the risk phrase R45: May cause cancer in humans

Among the epidemiological studies, one very large cohort mortality study on workers in styrene-butadiene rubber manufacturing plants in the USA and Canada is especially informative (Delzell *et al.*, 1995, 1996; Macaluso *et al.*, 1996). This study clearly demonstrated a clear excess of leukaemia associated with exposure to butadiene monomer. In the RAR an unpublished reanalysis of the exposure data in the Delzell study is cited. It is stated on page 151, second to last paragraph, that this reanalysis provides some indication of a dose-response relationship, but that the exposure data are not considered sufficiently robust to use as a reliable basis for determination of a quantitative dose-response relationship (also repeated on page 157). However, as also noted on page 157 and in Annex 2 of the RAR, human cancer risk estimates have been derived from the original Delzell study (Environment Canada, 2000; Stayner *et al*, 2000). The CSTEE cannot judge whether the reanalysis contradicts these risk estimates, but finds it appropriate to use the risk estimates as a rough guide, at least as long as the exposure reanalysis remains unpublished.

The statement on page 151, second to last paragraph, that the reanalysis raises the possibility that a theoretical threshold for the excess risk of cancer may exist, is biased in light of the overwhelming evidence of genotoxicity, and should be removed.

Risk characterisation

The CSTEE agrees that the main health effects of concern are mutagenicity and carcinogenicity, without a threshold for these effects, and consequently agrees with conclusion iii) for all exposure groups.

In 4.1.4.1.2, page 165, second paragraph, a comparison is made between occupational exposure and “short-term repeated exposure” studies, leading to conclusion ii). The meaning of this is confusing and the reasons for the conclusion are not clear. In the following paragraph, a comparison is made between occupational exposure and a NOAEL for gonadal damage in mice of 200 ppm after chronic exposure, also leading to conclusion ii). Although the NOAEL for testicular atrophy is 200 ppm, it should be noted that ovarian atrophy occurred at the lowest dose tested, 6.25 ppm. Thus, the comparison is misleading, and the conclusion is not obvious, especially if the exposure data expressed in chapter 4, table 4.15 would have been used.

For consumers and humans exposed indirectly via the environment it is concluded in the RAR that the exposures are very low and that there would be a negligible residual cancer risk. However, the CSTEE feels that such statements should be based on some sort of quantitative risk estimates, such as the ones presented in Annex 2 of the RAR. For workers, risk estimates have been calculated in Annex 2 both from the Delzell epidemiological study and from the cancer study with mice. These gave comparable results, around 4×10^{-3} as lifetime cancer risk at occupational exposure to 0.5 ppm and 4×10^{-2} at 5 ppm, which would support conclusion iii). For consumers the calculated lifetime risk is 3.6×10^{-5} and for indirect exposure via the environment 2.6×10^{-3} and 1.8×10^{-5} for local and regional scenario, respectively. Although these latter risk extrapolations contain large uncertainties and probably exaggerate the actual risk, it is not clear that they should be regarded as negligible². According to the CSTEE, all exposure to butadiene should be kept as low as possible, considering that it is a potent genotoxic carcinogen in mice.

References

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Delzell E, Macaluso M, Sathiakumar N, Matthews R. Leukemia and exposure to 1,3 butadiene, styrene and dimethyldithiocarbamate among workers in the synthetic rubber industry. Presented at an International Symposium on the Evaluation of Butadiene, Isoprene and Chloroprene Health Risks, London, 12-14 September 2000. Chem Biol Interact 2001 [In press].

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ECETOC, Document no 40: Comments on the Exposure Levels set by the Scientific Committee on Exposure on 1,3-Butadiene. January 2001

² Although risk management issues are outside the scope of the CSTEE, the Committee has reservations with the subdivision of conclusion iii) into type (a) and (b) for genotoxic and carcinogenic properties. It appears very difficult to have enough information to ascertain that existing controls in place are sufficient to ensure that exposures are continually driven downward and additional risk reduction measures over and above those already in place are not necessary.

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