# Tobacco Smoke–Related Health Effects Induced by 1,3-Butadiene and Strategies for Risk Reduction

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**1,3-Butadiene (BD) is a smoke component selected by the World Health Organization (WHO) study group on Tobacco Product Regulation (TobReg) for mandated lowering. We examined the tobacco smoke–related health effects induced by BD and possible health impacts of risk reduction strategies. BD levels in mainstream smoke (MSS) from international and Canadian cigarettes and environmental tobacco smoke (ETS) were derived from scientific journals and international government reports. Dose-response analyses from toxicity studies from government reports were evaluated and the most sensitive cancer and noncancer endpoints were selected. The risks were evaluated by taking the ratio (margin of exposure, MOE) from the most sensitive toxicity endpoint and appropriate exposure estimates for BD in MSS and ETS. BD is a good choice for lowering given that MSS and ETS were at levels for cancer (leukemia) and noncancer (ovarian atrophy) risks, and the risks can be significantly lowered when lowering the BD concentrations in smoke. Several risk reduction strategies were analyzed including a maximum level of 125% of the median BD value per milligram nicotine obtained from international brands as recommended by the WHO TobReg, tobacco substitute sheets, dual and triple carbon filters, and polymer-derived carbon. The use of tobacco substitute sheet with a polymer-derived carbon filter resulted in the most significant change in risk for cancer and noncancer effects. Our results demonstrate that MOE analysis might be a practical way to assess the impact of risk reduction strategies on human health in the future.**

***Key Words:*** **1,3-butadiene; risk reduction strategies; smoke components; mandated lowering; margin of exposure.**

Tobacco smoking is a global public health concern killing nearly 6 million people worldwide (Burns *et al.*, 2008; WHO, 2012). To help address the tobacco epidemic, the World Health Organization (WHO) Framework Convention on Tobacco Control (FCTC) was created to represent a treaty now signed by more than 167 countries to be responsible for establishing a framework for global tobacco regulation (FCTC, 2012).

Articles 9 and 10 of the FCTC deal specifically with regulation and disclosure, respectively, of the contents and emissions of tobacco products with emphasis on product attractiveness as a first step, followed by product addictiveness and toxicity (FCTC, 2010). Priority for regulating tobacco product attractiveness will be followed by guidance documents dealing with tobacco product addictiveness and toxicity (FCTC, 2010).

The WHO has put forth a strategy for regulation based on product performance measures with the goal of reducing the levels of toxic chemicals in mainstream cigarette smoke (MSS). The proposal is to establish levels for selected toxic substances in MSS per milligram of nicotine and to prohibit the sale or import of cigarette brands that have yields exceeding these levels (WHO, 2008). The smoking machine-generated levels of MSS are normalized to nicotine in order to better product comparison of MSS estimates generated under standardized conditions (WHO, 2008). The WHO study group on Tobacco Product Regulation (TobReg) has reviewed the literature on the amounts of various chemicals in MSS and their toxicological potency in order to make a selection of smoke components mandated for lowering (Burns *et al.*, 2008). Selection of toxicants was based on several factors such as known animal and human toxicity data and toxicity indices. In this list are 9 compounds representing different classes of chemicals: (1) volatile hydrocarbons (1,3-butadiene [BD] and benzene), (2) aldehydes (acetaldehyde, acrolein, and formaldehyde), (3) polycyclic aromatic hydrocarbons (benzo[a]pyrene), (4) tobacco-specific N-nitrosamines (4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone and N′-nitrosonornicotine), and (5) carbon monoxide (Burns *et al.*, 2008). This list of toxicants was selected based on a general toxicity assessment with key priority to smoke components implicated in cardiovascular and pulmonary toxicity, in addition to cancer. The variations in toxicant levels among different brands were also used as a first step toward the development of an overall strategy to reduce the levels of toxicants in tobacco smoke (WHO, 2008). For these toxicants, the

WHO TobReg has recommended a maximum level of 125% of the median value of the toxicant per milligram nicotine from brands on the market being regulated as a risk reduction strategy (Burns *et al.*, 2008).

Here, the applicability of risk reduction strategies in reducing the tobacco smoke–related effects (attractiveness, addictiveness, and toxicity) of BD was assessed. BD is generated from the combustion of natural precursors found in the tobacco leaf itself and precursors from additives including cellulose, paraffin, and sugars (Smith *et al.*, 2000). To date, there is no evidence for or against the contribution of BD to the enhancement of the attractiveness or addictiveness of cigarette smoke. For this reason, focus will be given to tobacco-related toxic health effects induced by BD. BD is a toxicant recommended for lowering by the WHO TobReg (Burns *et al.*, 2008; Counts *et al.*, 2005) because it has the highest cancer risk index per cigarette per day (Fowles and Dybing, 2003). The analysis by Fowles and Dybing (2003) was the first to provide a method for hazard prioritization of chemicals in cigarette smoke. Exposure standards for the exposure scenario of smoking are not available. Existing standards for inhalation exposure, such as the WHO Air Quality Guidelines, are meant for (lifetime) continuous exposure. The exposure scenario of smoking is rather complex. Smoking results in intermittent exposures during the day, and during smoking, the concentration in the lungs varies strongly. Immediately after a puff, the concentration in the lungs will be highest and will decrease until the next puff. Direct comparison of an airborne exposure concentration with an existing human limit value for inhalation exposure is therefore not feasible for the scenario of smoking.

At present, the margin of exposure (MOE) approach is believed to be the most appropriate approach to estimate the risks from smoke components because it is sufficiently flexible to deal with uncertainties involved in the risk assessment process as will be shown. The MOE is defined as the ratio of a critical toxicological estimate (eg, a NOAEL or benchmark dose) and an appropriate exposure dose metric. The higher an MOE, the lower the risk; if an MOE is sufficiently high, no or a low risk or priority for risk reduction measures is indicated. Judgement whether an MOE is sufficient will depend on the uncertainties associated with extrapolation issues involved (eg, interspecies and intraspecies differences, exposure scenario-related issues, etc.) or with the quality of the available database. Recently, Cunningham *et al.* (2011) has applied the MOE approach to evaluate risks for multiple endpoints for 15 tobacco smoke components (among which BD) where the human exposure estimate was based on smoking machine estimates (20 cigarettes per day). However, they made conservative assumptions for the human exposure estimate and recommended that future developments of their model should focus on refining the exposure estimate. In this article, a pragmatic approach is used to better account for some of the issues involved in the exposure scenario of smoking.

BD is a recognized human carcinogenic but is also known to cause serious nonneoplastic effects. In our analysis, both carcinogenic and noncarcinogenic points of departure (PODs) are considered. A POD is the concentration or dose of a chemical, either derived from human data or an experimental animal study, that best serves as starting point for the evaluation of a risk for health effects in humans in a given exposure scenario. A POD can be a benchmark dose (eg, $BMDL_{10}$) or a No-Observed-Adverse-Effect Level (NOAEL), but also a Lowest-Observed-Adverse-Effect level (LOAEL) in the absence of a NOAEL.

PODs for relevant health effects induced by BD were selected from government report publications and compared with the exposure in 2 scenarios: direct inhalation of MSS and via inhalation of environmental tobacco smoke (ETS). In addition, approaches for lowering BD levels in tobacco smoke were explored (WHO, 2008). These and other readily available tobacco alterations in tobacco processing or cigarette design were investigated for their effects in reducing BD emissions in tobacco products and how these effects can potentially contribute to a reduction in risk as illustrated by the MOE approach.

## MATERIALS AND METHODS

*Carcinogenicity Data*

BD is mutagenic and carcinogenic in animals and humans. The International Agency for Research on Cancer (IARC) has classified BD as a human carcinogen (Group 1) (IARC, 2008). Inhalation data in animals, particularly in mice, show that BD is a multisite carcinogen inducing tumors even at the lowest concentration tested (6.25 ppm) (NTP, 1993). Inhalation exposure to BD is characterized as carcinogenic to humans because there is sufficient evidence from epidemiological studies in styrene-butadiene production workers showing increased incidences in leukemias. Epidemiological studies of workers in styrene-butadiene rubber factories have reported an increase in incidence of respiratory, bladder, and stomach cancer. There is also sufficient evidence in laboratory animal studies demonstrating that BD is a multisite carcinogen inducing tumors in multiple organs in mice and in rats. The most sensitive sites observed were the lungs of female mice where tumors arose at the lowest concentration tested (EPA, 2002a). Altogether, BD's potential to cause cancer in humans has become an important public health issue.

*Nonneoplastic Endpoints*

Short-term (1–4 weeks) and long-term (104 weeks) inhalation animal studies and human occupational exposure assessments indicate that BD causes irritation and some neurotoxic effects at high concentrations. In addition, BD exposure has been linked to increased risk in cardiovascular disease, chronic obstructive pulmonary disease (COPD), and reproductive and developmental toxicity (ATSDR, 2012). Acute effects as a result of exposure to high levels of BD in humans include irritation of the eyes, nasal passages, throat, and lungs. At very high exposure levels (8000 ppm), neurological effects such as blurred vision, fatigue, headache, and vertigo have been reported (ATSDR, 2012). Chronic (long-term) effects from epidemiological studies included cardiovascular diseases (CVD) such as rheumatic and atherosclerotic cardiovascular disease developed due to formation of atherosclerotic lesions or plaques in the endothelium, which occlude the blood vessels and disrupt blood flow (ATSDR, 2012). Acute manifestations of CVD include myocardial infarctions and strokes where tissue oxygen and compromised nutrient supply to tissues. Inhalation of MSS or BD as a vapor phase smoke component was shown to promote atherosclerotic plaque development in cockerels (male young roosters), suggestive of a mechanism by which BD increases the risk of cardiovascular disease (Penn and Snyder, 1996, 2007). Cigarette smoke is a major risk

factor in the development of pulmonary emphysema, resulting in death in many patients with COPD (WHO, 2012). In terms of reproductive or developmental effects, none have been reported in humans, but animals (mice) exposed to BD via inhalation have reported developmental effects such as skeletal abnormalities, decreased fetal weight, and reproductive effects such as increased incidence in ovarian atrophy and testicular atrophy (ATSDR, 2012). Therefore, reproductive effects are considered the most critical nonneoplastic effect and are selected for POD determination.

*POD Selection for Neoplastic and Nonneoplastic Effects*

Assessing the risk for neoplastic and nonneoplastic effects after BD exposure is difficult because of the large species differences in responses, particularly between mice and rats (Hazelton-Laboratories-Europe, 1981; NTP, 1984, 1993). To better assess these differences, an understanding of metabolism and mode of action is necessary.

***Metabolism and toxicological mode of action.*** BD is metabolized into genotoxic metabolites in both animals and humans (EPA, 2002a). Briefly, BD is first oxidized primarily via cytochrome P450 (CYP) isozyme CYP2E1 (or CYP2A6) to generate 1,2-epoxy-3-butene (EB). EB can (1) undergo further oxidation to generate 1,2;3,4-diepoxybutane (DEB), (2) undergo detoxification by conjugation with glutathione (GSH) via glutathione-*S*-transferase, or (3) undergo detoxification by hydrolysis via epoxide hydrolase to generate 1,2-dihydroxy-3-butene metabolite (Kirman *et al.*, 2010). Both DEB and 1,2-dihydroxy-3-butene undergo further metabolism, epoxide hydrolase hydrolysis and CYP2E1 oxidation, respectively, to generate 1,2-dihydroxy-3,4-epoxybutane (EBdiol). EB, DEB, and EBdiol are presumed to be ultimate mutagenic and carcinogenic species (Kirman *et al.*, 2010). These metabolites differ significantly in their genotoxic potency (DEB >> EB > EBdiol) and play a significant role in carcinogenesis (Kirman *et al.*, 2010).

In murine lung, *in situ* hybridization experiments indicated that the CYP2E1 is mainly localized in nonciliated bronchiolar epithelial (Clara) cells, suggesting that the Clara cell is a preferential target for xenobiotics like BD metabolized by CYP2E1 (Forkert, 1995). Due to the high levels of CYP2E1 in mouse Clara cells, in comparison to rats, mice tend to be more sensitive to lung toxicity induced by xenobiotics requiring metabolic activation, whereas the rat is usually resistant (Hukkanen *et al.*, 2002), and CYP2E1 enzyme activity is about 7-fold higher in mouse than in human lung (Dowsley *et al.*, 1999). *In vivo* studies have shown higher EB circulating levels in mice in comparison to rats, suggesting that metabolism might be an important factor resulting in the high sensitivity of mice to develop lung tumors after BD exposure (Dahl *et al.*, 1990; Kreiling *et al.*, 1986). *In vitro* studies in lung microsomes comparing mice, rat, and human tissues indicate a rate of oxidation of BD to EB and EB to DEB of mice > rats ≈ humans (Csanády *et al.*, 1992; Schmidt and Loeser, 1985). Mice tend to form approximately 200 times more DEB than humans at exposures of 0.1–1.5 ppm BD (Swenberg *et al.*, 2011).The detoxification by hydrolysis via epoxide hydrolase to generate 1,2-dihydroxy-3-butene metabolite has a relative rate of humans > rats > mice (van Sittert *et al.*, 2000), whereas GSH conjugation has a relative rate of mice ≈ rats > humans (Csanády *et al.*, 1992). The overall activation/detoxification ratio was found to vary markedly between mice (72), rats (5.8), and humans (5.9) (Csanády *et al.*, 1992). In humans, there is a higher rate of hydrolytic metabolism of EB (van Sittert *et al.*, 2000). Careful consideration to these species differences need to be considered when extrapolating findings in mice to humans.

As a result of varying ways to account for species differences, various reports by agencies, authorizations and publications have been published (Anderson *et al.*, 1998; ATSDR, 1992, 2012; CEPA, 2000; ECB, 2002; EPA, 2002b; Grant *et al.*, 2010; Hackett *et al.*, 1987; Hazelton-Laboratories-Europe, 1981; IARC, 2008; IRIS, 2013; Kirman and Grant, 2012; Kirman *et al.*, 2010; NTP, 1984, 1993; Stayner *et al.*, 2000). For the present purpose, studies were selected from reports from Health Canada (CEPA, 2000), the European Union (ECB, 2002), the Dutch government (Hernández *et al.*, 2011), and the U.S. Environmental Protection Agency (U.S. EPA) (IRIS, 2013).

***Criteria for POD selection—Neoplastic effects.*** Factors taken into account for study selection included weight of evidence and human relevance with consideration to strength of association, consistency, and biological plausibility. The most relevant data are summarized in Table 1. The most pertinent animal study was the 2-year chronic bioassay performed by the National Toxicology Program (NTP, 1993). Various regulatory agencies have applied different modeling methods to analyze the data. Female mice had a lower 95% confidence limit of the concentration associated with a 10% increase ($BMCL_{10}$) in lung adenocarcinomas of 0.08 mg/m³ (Hernández *et al.*, 2011), and a tumorigenic concentration associated with a 5% increase in the incidence or mortality due to lung adenocarcinomas ($TC_{05}$) of 1.4 mg/m³ (CEPA, 2000). Rats, on the other hand, had a $TC_{05}$ for mammary tumors of 4.7 mg/m³ (CEPA, 2000). Epidemiology data of synthetic rubber industry workers resulted in a tumorigenic concentration associated with a 1% increase in the incidence or mortality due to cancer ($TC_{01}$) for leukemia of 1.4 mg/m³ for data independently analyzed by the Canadian government (CEPA, 2000) and 3.1 mg/m³ for data analyzed by Matanoski *et al.* (1997).

A series of epidemiological studies following synthetic styrene-butadiene rubber industry workers have been performed (Delzell *et al.*, 1995, 1996, 2001; Macaluso *et al.*, 1996; Sathiakumar and Delzell, 2007, 2009; Sathiakumar *et al.*, 1998, 2009; Sielken and Valdez-Flores, 2001; Sielken *et al.*, 2007). These raw studies were used by the Canadian government (Environment Canada/Health Canada) to derive cancer potency estimates from cumulative occupational exposures to BD and styrene at each year of the subject's life, beginning with the date the cohort begun until death (CEPA, 2000). A lifetime probability of death due to leukemia was computed taking into account the death rates in the Canadian population. The resulting occupational exposures per person per year were converted to environmental exposures by assuming the occupational exposures were for 8 h/day for 240 days/year (CEPA, 2000). Given the large species differences in biokinetics and in tumor response, epidemiological data are considered to be preferable for the assessment of the carcinogenic risk of BD to humans. Thus, the daily $TC_{01}$ of 1.4 mg/m³ derived by the Canadian government from data on human synthetic rubber industry workers (CEPA, 2000) was selected as a POD.

***Criteria for POD selection—Nonneoplastic effects.*** As with neoplastic effects, factors taken into account for study selection included weight of evidence and human relevance with consideration to strength of association, consistency, and biological plausibility. The most relevant data are summarized in Table 1. The most pertinent animal study was the 2-year chronic bioassay performed by the National Toxicology Program (NTP, 1993). There are several reproductive and developmental effects reported in mice exposed to BD via inhalation (ATSDR, 2012; EPA, 2002a; NTP, 1993). The most sensitive developmental effects were observed in a study of pregnant CD-1 mice exposed to BD for 6 h/day and exposed at gestational days 6–15. In this study, there was an increase in maternal toxicity and a decrease in fetal weight at the lowest concentration tested, which was 40 ppm (88 mg/m³) (Hackett *et al.*, 1987). In terms of reproductive effects, the most sensitive effects were ovarian atrophy in female and testicular atrophy in male mice, respectively, in a study with mice exposed to BD by inhalation for 6 h/day, 5 days/week for up to 103 weeks (NTP, 1993). Ovarian atrophy in female mice was observed at the lowest concentration tested (6.25 ppm; 13.8 mg/m³) (NTP, 1993), whereas testicular atrophy was observed at BD concentrations of 20 ppm (44 mg/m³) and higher (NTP, 1993). However, in the European Chemicals Bureau (ECB) Risk Assessment Report on BD, it was stated that it is not clear whether the effects on the gonads in mice is a direct effect on fertility or a secondary consequence of systemic toxicity (ECB, 2002). In the United States and Canada, a different evaluation was made. The consistent finding of ovarian and testicular atrophies in various studies, the presence of a clear dose-response relationship, and biological plausibility (Bevan *et al.*, 1996; NTP, 1984, 1993) were all factors that were considered (CEPA, 2000). It was concluded that studies have shown that BDE is toxic to the ovaries in both mice and rats with mice to be more sensitive than rats (Doerr *et al.*, 1996). In Canada, BD is considered to be a possible reproductive toxicant in humans given the qualitative similarities in the metabolism of BD in mice, rats, and humans, the likely variation in genetic polymorphisms for the relevant enzymes across the general population, and the observed ovarian toxicity in mice after BD exposure (CEPA, 2000). The Agency for Toxic Substances and

**TABLE 1**
**Summary of Most Relevant Dose-Response Studies Evaluated**

| Effects | Agency | Study Description | Concentration ($mg/m^3$) |
|---|---|---|---|
| **Neoplastic effects** | | | |
| Leukemia | Environment Canada, Health Canada | CEPA (2000), epidemiology data of synthetic rubber industry workers, $TC_{01}$ [a] (independent analysis) | 1.4 |
| Leukemia | Environment Canada, Health Canada | CEPA (2000), epidemiology data of synthetic rubber industry workers, $TC_{01}$ [a] (data from case control study of Matanoski et al.(1997) [b] | 3.1 |
| Lung adenocarcinoma | Environment Canada, Health Canada | CEPA (2000), F mice, $TC_{05}$ [c] | 1.4 |
| Mammary tumors | Environment Canada, Health Canada | CEPA (2000), F rats, $TC_{05}$ [d] | 4.7 |
| Leukemia | European Union | ECB (2002), Occupational $TC_{01}$ [e] | 7.8 |
| Lung adenocarcinoma | European Union | ECB (2002), F mice [f] | 13.8 |
| Mammary tumors | European Union | ECB (2002), F rats [f] | 2210 |
| Mixed tumors (lung adenocarcinomas) | RIVM (Hernández et al., 2011) | NTP (1993), F mice, 104 weeks, $BMCL_{10}$ | 0.003 |
| **Reproductive effects (nonneoplastic)** | | | |
| Ovarian atrophy | Environment Canada, Health Canada | CEPA (2000) mice, $TC_{05}$ | 2.5 |
| Ovarian atrophy | U.S. EPA | IRIS (2013), mice, human equivalent concentration $BMCL_{10}$ [f] | 1.94 |
| Ovarian atrophy | TCEQ | Grant et al. (2010), mice, $BMCL_{05}$ [f] | 1.02 |
| Ovarian atrophy | | Kirman and Grant (2012), mice, $BMCL_{01}$ [f] | 3.3 |

*Note.* $BMCL_{05}$, lower 95% confidence limit of the concentration associated with 5% increase in effect; $BMCL_{10}$, lower 95% confidence limit of the concentration associated with a 10% increase in effect; CEPA, Canadian Environmental Protection Act; IRIS, Integrated Risk Information System; NTP, National Toxicology Program; RIVM, National Institute for Public Health and the Environment in The Netherlands; $TC_{01}$, a tumorigenic concentration associated with a 1% increase in the incidence or mortality due to cancer; $TC_{05}$, a tumorigenic concentration associated with a 5% increase in the incidence or mortality due to cancer; TCEQ, Texas Commission on Environmental Quality.

[a]Carcinogenic potency estimates ($TC_{01}$) for models fitted to mean cumulative exposure per person-year based on study by Delzell et al. (1996).

[b]Analysis performed by Delzell et al. (1996).

[c]Carcinogenic potency estimates ($TC_{05}$) of BD based on results in mice (NTP, 1993).

[d]Carcinogenic potency estimates ($TC_{05}$) of BD based on results in rats (Hazelton-Laboratories-Europe, 1981).

[e]Carcinogenic potency estimates ($TC_{01}$) for lifetime exposure associated with a 1% increase in mortality due to leukemia calculated for occupational exposure on the basis of observed rate ratios and estimated cumulative exposure obtained from study by Delzell et al. (1996).

[f]Conversion factor used: 2.21 ppm/($mg/m^3$).

Disease Registry (ATSDR) reports that the U.S. EPA derived an inhalation reference concentration for BD based on a $BMCL_{10}$ of 0.88 ppm (1.94 $mg/m^3$) for ovarian atrophy in mice exposed to BD by inhalation for 6 h/day, 5 days/week for up to 103 weeks (ATSDR, 2012; EPA, 2002a; IRIS, 2013). Ovarian atrophy was selected as a critical effect because it yielded the lowest $BMCL_{10}$ from a high-quality 2-year study (ATSDR, 2012; EPA, 2002a). Although the overall differences in activation/detoxification ratio in BD metabolism between mice (72), rats (5.8), and humans (5.9) (Csanády et al., 1992) are acknowledged, ovarian atrophy is considered a possible relevant nonneoplastic effect for humans and an appropriate POD. For this reason, the U.S. EPA's $BMCL_{10}$ of 0.88 ppm (1.94 $mg/m^3$; Table 1) for ovarian atrophy in female mice (IRIS, 2013; NTP, 1993) was selected as a POD.

*Exposure Assessment: Scenario Description*

Two exposure scenarios were evaluated: (1) direct exposure to BD via cigarette smoking (MSS) and (2) indirect exposure via exposure to ETS while residing in a tavern or bar. For the first scenario, the BD content in cigarette smoke and insight in smoking dynamics are important determinants to determine the inhaled and absorbed amount of BD during smoking. The impact of risk reduction strategies were also evaluated for scenario 1. Because airborne BD concentrations adequately describing the exposure dynamics of smoking cannot be accurately calculated, the MOE analyses are performed on the internal (absorbed) dose of BD. For the second scenario, it sufficed to identify relevant BD concentrations in the environmental air in bars and taverns in combination with the dwell time in these locations.

*Exposure Assessment 1: Sources of BD in Cigarette Smoke*

BD is generated from the combustion of complex molecules such as carbohydrates, amino acids, phytosterols, paraffins, and many other tobacco components (Smith et al., 2000). BD is present in the gas phase of MSS and given that the primary formation of BD in smoke is via thermal cracking reactions in tobacco char; BD most likely shares a large number of natural tobacco leaf constituent precursors with benzene and polycyclic aromatic hydrocarbons (Ferguson, 2000). BD has natural precursors found in the tobacco leaf itself and precursors from additives. From the natural BD precursors, not all tobacco leaves are the same given that cigarettes made of pure Burley give lower BD yields than those made from flue-cured or oriental tobaccos (Adam et al., 2006). This difference in BD levels could be partly attributed to differences in curing methods and sugar content. Sugars in air-cured tobacco such as Burley are rapidly metabolized, thereby yielding lower sugar levels in comparison to flue-cured or sun-cured tobacco (oriental) (Talhout et al., 2006), which have very high sugar content (Baker et al., 2005; Sanders et al., 2002). Cellulose is another major BD precursor found in natural tobacco, and studies have shown that natural tobacco contains approximately 10% of polysaccharide cellulose (Leffingwell, 1999). Reducing BD levels in natural tobacco can be very challenging, but reducing BD levels from added precursors is more feasible. These added precursors include cellulose, paraffin, and sugars. An extensive pyrolysis study showed that BD was generated when cyclodextrins were mixed with cellulose in the tobacco blend and from casing materials and volatile top flavouring (Paschke et al., 2002). In the Netherlands, the average amount of cellulose and paraffin added to tobacco (from glue and cigarette paper and paraffin added to the ink of cigarette paper) was reported to

be 1.3% (wt/wt) per cigarette, with a maximum of 6.7% (analysis of data delivered to Dutch regulators up to September 2012 via the Electronic Model Tobacco Control (EMTOC) (EMTOC, 2012)). In the Netherlands, yearly submission of tobacco list of ingredients is required since 2003. The National Institute for Public Health and the Environment (RIVM) in The Netherlands coordinates the EMTOC project in a European consortium of 15 Member States. EMTOC is a European web application that enables safe submission of the tobacco ingredients list to the concerned authorities. The data submitted to EMTOC are only accessible to national authorities (regulators) and the European Commission (DG SANCO). The RIVM (2013) publishes the tobacco products ingredient information for the general public on their web site in a searchable database.

Regarding sugars, a study on the relationship of MSS constituents and sugar addition showed increased levels of BD, particularly in case of a high content of fructose corn syrup and sucrose in cigarettes (Roemer *et al.*, 2012). In the Netherlands, the average amount of added sugars was 1.5% (wt/wt), with a maximum of 10.9% (wt/wt) (EMTOC, 2012). Overall, this will be an underestimation of the amount of added BD precursors, given that not all compounds in cigarettes that may undergo pyrolysis to generate BD are known. Reducing the addition of BD precursors might be a way to reduce BD in MSS and ETS.

*Exposure Assessment 1: Methods for Deriving BD Estimates in Tobacco Smoke*

Human smoking behavior is a complex process influenced by factors such as puff volume, puff duration, interpuff interval, number of puffs per cigarette, and total puff volume (Marian *et al.*, 2009). Human smoking behavior can differ from commonly used smoking machine regimes such as the International Organization for Standardization (ISO) and the Canadian Intense (CI) protocol. ISO smoking machine method uses a puff volume of 35 ml, a puff frequency of 60 s, a total puff volume of 455 ml, and open ventilation. The CI smoking machine method, on the other hand, uses a puff volume of 55 ml, a puff frequency of 30 s, a total puff volume of 715 ml, and 100% blocked ventilation. Standardization of the machine-generated yields per nicotine has been suggested to minimize the variability between methods (Burns *et al.*, 2008). In contrast to smoking machines, smokers tend to adjust their puff volume and interpuff interval time to attain a desired level of nicotine in their system. A study investigating differences between human smoking behavior and smoking machine estimates found that the ratio between the measured nicotine in humans and the ISO differed by a factor of 2.5 for low nicotine yield cigarettes (≤ 0.8 mg of nicotine per cigarette) and by a factor of 2.2 for medium nicotine yield cigarettes (0.9–1.2 mg of nicotine per cigarette) (Djordjevic *et al.*, 2000). Therefore, by adjusting the toxicant level per milligram of nicotine as obtained from smoking machines to the smokers nicotine intake (2.5 mg nicotine/cigarette for low nicotine yield cigarettes, and 2.2 mg nicotine for medium nicotine yield cigarettes), we can obtain a better estimate of the actual level of toxicants that smokers are being exposed to (Djordjevic *et al.*, 2000).

*Exposure Assessment 1: Estimation of the Inhaled Amount of BD*

Due to the complexity of the smoking scenario, it will be difficult, if not impossible, to account for all factors involved in the estimation of the inhaled amount of a smoke component. Due to, among others, individual smoking behavior and respiration dynamics, the concentration of a smoke component in the respiratory tract, including the alveoli, changes continuously. Because in general data to account for these factors will be lacking, a pragmatic approach has been proposed that takes into account basic respiration characteristics to estimate the alveolar concentration and the absolute amount that reaches the alveoli and becomes available for absorption (Bos *et al.*, 2012). It is recommended to use chemical-specific data for refinement whenever available. The proposed approach accounts for the fact that approximately 70% of an inhaled dose reaches the alveoli where absorption takes place and thus can potentially be absorbed.

*Exposure Assessment 1: Estimation of the Absorbed Amount of BD*

BD is slightly soluble in water with a solubility of 735 mg/l at 20°C, and it is highly volatile with a high vapor pressure (1790 mmHg, 239 kPa at 20°C) (EPA, 2002a). These factors may affect the rate at which BD reaches the alveoli and enters the systemic circulation. In the case of BD, absorption is primarily dependent on the blood:air partition coefficient. Reported partition coefficients

of approximately 1 show that BD is moderately soluble in blood and indicate a theoretical alveolar retention of 50% in a single breath (EPA, 2002a). This was taken into account in the present evaluations.

*Exposure Scenario 1: Risk Reduction Strategies*

Several strategies were investigated for reducing toxicant levels in tobacco smoke. These include the modification of agricultural and curing practices, the selective removal of tobacco constituents, the addition of diluent materials to tobacco, the reduction or removal of BD precursors (cellulose, lipids, and/or wax), and the use of different filters such as cellulose acetate and carbon filters. These developing technologies might reduce BD levels in MSS and ETS. MOE analyses were performed before and after applying reduction measures to assess the possible health impacts of these reduction technologies. A literature search was performed in PubMed for the selection of published studies on risk reduction strategies to test their efficacy in reducing BD levels.

Several risk reduction strategies for BD have been proposed. The WHO TobReg recommends a maximum level of 125% of the median value of the toxicant per milligram of nicotine from brands on the market being regulated (Burns *et al.*, 2008). This strategy will result in yearly decreasing median BD values. This 125% estimate of the median value from international brands is used in the present exposure assessment as a risk reduction measure. Other risk reduction measures evaluated included the use of cigarettes composed of 50% tobacco-substitute sheet with a glycerol content of 12.5% and a double carbon filter (McAdam *et al.*, 2011), alterations of the tobacco blend by using a tobacco-substitute sheet, or a tobacco blend treatment consisting of a protease-treated tobacco with the potential to reduce protein nitrogen and polyphenols in the blend (McAdam *et al.*, 2012); cigarettes with amine-functionalized resin beads or high activity carbon were tested for their ability to reduce toxicant levels (McAdam *et al.*, 2012), and the use of a reduced toxicant prototype (80% U.S. blend) with 20% tobacco-substitute sheet and a 2-segment filter containing 80 mg of polymer-derived carbon (Fearon *et al.*, 2012).

*Exposure Scenario 2: BD Concentrations in ETS*

ETS primarily originates from the smoke emitted from the smouldering cigarette or sidestream smoke (SSS) and smoke exhaled by the smoker. Indoor concentrations of BD depend primarily on the presence of ETS (CARB, 1992). BD is an example where higher levels are present in SSS than in MSS (DHHS, 2010). BD emissions in SSS from cigarettes have been shown to range from 200 to 400 µg/cigarette, whereas ETS levels of BD in smoke-filled bars were found to range from 2.7 to 19 µg/m$^3$ (Brunnemann *et al.*, 1990; Löfroth *et al.*, 1989). In Canada, BD levels in SSS have been reported to range between 281 and 656 µg/cigarette, with an overall BD concentration of 375 µg/cigarette (CEPA, 2000). These numbers were approximately 10-fold higher than BD levels in MSS. Given the high levels of BD in SSS, it was concluded by the Canadian government that the presence of ETS contributes to significant levels of BD contamination in indoor air (CEPA, 2000).

The California Environmental Protection Agency (Cal EPA) performed measurements of BD in high tobacco smoke environments and estimated the amount of BD inhaled in 3 h to range from 0.01 to 0.02 mg/m$^3$ in a tavern and 0.003 to 0.005 mg/m$^3$ in a bar (Table 3) (EPA, 1992). In addition, the Canadian government reported a maximum mean indoor concentration in smoking environments of 0.019 mg/m$^3$ and a maximum of 0.037 mg/m$^3$. These 2 studies were used to assess BD exposure in smoking environments.

## RESULTS

*MOE Evaluation for Neoplastic and Nonneoplastic Endpoints*

The European Food Safety Authority (EFSA) has recommended an MOE approach for risk assessment of substances that are both genotoxic and carcinogenic and stated that an MOE of 10 000 or higher would be of low concern from a public health

perspective and be considered low priority for risk management when it is based on a $BMDL_{10}$ from a chronic animal study (EFSA, 2005). This MOE of 10 000 accounts for factors such as species differences, human variability, and uncertainties with regards to the nature of the carcinogenesis process and the reference point on the dose-response curve. For BD, the selected POD for carcinogenicity was derived from a 25-year follow-up human study where the $TC_{01}$ for leukemia was 1.4 mg/m³ (Table 1).

Because for BD the POD for carcinogenicity is derived from human data, there is no need to account for interspecies differences and thus an MOE lower than 10 000 can already be considered of low concern. Because generally a default assessment factor of 10 is applied for interspecies differences, an MOE of more than 1000 might be considered of low health concern for carcinogenic effects in the present evaluation. Furthermore, a $TC_{01}$ (1% additional tumor incidence) can be regarded as a more conservative POD than a $BMDL_{10}$ (10% additional tumor incidence). Therefore, an MOE of 1000 as a critical value is justifiable in the present evaluation.

For nonneoplastic endpoints, an MOE of 100 (which accounts for inter- and intraspecies variation) or greater is commonly acceptable (EFSA, 2012). Because there is no guidance on the judgement of the acceptability of an MOE for smoke components, risk assessment needs to be assessed on a case-by-case basis, at which it is to be judged whether additional uncertainties need to be accounted for.

For scenario 1, the internal dose of BD was calculated from the $TC_{01}$ using a default breathing volume of 20 m³/24 h and a default human body weight of 70 kg. The mouse $BMCL_{10}$ of 0.88 ppm (1.94 mg/m³) for ovarian atrophy was transferred into an internal daily dose assuming a body weight of 25 g and a breathing rate of 2.2 l/h (Paulussen et al., 1998). For both calculations, dead space volume (70%) and absorption rate (50%) were assumed to be similar and accounted for. The daily internal exposure dose for neoplastic effects was thus calculated to be 0.14 and 0.26 mg/kg for ovarian atrophy.

For scenario 2, the BD air concentrations of 1.4 and 1.9 mg/m³ were used for MOE analyses for neoplastic and nonneoplastic effects, respectively.

### Exposure Scenario No. 1: BD Exposure via MSS

In order to minimize variability between methods, all ISO and CI estimates were standardized per milligram of nicotine and by the average measured nicotine intake in human smokers (2.5 mg nicotine for low nicotine yield cigarettes and 2.2 mg nicotine for medium nicotine yield cigarettes) (Djordjevic et al., 2000). For ISO, the BD levels ranged from 76.4 to 259.6 µg/cigarette, and for CI, BD levels ranged from 78.7 to 188.8 µg per cigarette. The ISO BD minimum and maximum yields were selected for analysis because it encompassed a broader range inclusive of CI minimum and maximum yields.

Cigarette smoke is a major source of exposure to BD in the general population (EPA, 2002a). In Canada, BD levels in MSS have been reported to range from 14.3 to 59.5 µg/cigarette,

with an overall mean BD concentration of 30 µg/cigarette in 18 different Canadian and American cigarette brands (CEPA, 2000). Levels of BD have been reported to range from 6.4 to 34 µg/cigarette (corresponding to 35 to 104 µg/mg nicotine and 76.4 to 259.6 µg/cigarette after adjustment to human nicotine intake) in a study of international brands (Counts et al., 2005). This study representing BD levels from international brands was used to assess BD exposure via MSS. Given that the local effects induced by BD were only observed at extremely high concentrations, focus was given to systemic effects. The BD amount in MSS of 1 cigarette with and without the implementation of risk reduction strategies are presented in Table 2 as microgram BD per cigarette. BD amount absorbed per cigarette can be calculated by multiplying the total amount of BD in MSS of 1 cigarette ($BD_{MSS}$, obtained from smoking machine) by 0.7 (amount that reaches the alveoli) and subsequently by 0.5 (50% absorption). Assuming a person smoking an average of 20 cigarettes/day (Shepperd et al., 2013) and a default human body weight of 70 kg, the total amount of BD absorbed in milligram per kilogram body weight can be calculated by multiplying the BD in MSS (in mg) by $[(20 \times 0.7 \times 0.5)/70] = 0.1$ (Bos et al., 2012). The total absorbed doses from smoking 20 cigarettes/day (with and without risk reduction measures), which are used as exposure estimates for MOE analyses, are given in Table 2.

### Exposure Scenario No. 1: MOE for BD in MSS With and Without Risk Reduction Strategies

For neoplastic effects, the MOE without risk reduction measures from the mean BD concentration from international brands ranged between 5 and 18 (an MOE mean 11) (Table 2). Several risk reduction strategies for BD have been proposed. The WHO TobReg recommendation of a maximum level of 125% of the median value of the toxicant per milligram of nicotine from brands on the market being regulated (Burns et al., 2008) resulted in an MOE of 9. The MOE from cigarettes composed of 50% tobacco-substitute sheet with a glycerol content of 12.5% and a double carbon filter (McAdam et al., 2011) was 18. The tobacco-substitute sheet with a dual or triple carbon filter (McAdam et al., 2012) resulted in an MOE of 24 and 28, respectively. The tobacco blend treatment with a triple carbon filter (McAdam et al., 2012) resulted in an MOE of 18 (Table 2). Finally, the use of a reduced toxicant prototype (80% U.S. blend) with 20% tobacco-substitute sheet and a 2-segment filter containing 80 mg of polymer-derived carbon (Fearon et al., 2012) resulted in an MOE of 101 (Table 2).

For nonneoplastic effects (ovarian atrophy), the MOE from the mean BD concentration from international brands ranged between 10 and 34 (an MOE mean 20) (Table 2). The WHO TobReg risk reduction recommendation of a maximum level of 125% of the median value of the toxicant per milligram of nicotine from brands on the market being regulated (Burns et al., 2008) resulted in an MOE of 16. The MOE for cigarettes composed of 50% tobacco-substitute sheet with a glycerol content of 12.5% and a double carbon filter (McAdam et al., 2011)

**TABLE 2**

**Results of MOE Analyses for Neoplastic (Leukemia in Humans) and Nonneoplastic (Ovarian Atrophy) Endpoints due to BD in MSS Before and After Risk Reduction Strategy Implementation, Assuming Smoking of 20 Cigarettes/Day**

| | | | | Counts et al. (2005) Adjusted to Human Nicotine Intake (Djordjevic et al., 2000), MSS | | McAdam et al. (2011) | McAdam et al. (2012) | | | Fearon et al. (2012) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | MSS[a] Mean concentration (min–max range) | MSS[a] 125% of mean | MSS[a] 50% TSS + dual CF | TSS + dual CF | MSS[b] TSS + triple CF | TBT + triple CF | MSS[a] RP |
| | | | μg/cigarette | 128 (76.4–259.6) | 160 | 77 | 58 | 50 | 78 | 13.8 |
| | | *Absorbed dose from 20 cigarettes* → (mg/kg/day) | | 0.01 (0.008–0.026) | 0.016 | 0.008 | 0.006 | 0.005 | 0.008 | 0.001 |
| Effects | Agency | Reference | Internal daily dose (mg/kg/day) | | | MOE | | | | |
| **Neoplastic effects** | | | | | | | | | | |
| Leukemia | Environment Canada, Health Canada | CEPA (2000), epidemiology data of synthetic rubber industry workers, $TC_{01}$[c] (independent analysis of 25-year follow-up) | 0.14 | 11 (5–18) | 9 | 18 | 24 | 28 | 18 | 101 |
| **Nonneoplastic effects** | | | | | | | | | | |
| Ovarian atrophy | U.S. EPA | EPA (IRIS, 2013), mice, $BMCL_{10}$[d] | 0.26 | 20 (10–34) | 16 | 34 | 45 | 52 | 33 | 188 |

*Note.* TSS, tobacco-substitute sheet; CF, carbon filter; TBT, tobacco blend treatment; RP, reduced prototype consisting of 20% TSS and a 2-segment filter containing 80mg of polymer-derived carbon.

[a]ISO smoking machine regime adjusted to human nicotine intake (Djordjevic et al., 2000).

[b]CI smoking machine regime adjusted to human nicotine intake (Djordjevic et al., 2000).

[c]Internal doses were calculated using the following default parameters: human inhalation volume = 20 m$^3$/day, human body weight of 70kg, 0.7 to account for the fact that only 30% of inhaled air (dead space volume) does not reach the alveoli, and 0.5 to account for 50% absorption (1.4 × 20 m$^3$/day/70kg × 0.7 × 0.5).

[d]Internal doses were calculated using the following default parameters: 2.21 ppm/(mg/m$^3$), mouse default inhalation volume of 2.2 l/h, mouse body weight of 0.25 kg, 0.7 to account for the fact that only 30% of inhaled air (dead space volume) does not reach the alveoli, and 0.5 to account for 50% absorption (0.88 ppm × 2.21 ppm/(mg/m$^3$) × 2.2 l/h × 1 m$^3$/1000 l × 6h/day × 5 days/week/(0.025kg) (7 days/week) × 0.7 × 0.5).

was 34. The tobacco-substitute sheet with a dual or triple carbon filter (McAdam *et al.*, 2012) resulted in an MOE of 45 or 52, respectively. The tobacco blend treatment with a triple carbon filter (McAdam *et al.*, 2012) resulted in an MOE of 33 (Table 2). Finally, the use of a reduced toxicant prototype (80% U.S. blend) with 20% tobacco-substitute sheet and a 2-segment filter containing 80 mg of polymer-derived carbon (Fearon *et al.*, 2012) resulted in an MOE of 188 (Table 2).

### Exposure Scenario No. 2: ETS

The California Environmental Protection Agency (Cal EPA) performed measurements of BD in high tobacco smoke environments and estimated the amount of BD inhaled in 3 h to range from 0.01 to 0.02 mg/m$^3$ in a tavern and 0.003 to 0.005 mg/m$^3$ in a bar (Table 3) (EPA, 1992). In addition, the Canadian government reported a maximum mean 24-h indoor concentration in smoking environments of 0.019 mg/m$^3$ and maximum of 0.037 mg/m$^3$. These 2 studies were used to assess BD exposure in smoking environments. Therefore, 4 different exposure measures were considered in our exposure assessment: a 3-h indoor concentration in a tavern (0.01–0.02 mg/m$^3$), a 3-h indoor concentration in a bar (0.003–0.005 mg/m$^3$) (EPA, 1992), a 24-h maximum mean indoor concentration in a smoking environment (0.019 mg/m$^3$), and a 24-h maximum indoor concentration in a smoking environment (bingo hall) (0.037 mg/m$^3$) (CEPA, 2000). It must be kept in mind that the amount of BD inhaled in an ETS environment is, among others, dependent on the number of cigarettes smoked in the room, the size of the room, and the ventilation present (if any). A 3-h exposure was the only exposure duration provided by the Cal EPA, and the 24-h maximum mean and maximum indoor concentration of smoking environments as reported by the Canadian government were found to be appropriate given the limited indoor nonresidential smoking environments for which data are available. These assumptions can be easily adapted when more information about the daily exposure to heavy smoking environments is available.

### Exposure Scenario 2: MOE for BD in ETS

For neoplastic effects (leukemia in humans), the MOE derived from minimum and maximum BD levels in a tavern was 74 and 127, whereas the MOE derived from minimum and maximum BD levels in a bar was 311 and 424 (Table 3). The MOE derived from the maximum mean BD indoor concentration was 74 and from the maximum BD levels in smoking environments was 38 (Table 3).

For nonneoplastic effects (ovarian atrophy in female mice), the MOE derived from minimum and maximum BD levels in a tavern was 100 and 173, whereas the MOE derived from minimum and maximum BD levels in a bar was 422 and 576 (Table 3). The MOE derived from the maximum mean BD indoor concentration was 101 and from the maximum BD levels in smoking environments was 51 (Table 3).

## DISCUSSION

### Risk Assessment: MOE

MOE analysis is a well-accepted method to assess the risk of pesticides, food products, and other chemicals (Cunningham *et al.*, 2011). The MOE is the ratio of the most sensitive neoplastic or nonneoplastic effect to exposure levels; in this case, exposure to BD in MSS and ETS. Currently, there are no set of guidelines on an acceptable MOE for tobacco smoke components such as BD. It was previously reasoned that for evaluation of the carcinogenic risk of BD from cigarette smoke, an MOE of 1000 or greater can be considered as of low concern for human health. For nonneoplastic effects, the MOE should be at least 100 to account for interspecies differences and interindividual variability between humans.

### Exposure Scenario No. 1: MOE for BD in MSS With and Without Risk Reduction Strategies

For neoplastic effects (leukemia in humans), the BD levels in MSS from international brands (Counts *et al.*, 2005) resulted in an MOE ranging from 5 to 18, indicative that BD levels present in MSS from international brands are of concern for neoplastic effects. The changes in MOE after the application of risk reduction strategies were evaluated. First, we investigated the impact of the WHO TobReg proposed recommendation that BD levels should be reduced yearly to a maximum of 125% of the mean BD estimates for each region (Burns *et al.*, 2008). As illustrated in Table 2, the MOE was less than 1000 and fell in the range of the MOE derived from international brands and no significant change in risk was observed. Secondly, the use of a tobacco-substitute sheet releases glycerol when burnt, thus diluting the amount of toxicants such as BD in MSS and ETS (McAdam *et al.*, 2011). Experimental cigarettes were generated with varying levels of inclusion of tobacco-substitute sheet and glycerol in the final blend and the type of filter used (cellulose acetate or dual segment carbon). The MOE of an experimental cigarette composed of 50% tobacco-substitute sheet with a glycerol content of 12.5%, and a double carbon filter was selected for evaluation because this combination yielded the lowest BD levels (McAdam *et al.*, 2011). Nevertheless, the MOE was less than 1000 and still fell in the range of the MOE derived from international brands, and no significant change in risk was observed. Thirdly, activated carbon is very effective in absorbing toxicants such as BD. A subsequent study by the same group evaluated the potential use of altering the tobacco blend by using a tobacco-substitute sheet or a tobacco blend treatment consisting of a protease-treated tobacco with the potential to reduce protein nitrogen and polyphenols in the blend (McAdam *et al.*, 2012). In addition to variations in tobacco blend, experimental cigarettes with amine-functionalized resin beads or high activity carbon were tested for their ability to reduce toxicant levels (McAdam *et al.*, 2012). The use of a tobacco-substitute sheet and a dual or triple carbon filter, and tobacco blend treatment with a triple filter did not alter

**TABLE 3**
**Results From MOE Analyses for Neoplastic (Leukemia in Humans) and Nonneoplastic (Ovarian Atrophy) Endpoints due to Exposure to BD in ETS**

| Effects | Agency | References | mg/m³ Exposure concentration (mg/m³) | California Environmental Protection Agency (EPA, 1992) 3-h Indoor Concentration | | | | Environment Canada (CEPA, 2000) | | |
| | | | | Tavern | | Bar | | Nonsmoking Environment | Smoking Environment | Smoking Environment |
| | | | | Min 0.01 | Max 0.02 | Min 0.003 | Max 0.005 | Max 0.001 | Max mean 0.019 | Max 0.037 |
| | | | | | | | MOE | | | |
| Neoplastic effects Leukemia | Environment Canada, Health Canada | CEPA, (2000), epidemiology data of synthetic rubber industry workers, $TC_{01}$ (independent analysis of 25-year follow-up) | 1.4 | 127 | 74 | 424 | 311 | 1167 | 74 | 38 |
| Nonneoplastic effects Ovarian atrophy | U.S. EPA | EPA (IRIS, 2013), mice, $BMCL_{10}$ [a] | 1.9 | 173 | 100 | 576 | 422 | 1583 | 101 | 51 |

*Note.* [a] Conversion factor used: 2.21 ppm/(mg/m³).

the MOE significantly (Table 2). Finally, the use of a reduced toxicant prototype (80% U.S. blend) with 20% tobacco-substitute sheet and a 2-segment filter containing 80 mg of polymer-derived carbon (Fearon *et al.*, 2012) resulted in an MOE of 101. This prototype resulted in the highest level of reduction of BD levels. Although the MOE was still lower than 1000 and thus a concern for neoplastic effects remains, it was considered a significant reduction in BD exposure.

In summary, according to our analysis and assumptions, a risk for neoplastic effects (leukemia) exists from BD levels present in MSS as measured from international brands. Risk reduction strategies did not significantly alter the MOE, with the exception of the reduced toxicant prototype (80% U.S. blend) with 20% tobacco-substitute sheet and a 2-segment filter containing 80 mg of polymer-derived carbon (Fearon *et al.*, 2012). Nevertheless, the MOE for BD levels found in this prototype was still lower than 1000, and thus, a concern for neoplastic effects remains present. Importantly, the WHO TobReg proposed recommendation that BD levels should be reduced yearly to a maximum of 125% of the mean BD estimates for each region (Burns *et al.*, 2008) did not alter the MOE. It is expected that this risk reduction strategy will only slowly result in reduction of BD exposure and that it will take many years before a significant change in risk from BD exposure will be achieved.

For nonneoplastic effects (ovarian atrophy), the BD levels in MSS from international brands (Counts *et al.*, 2005) resulted in an MOE ranging from 10 to 34, indicative that BD levels present in MSS from international brands are of concern for nonneoplastic effects. The changes in MOE after the application of risk reduction strategies were evaluated. First, we investigated the impact of the WHO TobReg proposed recommendation that BD levels should be reduced to a maximum of 125% of the mean BD estimates for each region (Burns *et al.*, 2008). As illustrated in Table 2, the MOE was less than 100 and fell in the range of the MOE derived from international brands, and no significant change in risk was observed. Secondly, experimental cigarettes composed of 50% tobacco-substitute sheet with a glycerol content of 12.5% and a double carbon filter (McAdam *et al.*, 2011) resulted in an MOE that was less than 100 and fell in the range of the MOE derived from international brands, and no significant change in risk was observed. Thirdly, the use of a tobacco-substitute sheet and a dual or triple carbon filter and tobacco blend treatment with a triple filter (McAdam *et al.*, 2012) did not alter the MOE significantly (Table 2). Finally, the use of a reduced toxicant prototype (80% U.S. blend) with 20% tobacco-substitute sheet and a 2-segment filter containing 80 mg of polymer-derived carbon (Fearon *et al.*, 2012) resulted in an MOE higher than 100 and was the only risk reduction strategy that significantly altered the risk for nonneoplastic effects to a level that can be considered of no concern.

In summary, according to our analysis and assumptions, a risk cannot be excluded for nonneoplastic effects (ovarian atrophy) from BD levels present in MSS as measured from international brands. Risk reduction strategies did not significantly

alter the MOE, with the exception of the reduced toxicant prototype (80% U.S. blend) with 20% tobacco-substitute sheet and a 2-segment filter containing 80 mg of polymer-derived carbon (Fearon *et al.*, 2012). This was the only risk reduction strategy that resulted in an MOE indicating no concern for nonneoplastic effects. Importantly, as found with neoplastic effects, the WHO TobReg proposed recommendation that BD levels should be reduced yearly to a maximum of 125% of the mean BD estimates for each region (Burns *et al.*, 2008) did not alter the MOE significantly.

Even though the above mentioned risk reduction strategies do not reduce BD in MSS significantly at the moment, new technologies might be more efficient. New studies have shown that a macroporous, polystyrene-based iron-exchange resin (DiaionCR20) with surface amine group functionality is highly selective and efficient in absorbing volatile species such as formaldehyde, acetaldehyde, and hydrogen cyanide in cigarette smoke. More studies are needed to explore whether this new macroporous, polystyrene-based iron-exchange resin (DiaionCR20) can reduce BD levels significantly in cigarette smoke (Pacchierotti *et al.*, 1998).

*Exposure Scenario 2: MOE for BD in ETS*

For neoplastic effects, 4 different exposure measures were considered in our exposure assessment: a 3-h indoor concentration in a tavern ($0.01–0.02 \, mg/m^3$), a 3-h indoor concentration in a bar ($0.003–0.005 \, mg/m^3$) (EPA, 1992), a 24-h maximum mean indoor concentration in a smoking environment ($0.019 \, mg/m^3$), and a 24-h maximum indoor concentration in a smoking environment (bingo hall) ($0.037 \, mg/m^3$) (CEPA, 2000). An important consideration in the interpretation of the MOE for the bar and tavern is that the exposure measured by the Cal EPA was a 3-h indoor concentration in comparison to the 24-h $TC_{01}$ obtained from the epidemiology study on synthetic rubber industry workers (Table 3) (CEPA, 2000). Secondly, it can be assumed that people in general will not visit bars or taverns on a daily basis. For instance, if we assume that people go to a bar or tavern thrice per week and spend on average 5 h in the bar, they will be exposed to ETS for 15 out of 168 h/week (ie, approximately 10% of their time). In that case, their internal dose will also be 10-fold less than when exposed continuously. This 10-fold difference should be weighed in the interpretation of the MOE that is based on a $TC_{01}$ that counts for continuous daily exposure. For neoplastic effects, the MOE for taverns ranges from 74 to 127 and for bars from 311 to 424. For the tavern, the lower MOE will be close to the critical value of 1000 and might be of low concern if this 10-fold difference in exposure duration is taken into account. However, further refinement (eg, by replacing assumptions by actual data), is needed for a definitive conclusion. More information on the frequency and duration of dwell time in a tavern might for instance be helpful. As to the bar, the MOEs are close to 1000, and considering the presumed limited dwell time, it can be concluded that the risk for neoplastic effects will be of

low concern. Actual data on people's habits for bar visiting can confirm this conclusion.

However, the maximum mean concentration or maximum concentration of BD in smoking environments as reported by the Canadian government results in an MOE of significantly less than 1000 and for this reason a risk for neoplastic effects cannot be excluded. Refinement of the assessment can be made when more information on how often people are exposed to smoking environments is available.

In summary, following our analysis, a risk cannot be excluded for neoplastic effects (leukemia) from 24-h maximum mean and maximum indoor BD concentrations in smoking environments as reported by the Canadian government. The risk derived from BD obtained from 3-h indoor concentrations of a tavern or bar might be of low concern. More information on the duration of exposure to smoking environments is needed to refine our exposure assessment.

For nonneoplastic effects (ovarian atrophy), the MOEs derived from BD indoor concentration in a tavern and bar are above the critical value of 100, indicating no concern for a health risk. When evaluating these MOEs, it should be weighed that the exposure scenario in the mouse study was 6 h/day for 5 days/week. The dwell time in a bar or tavern per week might be less, introducing an additional margin of exposure. The maximum mean concentration of BD in smoking environments derived by the Canadian government had an MOE approximately equal to the critical value of 100. Especially when it can be assumed that the dwell time of people in such an environment is limited, it can be concluded that there is no risk for nonneoplastic effects. However, the maximum concentration of BD in smoking environments derived by the Canadian government results in an MOE of 51, and a risk for nonneoplastic effects then cannot be excluded. Better insight in the actual dwell time of people in these environments and on their exposure is needed to draw further conclusions. In summary, according to our analysis and assumptions, BD levels in smoking environments will generally be of no concern for nonneoplastic health effects. However, in incidental situations with high concentrations of BD due to ETS, a health risk might be indicated, depending on the actual dwell time in these environments.

*Assumptions and Uncertainties*

Currently, there are no set guidelines on an acceptable MOE for tobacco smoke components such as BD. The present approach has several assumptions and uncertainties. An important aspect is that BD will not be the only source of adverse effects. Given that cigarette smoke is a complex mixture, the increase or decrease of the levels of other compound(s) as a result of BD reduction cannot be excluded. Nevertheless, risk reduction strategies such as carbon filters and new technologies such as the macroporous, polystyrene-based iron-exchange resin (DiaionCR20) are not specific for BD and can trap other volatile species. Although it is acknowledged that risk assessment of smoking should preferably aimed at the entire mixture

of components, a method for health risk assessment of complex mixtures is not available yet. Risk analysis of a complex mixture such as tobacco smoke was beyond the scope of this study. The purpose of the risk analysis was to estimate the health risks of BD in MSS and ETS and how these were altered with risk reduction strategies. Risk assessment of BD is a complex process considering the many issues involved, such as interspecies differences in biokinetics and toxicity. For the present evaluation, a number of pragmatic choices had to be made. Our analysis was based on evaluations of well-known, recognized international organizations. POD studies for cancer endpoints (leukemia) were obtained from synthetic BD-styrene rubber industry, and cancer effects could have also been attributed to styrene or benzene exposure. Nevertheless, a detailed and quantitative exposure estimation was made for BD, styrene, and benzene for each of the 16610 styrene-butadiene production workers (Macaluso *et al.*, 1996), and human studies were preferred over animal studies given the large species difference in BD metabolism and toxicity. For noncancer endpoints, ovarian atrophy was observed in both long-term (2-year) (NTP, 1984, 1993) and short-term (13-weeks) (Bevan *et al.*, 1996) animal studies, and although the etiology of the observed ovarian atrophy in mice is not fully understood, it was considered the most sensitive noncancer endpoint and appropriate for dose-response characterization. Ovarian atrophy in mice has also been selected as the most sensitive nonneoplastic effect by various international agencies (CEPA, 2000; EPA, 2002a) even though uncertainties are always present when extrapolating from animal to humans. It is important to keep in mind that although smoking is a risk factor for heart and respiratory diseases (ie, COPD), increased mortality due to arteriosclerotic or ischemic heart disease or circulatory disease were only observed in some subgroups of workers (Delzell *et al.*, 1995; Matanoski *et al.*, 1990; McMichael *et al.*, 1974, 1976), and the potential association to BD exposure could not be accounted for and need thorough investigation. Due to the lack of data, an MOE analysis for heart and respiratory diseases could not be performed. For the exposure scenario, default values from human smoking behavior studies were used for the exposure assessment (Djordjevic *et al.*, 2000). More details on the exposure assessment can be found in Bos *et al.* (2012).

Several assumptions were made for the present analyses. For instance, the average smoker was assumed to smoke 20 cigarettes/day. These were just approximations as smokers vary in their smoking habits. Refinement of the analyses can be achieved by using actual data on smoking behavior if available for the scenarios of interest. For ETS exposure, exposure depends mainly on the (weekly) duration of the exposure. Further, the concentration of ETS depends on many factors, and it would be of help if these situations would be described into more detail or that broadly accepted standard scenarios are developed. Because it is recognized that several assumptions have been made, it is obvious that the risk (MOE) analysis can be refined by addressing these assumptions and uncertainties

(Cunningham *et al.*, 2011). Irrespective of these, we have put forth a pragmatic approach for assessing the risk of toxicants in MSS and ETS where chemical-specific data should be used whenever available. It would be of help if a broadly accepted methodology with adequately defined assumptions and well-described exposure scenarios for smoking will be developed.

*Implications for Tobacco Control*

MOE analysis is a flexible quantitative method to assess human health risk in a given exposure scenario. Scenario-specific factors can be weighted and taken into account in the interpretation of the MOE. For example, differences in exposure duration between the human exposure scenario and the POD study were considered in the interpretation of the results when evaluating possible risks of neoplastic effects induced by BD in ETS. Further, the MOE approach allows evaluation of the impact of risk reduction strategies on human health. The MOE analysis is a common and accepted risk assessment methodology, which is often applied in other frameworks.

A step further for the assessment of the efficacy of risk reduction strategies might be monitoring and determining whether the levels of reduction required for achieving a biologically relevant decrease in BD levels to smoker are achieved. For this, biomarkers of risk/injury might be useful indicators. Currently, there are few biomarkers of BD exposure available, generally taken from urine, blood, or breath samples. The major urinary metabolites of BD are monohydroxybutenyl-mercapturic acids (MHBMAs) and dihydroxybutyl-mercapturic acid (DHBMA). Urinary MHBMA and DHBMA have been found to correlate with BD exposure (van Sittert *et al.*, 2000). Levels of MHBMA have been reported to be $86.4 \pm 14.0$ (SD) µg/24 h in smokers and $12.5 \pm 1.0$ µg/24 h in nonsmokers, with a significant difference in MHBMA levels observed between smokers and nonsmokers (Urban *et al.*, 2003). Reported DHBMA levels between smokers ($644 \pm 90$ [SD] µg/24 h) and nonsmoker ($459 \pm 72$ µg/24 h), on the other hand, have not been significant (Urban *et al.*, 2003), and DHBMA does not appear to be specific for BD exposure. Hemoglobin adducts have also proven useful markers of long-term exposure to BD and might be good biomarkers of risk. The long half-lives of these adducts result in an average measurement that is more time weighted than that for some other metabolites such as those derived from urine (Boysen and Hecht, 2003; Swenberg *et al.*, 2001). Breath samples collected can measure volatile compounds such as benzene and BD because they have a short residence time in the body and their concentrations in breath have been found to be a function of the number of cigarettes smoked and the time between when the smoker takes a puff and when the breath sample is collected (Gordon *et al.*, 2002). A recent study showed that MHBMA had a greater selectivity than DHBMA for BD exposure. Studies measuring urinary MHBMA and other biomarkers of exposure in people smoking cigarettes with either a charcoal or cellulose-tipped filter showed that the charcoal filter–tipped cigarettes did not modify the uptake of carbon monoxide or nicotine in comparison to the cellulose filter, but the charcoal filter significantly reduced the levels of BD and other compounds such as acrolein, crotonaldehyde, and benzene (Scherer *et al.*, 2006). Levels of MHBMA were shown to be reduced by 63% in people smoking cigarettes with an ISO tar level of 6 mg, a tobacco-substitute sheet (20%) and a 2-stage filter; by 46% percent in people smoking cigarettes with an ISO tar level of 1 mg, a tobacco-substitute sheet (20% of cigarette) and a 3-stage filter; and by 55% in people smoking cigarettes with an ISO level of 1 mg, tobacco that had been blend treated (75.4% washed, extracted, and enzyme treated) and a 3-stage filter (Shepperd *et al.*, 2013). Interestingly, for vapor phase toxicants such as BD, similar reductions were observed in both smoke chemistry and MHBMA levels, suggesting that the changes in smoking machine BD estimates corresponded to changes in MHBMA levels. In summary, urine MHBMA, hemoglobin adducts, and breath samples measured using real-time breath measurement technology are good biomarkers of risk candidates, which can be used for assessing the levels of BD in humans. Biomonitoring is very expensive and time consuming and might not be a realistic option for routine screening. For tobacco control, MOE analysis might be a practical way to assess the health effects of risk reduction strategies. For this, research investigating whether a reduction in toxicant levels in MSS measured using smoking machines results in a reduction of toxicant biomarkers is needed. For BD, this has already been demonstrated by Shepperd *et al.* (2013).

## CONCLUSIONS

This study used a risk analysis using the MOE approach for the assessment of health risks due to BD exposure via MSS and ETS. In addition, a risk analysis was performed for the assessment of health risks after implementation of risk reduction strategies for BD in MSS. Here, we confirm that BD was a good choice for mandated lowering, given that a risk for neoplastic (leukemia) and nonneoplastic (ovarian atrophy) endpoints exists for BD levels present in MSS. Risk reduction strategies such as the use of tobacco-substitute sheets, tobacco blend treatment, and various carbon filters do not reduce BD in MSS significantly at the moment for BD levels to be of low concern for neoplastic (leukemia) effects. According to our analysis and assumptions, a significant change in MOE was observed with the reduced toxicant prototype (80% U.S. blend) with 20% tobacco-substitute sheet with a polymer-derived carbon filter, but this change was not sufficient to reach a level of low concern, and a health concern for neoplastic effects remains. For nonneoplastic effects, this prototype resulted in an MOE, indicating no concern for nonneoplastic effects (ovarian atrophy). Of importance is that the WHO TobReg proposed recommendation that BD levels should be reduced yearly to a maximum of 125% of the mean BD estimates for each region (Burns *et al.*, 2008) did not alter the MOE significantly

for both neoplastic and nonneoplastic effects. According to our analysis and assumptions, a risk cannot be excluded for neoplastic effects (leukemia) from 24-h maximum mean and maximum BD indoor concentrations (ETS) as reported by the Canadian government. On the other hand, BD levels in ETS from smoking environments tend to generally be of no concern for nonneoplastic effects. For tobacco control, MOE analysis as demonstrated here might be a practical way to assess the health effects of the various risk reduction strategies and should be evaluated on a case-by-case basis using chemical-specific data when available.

## FUNDING

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