APPENDIX A6.1.3.2 1,3-BUTADIENE - FACTSHEET

	HPHC	BoExp assessed by PMI
IUPAC name	Buta-1,3-diene	Monohydroxybutenyl-mercapturic acid
Usual abbreviation	BDE, BD	MHBMA
Other names	1,3-Butadien, divinyl; vinylethylene, biethylene, bivinyl erythrene, alpha,gamma-butadiene, pyrrolylene, butadiene monomer	Monohydroxy-3-butenyl mercapturic acid
CAS number	106-99-0	None
Formula and molecular weight	C_4H_6 MW: 54.09	$C_9H_{15}NO_4S$ MW: 233.29

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Main characteristics

Chemical class	Aliphatic dienes
HPHC lists	FDA-93 [1]; FDA-18 [2]; HC [3, 4]; PMI-58; WHO-39 [5]; WHO-18 [6]; WHO-9 [6]
Source in cigarettes	Amount in MSS (3R4F): 38.5µg/cigarette (ISO); 75.5µg/cigarette (HCI) [7] Smoke phase: gas/vapor phase [8-10] Presence in tobacco / pyrosynthesis (T°): not present in uncombusted tobacco [11]. Precursors in tobacco are many organic compounds, very similar to benzene precursors [10, 12, 13] / 500-800°C [14]
Other sources [15]	The main source of 1,3-butadiene exposure is the incomplete combustion of petroleum-derived fuels. Two main potential sources of exposure are from indoor air (primarily due to release from carpet backings) and from butadiene-based food packaging materials [16]. Sources of 1,3-butadiene released into the air include motor vehicle exhaust, manufacturing and processing facilities, forest fires or other combustion, and cigarette smoke [17]. Higher levels of 1,3-butadiene may be found in highly industrialized cities or near oil refineries, chemical manufacturing plants, and plastic and rubber factories. 1,3-Butadiene has been found in drinking water and in plastic or rubber food containers, but not in food samples. Occupational exposure to 1,3-butadiene may occur in the rubber, plastics, and resins industries [17, 18].
Toxicity class (FDA) [1]	CA, CT, RDT
Toxicity – CA	IARC: group 1: carcinogenic to humans [19] Epidemiological study looking at the association of exposure to 1,3-butadiene and non-Hodgkin lymphoma show a six-fold statistically significant excess risk in one of three studies (18'000 male styrene-butadiene workers in Canada and US) [19] (p. 160) and excess of leukemia [20]. No evidence was found of an effect of exposure to 1,3-butadiene on lung cancer-related mortality [21]. Data on exposure of the study population have been sufficiently characterized to permit quantitation of exposure–response in only one epidemiological investigation of the

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	association between butadiene and leukemia [22].
Toxicity – RT	Workers exposed to 1,3-butadiene during the manufacture of rubber complained of irritation of the eyes, nasal passages, throat, and lungs [23].
Toxicity – CT [23]	In a retrospective epidemiological study of middle-aged workers in the rubber industry, excessive mortality was noted for certain types of cardiovascular diseases, mainly chronic rheumatic and arteriosclerotic heart diseases [24]. Furthermore, increased mortality for arteriosclerotic heart disease was reported among black males in the rubber industry. However, increased mortality from cardiovascular disease was not observed in three other cohorts of styrene-butadiene rubber and 1,3-butadiene monomer workers. Thus, it is unclear if cardiovascular disease is likely to be caused by 1,3-butadiene exposure [23].
Toxicity – RDT	Dose-related maternal and fetal toxicity was observed in rodents after exposure to 1,3-butadiene during gestation, as well as decreased fetal weight and skeletal defects [23]
Toxicity – AD	-
Toxicity - others	All available toxicity data can be found on the Website Aggregated Computational Toxicology Resource (ACToR) [25].

ADME properties

Absorption [23, 26] - In human volunteers inhaling 2 ppm 1,3-butadiene for 20 minutes, the absorbed fraction varied from 18 to 74% [27]. Neither sex nor age were factors in this variation. Fractional absorption in Asian volunteers was about 20% greater than in Caucasians, African-Americans, or Hispanics. Blood triglyceride levels may influence absorption, as blood:air partition coefficients increased by 20–40% in humans having borderline higher triglyceride levels after ingestion of fat in the diet [28].

Distribution [23, 26] - Reported partition coefficient of approximately 1 show that 1,3-butadiene is moderately soluble in blood of rats and mice [29] and indicate a theoretical alveolar retention of 50% in a single breath [30]. *In vitro* measurements of tissue:blood equilibrium partition coefficients suggest that 1,3-butadiene distributes to a variety of tissues. In volunteers inhaling 2 ppm 1,3-butadiene for 20 minutes, blood levels approached equilibrium by 5 minutes. A potential gender differences in the kinetics of 1,3-butadiene has been researched to a limited extent, and suggest that distribution of 1,3-butadiene differs between male and female rats, but this was not found in mice, and thus cannot be extended to all species [29].

Metabolism [23, 30] - The liver is the predominant site of 1,3-butadiene metabolism. Metabolism of EB is mediated by three competing oxidative, hydrolytic, or conjugation

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pathways (Figure 1) [23]. The flux of 1,3-butadiene through the various pathways is concentration- and species-dependent [23]. 1,3-Butadiene is first oxidized via cytochrome P-450 enzymes (primarily P-450 2E1 in humans, although other isoforms may also be involved) to the monoepoxide, 1,2-epoxy-3-butene (EB) and can then oxidized to its diepoxide, 1,2,3,4-diepoxybutane (DEB) (Figure 1) [23, 31, 32]. Hydrolysis of DEB and possibly EB via epoxide hydrolase (EH) or oxidation of the butenediol via cytochrome P-450 will result in the formation of 3,4-epoxy-1,2-butanediol (EBD). These epoxides have the potential of reacting with biomolecules such as DNA and proteins or will be inactivated via enzyme-catalyzed hydrolysis and conjugation with glutathione. Two well-studied hemoglobin adducts are N-(2,3,4-trihydroxybutyl)-valine adduct (THBVal) and 1- and 2-(hydroxybutenyl)valine (MHBVal), but little has been done in smokers and non-smokers. Hemoglobin adducts have proven useful markers of long-term exposure to 1,3-butadiene and might be good biomarkers of risk for occupational exposure. 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 [33, 34].

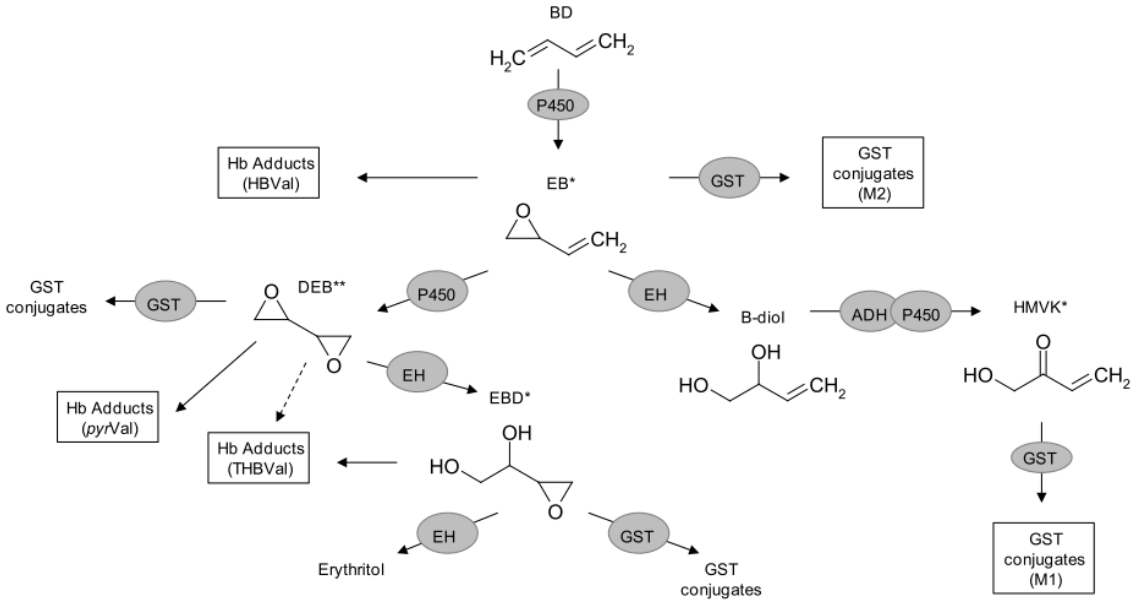


Figure 1 Metabolism of 1,3-butadiene (BD) [23]

□ = boxes indicate biomarkers of exposure that have been measured in exposed workers; * = monofunctional alkylating agent; ** = bifunctional alkylating agent

ADH = alcohol dehydrogenase; B-diol = butanediol; BD = 1,3-butadiene; DEB = diepoxybutane; EB = epoxybutene; EBD = epoxybutane diol; EH = epoxide hydrolase; GST = glutathione S-transferase; HBVal = N-(2-hydroxy-3-butenyl)-valine; HMVK = hydroxymethylvinyl ketone; M1 = 1,2-dihydroxy-4-(N-acetylcysteinyl)-butane (**urinary metabolite, DHBMA**); M2 = 1-(N-acetylcysteinyl)-2-hydroxy-3-butene (**urinary metabolite, MHBMA**); P450 = cytochrome P450; pyrVal = N,N-(2,3-dihydroxy-1,4-butadiyl)-valine; THBVal = N-(2,3,4-trihydroxybutyl)-valine

Figure Source: Agency for Toxic Substances and Disease Registry

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Alternatively, EB can be hydrolyzed by epoxide hydrolase (EH) to B-diol, which can be metabolized by CYP or aldehyde dehydrogenase (ADH) to hydroxymethylvinyl (HMVK). HMVK can subsequently be conjugated by GST to glutathione to form N-acetyl-S-(3,4-dihydroxy- butyl) cysteine (DHBMA, or M1, Figure 1) [23]. On the other hand, EB can also be conjugated to glutathione by GST to form monohydroxybutenylmercaptic acid (MHBMA, or M2, Figure 1), a mixture of N-acetyl-S-([1-hydroxymethyl]-2-propenyl)cysteine and N-acetyl-S-([2-hydroxymethyl]-3-propenyl)cysteine [23]. Both mercapturic acids are excreted in the urine.

Genetic polymorphisms should be taken into account to better understand the metabolism of butadiene, as levels of THBVal, for example, are influenced by the genotypes of CYP2E1(G-$_{35}$T), GSTT1, and GSTM1 [35].

A new MHBMA isomer, i.e., 1-hydroxybutene-2-yl mercapturic acid (1-MHBMA), has been recently identified, and has been suggested to be a potentially more reliable biomarker for 1,3-butadiene exposure than the other isomer, but this needs to be further validated in the context of tobacco smoke exposure [36]. Urinary MHBMA and DHBMA have been found to correlate with 1,3-butadiene exposure [31].

In molecular epidemiological studies of occupational exposure to 1,3-butadiene, the ratio of DHBMA to DHBMA + MHBMA was lower in workers who were homozygous for GSTM1-null and GSTT1-null [37, 38]. The formation of epoxybutanediol diepoxybutane requires a second oxidation of either butenediol or epoxybutene, respectively. At increasing exposure concentrations of butadiene, competition between butadiene and butenediol or epoxybutene for CYP may limit the extent to which the second oxidation reaction may occur.

Gender may also have an effect on the metabolism of 1,3-butadiene, as shown by a study by Albertini et al. [39]. Occupationally exposed females (polymerization of 1,3-butadiene monomer production) showed lower concentrations of both MHBMA and DHBMA in the urine per unit of 1,3-butadiene exposure than did males while exhibiting the same ratio. The same gender effect was observed for the THB-Val hemoglobin adduct [40].

Metabolism is overall qualitatively similar between mice, rats and humans, although there may be quantitative differences in the amount of butadiene absorbed as well as in metabolic rates and the proportion of metabolites generated [32]. Humans have a much higher hydrolytic enzymatic activities for the 1,3-butadiene derived epoxides than animals [32], but rodents, mice in particular, are more likely to form epoxides than humans [41-44]. A recent publication has shown that some metabolites may still differ between species, as found for example with 4-bis-(N-acetyl-l-cystein-S-yl) butane-2,3-diol (bis-BDMA), which has been detected in the urine of rats exposed to 1,3-butadiene by inhalation as a minor metabolite, but not of humans [45].

Metabolism of 1,3-butadiene exhibits nonlinear kinetics [44], with both dose- and duration-dependent effects [23].

Metabolism of butadiene and subsequent conversion may also take place to a more limited degree in the bone marrow by means other than P-450 oxidation (possibly via myeloperoxidase based on *in vitro* observations and the detection of the epoxides in the

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bone marrow of rodents, although this potential pathway has not yet been extensively investigated [32].

The following findings are reviewed in the IARC monograph on 1,3-butadiene [37]. Rates of butadiene metabolism have been reported for human tissues cultured in vitro. However, the range of observed rates is limited by the extent of the inter-individual variability in CYP, EH and GST activities of the tissues sampled, and by the fact that only small numbers of human liver and lung samples were analyzed. Thus, the actual kinetic range of the metabolism of butadiene in the human population is unknown.

The metabolism of 1,3-butadiene in mice and rats shows linear elimination kinetics at exposures of up to about 1000 ppm [2210 mg/m^3]. Responses that increase proportionally above the levels of metabolic saturation probably represent effects of the parent compound. In the range of linear kinetics, mice metabolize 1,3-butadiene about twice as rapidly as do rats. Although epoxybutene is formed primarily through CYP-mediated oxidation of butadiene, the formation of this alkylating agent by a myeloperoxidase-catalyzed reaction in bone marrow cells may be relevant to the induction of haematopoietic cancers in mice, rats and humans [29]. Metabolism of 1,3-butadiene is qualitatively similar among species, but not quantitatively [29], leading to significant differences in the tissue distribution and concentrations of metabolites. This has an impact on the susceptibility of various species to develop tumors. Several studies have shown that mice are more sensitive to the induction of carcinogenic effects than are rats, which is likely related to the fact that the uptake of 1,3-butadiene by inhalation in mice is approximately twice the uptake of rats, partly because of higher ventilation per kg of body weight, higher tissue perfusion, and higher rates of metabolism. On the other hand, the uptake of butadiene as a result of metabolism was much lower in monkeys than in rodents [46].

Excretion - 1,3-Butadiene can be excreted via the respiratory system as the parent compound or as a metabolite, in urine or feces [29]. The enzymatic rates for specific metabolic routes differ among species (and tissues). Data on urinary metabolites indicate that the elimination of epoxybutene in mice occurs to a greater extent by conjugation with GSH than by hydrolysis. Although no studies have been reported that characterize the full profile of urinary metabolites of butadiene in humans, the high ratio of 1,2-dihydroxybutylmercapturic acid (DHBMA) vs monohydroxy-3-butenyl-mercapturic acid (MHBMA) in exposed workers indicates that epoxybutene is preferentially metabolized by hydrolysis in humans. In rats, metabolic elimination of epoxybutene formed from butadiene occurs to a similar extent by hydrolysis or GSH conjugation.

Breath samples collected can measure volatile compounds such as 1,3-butadiene 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 [47]. The following data were generated by Kotapati et al. [45]. Mean urinary concentrations of MHBMA, DHBMA and THBMA in workers exposed to butadiene (0.1–2 ppm) were 82 ± 138, 3094 ± 4162 and 157 ± 158ng/mg creatinine, respectively. In smokers, the corresponding concentrations were 11 ± 12, 631 ± 452 and 31 ± 20ng/mg creatinine, respectively.

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DHBMA was the major 1,3-butadiene metabolite excreted in human urine (93%), followed by THBMA (5%) and MHBMA (2%) [45]. These proportions clearly differed from the rat findings [45], where the relative molar concentrations of 1,3-butadiene-mercapturic acids in urine of 1,3-butadiene-exposed rats were in the order of 47% for DHBMA, 37% for THMA and 15% for MHBMA. Bis-BDMA, (bis-butanediol-mercapturic) found only in rats, represented about 1% of the mercapturic acids [45]. The determination of MHBMA was shown to be more specific than that of DHBMA [38], thus even though DHBMA represents the major urinary metabolite of 1,3-butadiene in humans, MHBMA has often been used as a reliable biomarker of exposure to this toxicant. Its background value was also shown to be lower than for DHBMA, increasing its dynamic effect [48].

Several studies reported values of DHBMA that were quite similar in smokers and non-smokers [49-53]. In a study aiming at evaluating urinary excretion MHBMA and DHBMA as potential biomarkers of 1,3-butadiene, the half-life of MHBMA was estimated to be 8.6 hours [54]. The high background levels of DHBMA in subjects abstinent from smoking suggests that this metabolite is also formed from ambient sources that are not related to cigarette smoke, as the levels of DHBMA did not decrease after 8 days of smoking abstinence. Half-life of DHBMA could thus not be estimated. Another smoking cessation study of up to 56 days also showed no decrease of DHBMA [55, 56].

The species differences in 1,3-butadiene metabolism and gaps in the knowledge of the toxicokinetics of 1,3-butadiene in humans limit the ability to make precise assessments of the risk to humans from exposure to 1,3-butadiene [29].

Exposure levels in smokers and non-smokers

MHBMA

In non-smokers	Ranges: 0.65-7.5 nmol/24h [151.64-1749.68 ng/24h] [57]
In smokers	Ranges: 15.5-322 nmol/24h [3616-75.119.38 ng/24h] [57]

Levels of MHBMA have been reported to be 86.4 ± 14.0 (SD) µg/24 h [86400 ± 14000 (SD) ng/24h] in smokers and 12.5 ± 1.0 µg/24 h [12500 ± 1000 ng/24h] in nonsmokers, which is statistically significant [48]. Reported DHBMA levels between smokers (644 ± 90 (SD) µg/24 h) and nonsmoker (459 ± 72 µg/24 h), on the other hand, have not been significant [48], and DHBMA does not appear to be specific for 1,3-butadiene exposure [55, 56].

Reported urinary levels of MHBMA in smokers and non-smokers differ by 2- to almost 40-fold difference (Table 1), but are generally around 10-fold higher in smokers. A recent study in which smokers consuming 20 cigarettes per day (cpd) or more were forced to smoke exactly 20, 15, 5 or 0 cpd during a 5-day confinement period showed a dose-relationship between the number of cigarettes and the levels of urinary MHBMA (i.e. 6.3, 4.3, 2.2 and 0.5 µg/24h, respectively) [58]. Furthermore, a study comparing levels of urinary MHBMA levels in different ethnicities found that nicotine equivalents were the strongest predictors of MHBMA levels [59]. Also, a significant correlation between the number of cigarettes smoked per day and the levels of urinary MHBMA was shown in a

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European population [60]. On the other hand, MHBMA levels did not differ significantly between smokers of different tar yield categories, most likely because of other non-smoking related exposure, metabolic variability or other factors [61].

Most studies focussed on adult Caucasian subjects. However, in a recent study, the levels of MHBMA and DHBMA in overnight urine samples were compared among 584 Native Hawaiians, Japanese Americans, and white smokers in Hawaii [59]. The results of this study showed that mean urinary MHBMA differed by race/ethnicity, with the highest values in whites and lowest in Japanese Americans. This difference was only observed in individuals with the GSTT1-nullgenotype. Mean urinary DHBMA did not differ across racial/ethnic groups.

Overall, smoking cessation studies over periods of 3 to 8 days showed reductions of urinary levels of MHBMA of 6- to 30-fold, as compared to baseline levels, with an average of around 10-fold reductions (Table 2). In one study, MHBMA levels decreased by 18% when smokers switched from cellulose acetate to charcoal filtered cigarettes [62], while a second set of studies demonstrated a decrease of around 5-fold [63].

In a smoking cessation study, where different BoExp were measured at 3, 7, 14, 21, 28, 42 and 56 days, all decreases in biomarker levels from baseline to day 3 were significant, with the exception of DHBMA [55]. After the initial reduction from baseline to day 3, the rate of decline from day 3 to day 56 was still significant for MHBMA. These data clearly demonstrate different fates of metabolites of 1,3-butadiene after smoking cessation, with MHBMA, but not DHBMA, decreasing significantly. Consistent with those results, other studies have shown that MHBMA is related to 1,3-butadiene exposure while DHBMA, which is present in far higher concentrations, is not related to exposure [41, 54]. Apparently there are significant sources of exposure to ketone or other precursors to DHBMA other than metabolism of 1,3-butadiene, which could explain this lack of correlation with exposure to cigarette smoke [55, 56].

Only a very slight difference of THBVal between smokers and non-smokers could be observed, making those adducts not very reliable biomarkers of exposure for cigarette smoke [64]. For MHBVal, no difference was detected between smokers and non-smokers [31].

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Table 1 Representative urinary MHBMA levels in smokers and non-smokers

Author (year)	Levels in smokers	Levels in non-smokers	Units; variability
Lindner et al. (2011) [60]	3.27 ± 3.02 [3270 ± 3020]	0.30 ± 0.42 [300 ± 420]	µg/24h [ng/24h]; mean ± SD
Sarkar et al. (2005) [54]	3.02 ± 2.17 [3020 ± 2170]	0.16 ± 0.20 [160 ± 200]	µg/g creat [pg/mg creat]; mean ± SD
Schettgen et al. (2009) [65]	< 2 (8.6)	< 2 (<2)	µg/L; median (95th percentile)
Roethig et al. (2009) [66]	3.61 (0.1) [3610 (100)]	0.30 (0.02) [300 (20)]	µg/24h [ng/24h]; weighed mean (SE)
Scherer et al. (2006) [62]	75.0 ± 50.0 [75000 ± 50000]	NM	µg/24h [ng/24h]
Ding et al. (2009) [67]	ND-59.7 [59700]	ND-122 [122000]	µg/g creat [pg/mg creat] (range)
Shepperd et al. (2013) [68]	4501 ± 3614 (376–18739)	117 ± 78 (40–447)	ng/24h; LS means ± SD (ranges)
Jain (2015) [53]	25.1 (20.0-31.4)	11.5 (10.0-13.4)	ng/mL; adjusted geometric mean (95% CI)
Eckert (2011) [51]	<5.0-12.0 (<5.0-11.9) [<5000-12000 (<5000-11900)]	<5.0 (<5.0) [<5000 (<5000)]	µg/24h [ng/24h]; median (range)
Bergen (2015) [50]	3.52 ± 3.3 [3520 ± 3300]	0.49 ± 1.0 [490 ± 1000]	µg/24h [ng/24h]; mean ± SD

Table 2 Representative urinary MHBMA levels after smoking cessation

Author (year)	Levels in smokers	Levels in non-smokers	Units; variability
Sarkar et al. (2008) [63]	2.70 ± 1.59 [2700 ± 1590]	0.09 ± 0.10 [90 ± 100] [a]	µg/g creat [pg/mg creat]; mean ± SD
Carmella et al. (2009 and 2012) [55, 56]	33.05 ± 69.4 [7710.23 ± 16190.33]	2.71 ± 4.35 [632.22 ± 1014.81] [b]	nmol/24h [ng/24h]; mean ± SD
Tricker et al. (2012) [69]	3.75 ± 3.15 [3750 ± 3150]	0.27 ± 0.14 [270 ± 140] [a]	µg/24h [ng/24h]; mean ± SD
Tricker et al. (2012) [70]	1.63 ± 1.40 [1630 ± 1400]	0.28 ± 0.27 [280 ± 270] [a]	µg/24h [ng/24h]; mean ± SD
Tricker et al. (2012) [71]	3.01 ± 3.22 [3010 ± 3220]	0.44 ± 0.32 [440 ± 320] [a]	µg/24h [ng/24h]; mean ± SD
Tricker et al. (2012) [72]	2.51 ± 2.33 [2510 ± 2330]	0.36 ± 0.58 [360 ± 580] [c]	µg/24h [ng/24h]; mean ± SD
Theopilus et al. (2015) [58]	5.8 ± 4.1 [5800 ± 4100]	0.5 ± 0.2 [500 ± 200] [c]	µg/24h [ng/24h]; mean ± SD
Frost-Pineda et al. (2008) [73]	4.7 ± 3.2 [4700 ± 3200]	0.22 ± 0.2 [220 ± 200] [a]	µg/24h [ng/24h]; mean ± SD

[a] Smoking cessation for 8 days

[b] Smoking cessation for 3 days

[c] Smoking cessation for 5 days

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List of abbreviations

3R4F	Kentucky 3R4F reference research cigarette [74]
ACToR	Aggregated Computational Toxicology Resource
AD	Addictive
ADME	Absorption and Distribution, Metabolism, and Excretion
CA	Carcinogen
CAS number	Chemical Abstracts Service number
CT	Cardiovascular toxicant
FDA	Food and Drug Administration
HC	Health Canada
HCI	Health Canada Intense regime [75]
HPHC	Harmful and Potentially Harmful Constituents
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization smoking regime [76]
IUPAC	International Union of Pure and Applied Chemistry
MSS	Mainstream smoke
MW	Molecular weight
ND	Not detectable
PMI	Philip Morris International
RDT	Reproductive or developmental toxicant
RT	Respiratory toxicant
SD	Standard deviation
SE	Standard error
WHO	World Health Organization

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