APPENDIX A6.1.3.4 CROTONALDEHYDE - FACTSHEET

	HPHC	BoExp assessed by PMI
IUPAC name	But-2-enal	3-hydroxy-1-methylpropylmercapturic acid
Usual abbreviation	-	HMPMA; 3-HMPMA; HBMA
Other names	Crotonaldehyde, 2-butenal, (2E)-but-2-enal, 2-butenaldehyde, crotoinic aldehyde, crotonal, trans-crotonaldehyde, crotonic aldehyde, crotylaldehyde, 1-formylpropene, methylpropenal, trans-crotonaldehyde, β-methacrolein, 3-methylacrolein, propylene aldehyde	4-hydroxybut-2-yl mercapturic acid (HBMA) N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine
CAS number	4170-30-3 (mixed isomers) 123-73-9 (trans isomer; (E)-2-butenal) 15798-64-8 (cis isomer; (Z)-2-butenal)	33164-64-6
Formula and molecular weight	C_4H_6O MW: 70.09	$C_9H_{17}NO_4S$ MW. 235.3

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Main characteristics

Chemical class	α,β-unsaturated aldehyde
HPHC lists	FDA-93 [1]; FDA-18 [2]; HC [3, 4]; WHO-39 [5]; WHO-18 [6]; PMI-58
Source in cigarettes	Amount in MSS (3R4F): 10.1 µg (ISO); 43.1 µg (HCI) [7] Smoke phase: gas/vapor phase [8] Presence in tobacco / pyrosynthesis (T°): present in uncombusted tobacco [9] / Main generation range 25-500°C [10]. Possible precursors of crotonaldehyde are carbohydrates (sugar, starch, cellulose) [9]. Crotonaldehyde in smoke can be also formed from 1,3-butadiene and acetaldehyde [11].
Other sources [12]	Crotonaldehyde is produced endogenously and is found in many food products up to the lower milligram per kilogram range as a result of enzymatic and abiotic (autoxidative, thermal) processes. Emissions into the atmosphere are from combustion, in particular, combustion of vehicle fuels, wood combustion, and tobacco smoking. Crotonaldehyde's production and use as a chemical intermediate and in organic synthesis may result in its release to the environment through various waste streams. It is also released to the environment from tobacco smoke, polymer combustion, and turbine exhaust. Crotonaldehyde has been identified as a volatile emission product from the arboreous plant Chinese arbor vitae and in ground musty sorghum. Crotonaldehyde has also been detected in gases emitted from volcanoes. In food, (E)-crotonaldehyde has been found as a volatile component of raw beef, chickpea, mutton, chicken, and pork, but it also occurs naturally in fish, fruits, vegetables and alcoholic beverages, such as wine and whisky. (E)-Crotonaldehyde's production and use in the manufacture of butyraldehyde, in locating breaks and leaks in pipes, in the manufacture of maleic acid, crotyl alcohol, butyl chloral hydrate, in rubber accelerators, in organic syntheses, as solvent in purification of mineral oils, manufacture of resins, rubber antioxidants, in chemical warfare, and as an intermediate for 2-ethylhexyl alcohol may result in it release to the environment through various waste streams. Crotonaldehyde (steric form not reported) has been identified as a volatile emission product from the arboreous plant Chinese arbor vitae. It has also been detected in gases emitted from

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	volcanoes. It is also released to the environment from tobacco smoke, polymer combustion, and turbine exhaust. (E)-Crotonaldehyde has been detected in drinking water and wastewater, and in human milk and expired air. Crotonaldehyde exists in two isomeric forms (cis and trans) that have similar properties and effects. It was identified as a rat hepatic microsomal metabolite of the hepatocarcinogen N-nitrosopyrrolidine, a minor smoke constituent [13] that is a probable human carcinogen.
Toxicity class (FDA) [1]	CA
Toxicity – CA [14]	IARC: group 3: not classifiable as to its carcinogenicity to humans Crotonaldehyde was tested for carcinogenicity in one study in male rats by administration in the drinking-water. Increased incidences of hepatic neoplastic nodules and altered liver-cell foci were seen, but these were not dose-related [14].
Toxicity – RT	Crotonaldehyde is irritating to the upper respiratory tract even at low concentrations. It can cause pulmonary edema [15].
Toxicity – CT	-
Toxicity – RDT	-
Toxicity – AD	-
Toxicity - others	Crotonaldehyde is highly toxic by all routes [15]. There is increasing evidence for the cytotoxicity of 2-butenal and other alkenals, which induce cell death by acute exposure of cells to oxidative stress through consumption of the antioxidant glutathione [16]. All available toxicity data can be found on the Website Aggregated Computational Toxicology Resource (ACToR) [17].

ADME properties

Absorption and distribution [16]- No information on the absorption and distribution after administration by any route could be identified in an assessment on crotonaldehyde by WHO [16]. There were no studies specifically investigating the absorption and distribution of crotonaldehyde in experimental animals after its exogenous administration by any route.

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Metabolism [16] – Crotonaldehyde is formed endogenously during lipid peroxidation and forms protein and DNA adducts. Protein and DNA adducts of crotonaldehyde have been found and studied in a large number of tissues in the body [16]. DNA adducts have been found in almost all investigated tissues (skin, liver, lung, kidney, brain, intestinal epithelial cells, and leukocytes) from rats and mice, showing the wide distribution of crotonaldehyde in the body with and without external administration of the compound. DNA adducts have also been detected in human liver, leukocytes, mammary glands, and oral tissues [16].

In general, aldehydes are readily metabolized by three principal routes [16, 18]: 1) oxidation to acids; 2) reduction to alcohols; and 3) conjugation with sulfhydryls, such as glutathione. The reaction of alkenals with glutathione to form glutathione conjugates by Michael addition (i.e. nucleophilic addition of a carbanion or another nucleophile to an α,β-unsaturated carbonyl compound) is a major detoxification pathway. Crotonaldehyde is not easily oxidized by aldehyde dehydrogenase. In vivo, the subcutaneous injection of crotonaldehyde leads to reduction of the glutathione level in the liver. In vitro, crotonaldehyde reacts rapidly with cellular sulfhydryl groups and glutathione directly and to a small degree by enzyme catalysis, such as glutathione *S*-transferase–catalysed conjugation of glutathione with crotonaldehyde, leading to the formation 3-HMPMA (also called 4-hydroxybut-2-yl mercapturic acid (HBMA) [19, 20]), and smaller amounts of 2-carboxy-1-methylethylmercapturic acid (CMEMA; CAS no 33164-65-7) [18, 21], even though amounts measured in the human urine can be within the same range than 3-HMPMA [18].

Excretion [16] - Data on the pharmacokinetic properties of crotonaldehyde are not available are scarce. The presence of 3-hydroxy-1-methylpropylmercapturic acid (3-HMPMA) (6–15% of the applied quantity) and small amounts of 2-carboxy-1-methylethylmercapturic acid (CMEMA) in rat urine 24 h after subcutaneous injection of crotonaldehlyde (0.7 nmol/kg body weight, corresponding to about 53 mg/kg body weight), indicates the addition of a “thio” group onto the double bond of crotonaldehyde, probably via a Michael addition. Half-life of 3-HMPMA is estimated to be of 5-9h [22-24]. No information on the half-life of CMEMA has been found.

Exposure levels in smokers and non-smokers

Levels of urinary 3-HPMA was consistently found to be higher in smokers than in non-smokers (Table 1). A recent study by Park et al. [25] has shown that 3-HMPMA urinary levels of 2200 smokers of five different ethnicities (i.e., African Americans, native Hawaiians, whites, Latinos and Japanese Americans) were significantly different in the five groups, which may partly explain the differing risks of native Hawaiian and Latino smokers in developing lung cancer. Hydroxy-1-methylpropylmercapturic acid was detected in human urine in 39 regular cigarette smokers using a method developed by Scherer et al. [23]. It was found that cigarette smokers excreted about three to seven-fold more 3-HMPMA, and only slightly elevated amounts of CMEMA (Table 1 and Table 3), in their urine compared to non-smokers. In smokers, significant correlations were found between the urinary excretion levels of 3-HMPMA (but not CMEMA) and several markers of exposure for smoking, including the daily cigarette consumption, carbon monoxide in exhaled breath, salivary cotinine, and nicotine plus five of its major

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metabolites in urine [18]. It was noted that acetaldehyde formed from ingested ethanol does not increase the urinary excretion of 3-HMPMA or CMEMA [18].

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 HMPMA (i.e. 598, 406, 319 and 213 µg/24h, respectively) [26]. Park et al. have further shown that 3-HMPMA levels correlate well with cpd and with levels of 3-hydroxypropyl mercapturic acid (3-HPMA, a metabolite of acrolein), while correlation with total nicotine equivalents were somewhat weaker [25]. 3-HMPMA was shown to be associated with statistically significantly increased risk of developing lung cancer among Shanghai smokers after adjustment for smoking intensity and duration [27].

In smoking abstinence studies, levels of 3-HMPMA decreased by 3- to 9-fold, after 3 to 8 days (Table 2). 3-HMPMA in urine decreased by 17% when switching from CC to charcoal filter tipped cigarettes [23]. For CMEMA, on the other hand, no significant change was found upon smoking cessation (Table 4). Data on this biomarker of exposure are, however, scarce.

Altogether, there are sufficient data indicating that HMPMA is a fit-for-purpose biomarker for smoking-related exposure to crotonaldehyde, while CMEMA does not show the expected sensitivity.

3-HMPMA (or HBMA)

In non-smokers 1,200–3,200 nmol/24h [282.36-752.96 µg/24h] [20]

In smokers 9,800–26,000 nmol/24h [2305.94-6117.80 µg/24h] [20]

Table 1 Representative urinary 3-HMPMA levels in smokers and non-smokers

Author (year)	Levels in smokers	Levels in non-smokers (or ex-smokers)	Units; variability
Hecht (2014) [28]	4990 (2930–8490)	705 (456–1090)	pmol/ml; geometric mean (95% CI)
Zhang (2014) [29]	1287.83 (100.53–6975)	191.9 (0.00–785)	ng/mL; mean (range)
Scherer (2007) [18]	2122 ± 1286	752 ± 457	µg/24h; mean ± SD
Pluym (2015) [30]	121.7 (57.0-220.0)	18.9 (9.7-64.4)	µg/g creat [ng/mg creat]; median (min-max)
Miura (2015) [31]	1616 ± 793	257 ± 58.1	µg/24h; mean ± SD
Alwis (2015) [32]	1546 ± 1643	406 ± 487	ng/mL; mean ± SD

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Table 2 Representative urinary 3-HMPMA levels after smoking cessation or switching to a reduced toxicant prototype

Author (year)	Levels in smokers	Levels in ex-smokers or switchers	Units; variability
Carmella (2009 and 2012) [33, 34]	9825 ± 1001	1325 ± 113 [a]	nmol/24h; mean ± SD
	[2312 ± 236]	[312 ± 27]	[converted into µg/24h]
Frost-Pineda (2008) [35]	2695.7 ± 962	299 ± 166.3 [b]	µg/24h; mean ± SD
Scherer (2007) [18]	1890 ± 889	394 ± 200 [b]	µg/24 hours; mean ± SD
Theophilus (2015) [26]	715 ± 230	213 ± 131 [c]	µg/24h; mean ± SD

[a] After 3 days of smoking cessation
[b] After 8 days of smoking cessation
[c] After 5 days of smoking cessation

CMEMA

In non-smokers Ranges: 558-1584 µg/24h [18]

In smokers Ranges: 0-4470 µg/24h [18]

Table 3 Representative urinary CMEMA levels in smokers and non-smokers

Author (year)	Levels in smokers	Levels in non-smokers (or ex-smokers)	Units; variability
Scherer 2007 [18]	1970 ± 2500	1071 ± 513	µg/24h; mean ± SD

Table 4 Representative urinary CMEMA levels after smoking cessation

Author (year)	Levels in smokers	Levels in ex-smokers or switchers	Units; variability
Scherer 2007 [18]	800 ± 424	705 ± 294 [a]	µg/24h (mean ± SD)

[a] After 8 days of smoking cessation

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List of abbreviations

3R4F	Kentucky 3R4F reference research cigarette [36]
ACToR	Aggregated Computational Toxicology Resource
AD	Addictive
ADME	Absorption and Distribution, Metabolism, and Excretion
CA	Carcinogen
CAS number	Chemical Abstracts Service number
CC	Conventional Cigarette
cpd	Cigarette per day
Creat	Creatinine
CT	Cardiovascular toxicant
FDA	Food and Drug Administration
HC	Health Canada
HCI	Health Canada Intense regime [37]
HPHC	Harmful and Potentially Harmful Constituents
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization smoking regime [38]
IUPAC	International Union of Pure and Applied Chemistry
MSS	Mainstream smoke
MW	Molecular weight
NM	Not measured
PMI	Philip Morris International
RDT	Reproductive or developmental toxicant
RT	Respiratory toxicant
SD	Standard deviation
WHO	World Health Organization

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