## APPENDIX A6.1.3.3 ACROLEIN - FACTSHEET

| | HPHC | BoExp assessed by PMI (matrix) |
|---|---|---|
| **IUPAC name** | Prop-2-enal | 1) S-(3-hydroxypropyl)mercapturic acid (in urine)<br>2) 2-carboxyethyl-N-acetylcysteine (in urine) |
| **Usual abbreviation** | ACR; ACRL | 1) 3-HPMA<br>2) CEMA [1], CMA or CNEMA |
| **Other names** | Acraldehyde, acrylic aldehyde, allyl aldehyde, ethylene aldehyde | 1) N-acetyl-S-3-hydroxypropylcysteine, S-(3-hydroxypropyl)cysteine N-acetate, N-Acetyl-S-(3-hydroxypropyl)cysteine<br>2) carboxyethyl mercapturic acid |
| **CAS number** | 107-02-8 | 1) 23127-40-4<br>2) 51868-61-2 |
| **Formula and molecular weight** | $C_3H_4O$<br>MW: 56.06 | 1) $C_8H_{15}NO_4S$<br>MW: 221.27<br>2) $C_8H_{13}NO_5S$<br>MW: 235.26 |

[1] CEMA is also a common abbreviation of N-acetyl-S-(2-cyanoethyl)-L-cysteine (2-cyanoethylmercapturic acid), a metabolite of acrylonitrile

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**Main characteristics**

| | |
|---|---|
| **Chemical class** | Aldehydes; carbonyls |
| **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 main stream smoke (3R4F): 56.7µg/cigarette (ISO); 155µg/cigarette (HCI) [7]<br><br>Smoke phase: gas/vapor phase [8]<br><br>Presence in tobacco / pyrosynthesis (T°): present in uncombusted tobacco / Main generation range is between 200-400°C [9]. Precursos of acrolein in tobacco are cellulose, fructose, glucose, sucrose, triglycerides and glycerol [10, 11]. |
| **Other sources** | Acrolein has been produced commercially since the 1940's. It is used mainly in the production of acrylic acid, a starting material for acrylate polymers. It is also used in the production of DL-methionine and as a herbicide and slimicide. Acrolein occurs naturally in foods and is formed during the combustion of fossil fuels (including engine exhausts), wood and tobacco and during the heating of cooking oils. Human exposure occurs from these sources and during its production and use [12].<br><br>Small amounts of acrolein are produced endogenously during the normal intermediary catabolism of various amino acids and polyamines and during the peroxidation of membrane lipids [13, 14].<br><br>Acrolein is a metabolite of the antineoplastic drug cyclophosphamide, and has been suspected of being the cause of hemorrhagic cystitis, one of the major toxic effects of cyclophosphamide [15]. |
| **Toxicity class (FDA)** [1] | RT, CT |
| **Toxicity – CA [13]** | IARC lists acrolein in the group 3 agents, i.e. not classifiable as to its carcinogenicity to humans [12]. Informative epidemiological studies on the long-term effects of acrolein have not been identified. Available data are inadequate to serve as a basis for assessment of the carcinogenicity of acrolein following inhalation. In the limited studies concerning the chronic toxicity/carcinogenicity of acrolein following oral exposure in rats and dogs, there have been no increases in the incidence of tumors of any type, although mortality, the cause of which is |

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| | |
|---|---|
| | unclear, was increased in rats and mice. Acrolein is mutagenic *in vitro*, but limited available data do not indicate genotoxic effects in the nasal mucosa (i.e., the site of contact) in rats exposed by inhalation, although *in vitro* studies indicate that acrolein can interact directly with DNA and induce DNA damage, through generation of DNA adducts and generation of oxidative stress [13, 16]. |
| **Toxicity – RT** | Based upon studies conducted primarily in laboratory animals, adverse health effects associated with exposure to acrolein are mostly confined to the tissue of first contact (i.e., the respiratory and gastrointestinal tracts after inhalation and ingestion, respectively) and are concentration related. Studies of the systemic effects of acrolein in humans have not been identified, with available data relevant to the assessment of the potential adverse effects in humans limited primarily to irritation [13]. In humans and experimental species, acrolein is an upper respiratory tract and eye irritant [13]. Histological changes in respiratory and gastrointestinal epithelium have been observed from both inhalation and oral exposures, respectively [17] |
| **Toxicity – CT** | No studies were located regarding cardiovascular effects in humans following inhalation exposure to acrolein. Non-specific inflammatory lesions in the heart were reported in rats, dogs, monkeys, and guinea pigs after a continuous 90-day exposure to acrolein. Also, an increase in relative heart weight was observed in hamsters and rats exposed to acrolein. The non-specific cardiovascular effects may be secondary effects of acrolein on respiratory tissues [17]. Elevated urinary 3-HPMA levels have been associated with low abundance of specific circulating angiogenic cell subpopulations, increased circulating levels of platelet–leukocyte aggregates, and increased risk of cardiovascular disease [18]. |
| **Toxicity – RDT** | An increased relative frequency of bronchial and oral cavity tumors was found among nine cancer cases in one study of chemical workers exposed to various aldehydes. Esophageal tumors have been associated with genetically determined, high metabolic levels of acetaldehyde after drinking alcohol. It produced tumors of the respiratory tract following inhalation, particularly adenocarcinomas and squamous-cell carcinomas of the nasal mucosa in rats and laryngeal carcinomas in hamsters |
| **Toxicity – AD** | - |
| **Toxicity - others** | Acrolein is a metabolite of the antineoplastic drug cyclophosphamide, and has been suspected of being the cause of |

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hemorrhagic cystitis, one of the major toxic effects of cyclophosphamide [15].

All other available toxicity data can be found on the Website Aggregated Computational Toxicology Resource (ACToR) [19].

**ADME properties**

*Absorption* [20] - Respiratory uptake studies with acrolein in mongrel dogs of both sexes indicate that acrolein is retained at rates of 75-80% in the upper respiratory tract with a lesser rate of retention (65-70%) for the lower respiratory tract. At inhaled concentrations of 176-264 ppm (400-600 mg/m3), 80-85% was retained in the total respiratory track at varying ventilation rates, suggesting little distribution elsewhere [21]. Acrolein's strong reactivity with tissues is proposed to result in little systemic distribution [22]. Controlled experiments on systemic absorption and kinetics have not been conducted, but there are indications that acrolein is not highly absorbed into the system since toxicological findings are restricted to the site of exposure [23]. The fact that no reduction in liver glutathione is seen following inhalation exposure also suggests that inhaled acrolein does not reach the liver to any great extent [23].

*Distribution* [20] - The distribution of radiolabeled acrolein administered to rats by intravenous or oral routes has been evaluated. Radiolabel in expired air, urine, and feces was measured at 4, 12, and 16 and 24 hours post-dosing, then every 24 hours for the next 6 days. The large majority of label (>96%) was recovered in excreta within the first 24 hours. Tissue concentrations (including blood) of radioactivity were minimal (<1.2% from the iv dosing and <0.7% from the oral dosing) and time course of excretion for all groups was similar except for delayed excretion in the high-dose group. Radiolabel measured in excreta and in tissues was associated with various acrolein metabolites (including 3-HPMA [24]) and not attributed to parent compound. These studies indicate little systemic distribution of acrolein.

The high reactivity of acrolein is due to the polarization of the double bond by the aldehyde group, and the resulting increased potential for nucleophilic addition. Because acrolein readily reacts with sulfhydryl and amino groups on proteins, it is unlikely to distribute systemically, and thus its adverse effects are characterized in terms of cytotoxicity at the site of entry, i.e., the respiratory system, the gastrointestinal tract, mucous membranes, and skin.

*Metabolism* [14, 20] - Absorbed acrolein reacts directly with protein and non-protein sulfhydryl groups, and with primary and secondary amines found in proteins and nucleic acids. In proteins, it preferentially attacks free thiol (SH) groups of cysteine residues - amino groups of lysine residues and histidine residues. *In vitro*, acrolein binds to serum albumin and low-density lipoproteins. It is well-documented that the conjugation of the β-carbon of acrolein with sulfhydryl groups is rapid and essentially irreversible, and leads to thiazolidine derivatives and a decrease in glutathione (GSH) stores without an increase in oxidized GSH (GSSG) [25]. This pathway results in an acrolein-GSH adduct which is then further metabolized by both high- and low-affinity forms of mitochondrial and cytosolic aldehyde and alcohol dehydrogenase; one resultant product has been identified

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as 3-HPMA. This product has been isolated from urine of rats after subcutaneous injection of acrolein and after inhalation and intraperitoneal injection of Wistar rats [20]. The reduction of the acrolein-GSH adduct by alcohol dehydrogenase to 3-hydroxypropylmercapturic acid was postulated as a potentially important pathway.

There is increasing evidence that aldehydes such as acrolein are generated endogenously during the process of lipid peroxidation [20]. Among all α, β-unsaturated aldehydes, acrolein is the strongest electrophile, which accounts for its high reactivity with nucleophiles. Thiol adducts of acrolein are considerably more stable than adducts formed by all other α, β-unsaturated aldehydes.

The main pathway for metabolism of acrolein (Figure 1) is conjugation with glutathione (GSH), followed by enzymatic cleavage of the γ-glutamic acid and glycine residues, respectively, in the liver and in the kidney, and N-acetylation of the resultant cysteine conjugate to form S-(3-oxopropyl)-N-acetylcysteine (OPMA) in the kidney. Reduction of this aldehyde yields S-(3-hydroxypropyl)-N-acetylcysteine (3-HPMA), the main metabolite of acrolein found in urine while oxidation of the aldehyde group produces S-carboxyethyl-N-acetylcysteine (carboxyethylmercapturic acid, CEMA).

*Excretion* [23] – 3-HPMA was estimated to be around 80% of the total amount of acrolein-related mercapturic acids metabolites recovered in the urine, whereas CEMA was estimated to represent less than 10% [14]. Oxidation of acrolein may precede conjugation with GSH, thus forming acrylic acid. Enzyme-mediated epoxidation of acrolein produces glycidaldehyde, an instable intermediate that can react with water to yield glyceraldehyde or it can form a conjugate with GSH. Another pathway of metabolism is that of epoxidation of the double bond followed by attack of GSH on the epoxide. A third pathway involves addition of water to acrolein to form 3-hydroxypropionaldehyde, which is further oxidized to malonic acid and ultimately oxalic acid. Some of these compounds can be incorporated into normal metabolic pathways. For example, glycidaldehyde can be hydrated to glyceraldehyde.

3-HPMA has been identified as a minor metabolite of allyl chloride (3-chloropropene; used in the production of epoxy resins) in urine samples from the maintenance workers exposed to allyl chloride, but detected at traces levels [26].

Half-life of 3-HPMA has been described to be of 5-9h [27].

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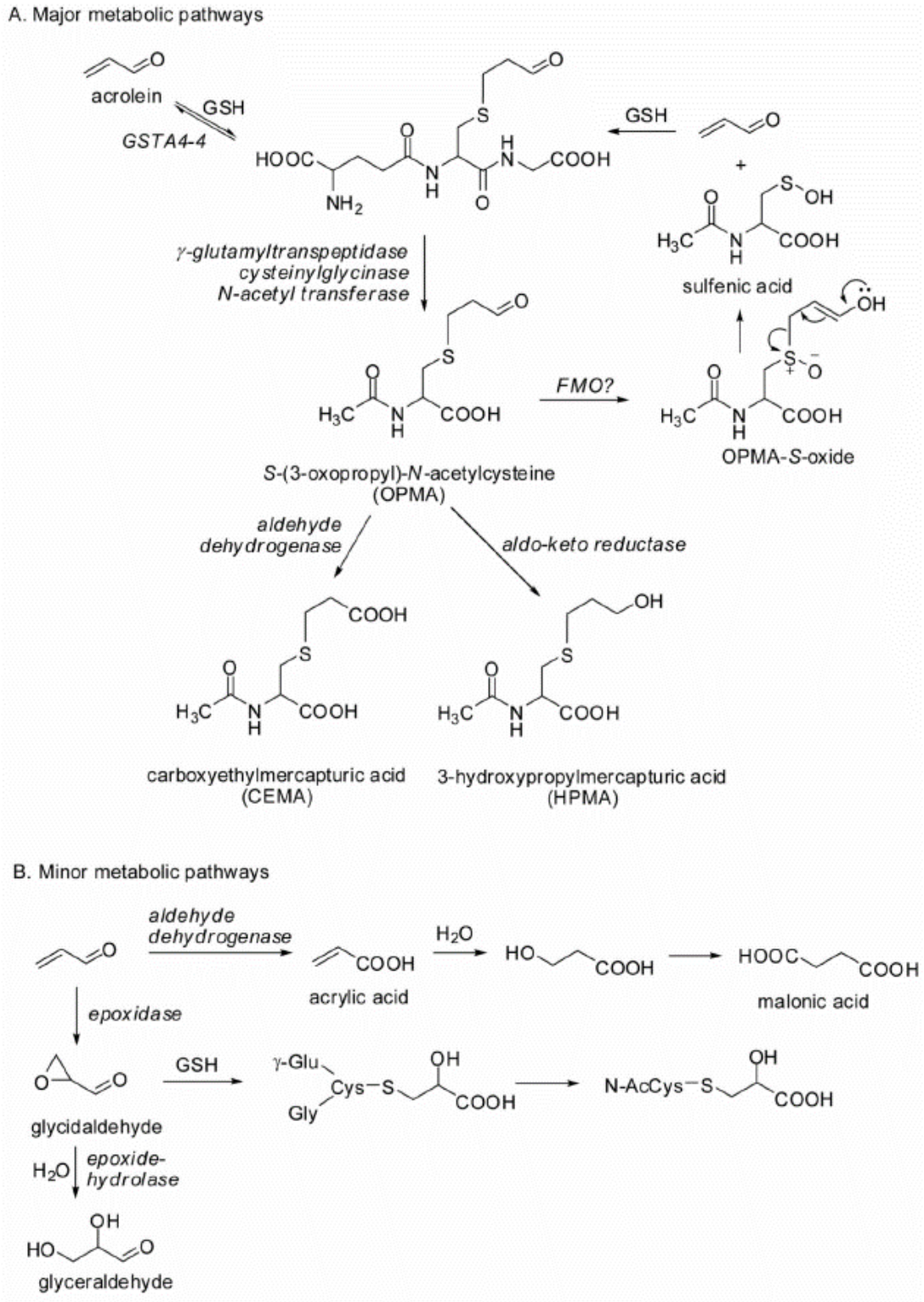



**Figure 1 Metabolism of acrolein [14]**

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**Exposure levels in smokers and non-smokers**

**3-HPMA**

| | |
|---|---|
| In non-smokers | Range: 1131-1847 nmol/24h [250.26-408.69 µg/24h] [28] |
| In smokers | Range: 5869-11190 nmol/24h [1298.63-2476.01 µg/24h] [28] |

The excretion of 3-HPMA in urine has been used in academic, governmental, and tobacco industry studies for the biomonitoring of exposure to MS (e.g. [29-31]). A significant increase (1.4-fold) in the level of this marker could be detected in Chinese women who regularly cook at home [32], though not nearly as high as in smokers, whose levels are 5 to 6-fold higher than non-smokers [28, 33, 34].

Levels of 3-HPMA among non-smokers and smokers can overlap (Table 1), but the differences between the groups have largely been described as statistically significant. A recent study by Park et al. [35] has shown that 3-HPMA 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, despite the debate about the role of acrolein in lung carcinogenesis by cigarette smoke, as there is little evidence for its carcinogenicity in laboratory animals.

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 3-HPMA (i.e. 2.0, 1.4, 0.9 and 0.5 mg/24h, respectively) [39]. In a study by Moldoveanu et al. acrolein respiratory retentions of 98–100% following the inhalation of cigarette smoke were reported [36]. The correlation between acrolein mouth level exposure and its urinary metabolite 3-HPMA ($r = 0.82$) is similar to that seen for nicotine and nicotine equivalents [37].

Levels of 3-HPMA decreased by 8% when smokers switched from cellulose acetate to charcoal filter cigarettes [38], but in another study, those levels decreased by 50 – 75% [33]. Small decreases in 3-HPMA were observed in a study in which smokers switched from full-flavor to light or ultra-light cigarettes (15 to 6 mg ISO tar yields and 1.1 to 0.5 mg nicotine) [39]. The smoking cessation study by Carmella et al. [40] showed that 3-HPMA levels decreased significantly by 78% in smokers who abstained for 4 weeks, thus confirming some of their earlier findings [31]. After 5 to 8 days of smoking abstinence, levels of 3-HPMA decreased in ranges of 1.5-to 8-fold [41-46] (Table 2). Significant decrease was observed earlier, i.e., after 3 days [40]. A significant 35% decrease in urinary 3-HPMA was noted in smokers who switched to a second-generation electrically heated cigarette smoking system compared to those who continued smoking conventional cigarettes for 12 months [29].

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**Table 1 Representative urinary 3-HPMA levels in smokers and non-smokers**

| Author (year) | Levels in smokers | Levels in non-smokers | Units; variability |
|---|---|---|---|
| Lindner (2011) [47] | 1.81 ± 1.26 [1810 ± 1260] | 0.63 ± 0.80 [630 ± 800] | mg/24h [µg/24h]; mean ± SD |
| Carmella (2007) [31] | 3950 ± 2950 [874 ± 653] | 1900 ± 3000 [420 ± 664] | pmol/mg creat [ng/mg creat]; mean ± SD |
| Ding (2009) [30] | 75-3678 | ND-245 | µg/g creat or ng/mg creat; mean ± SD |
| Shepperd (2013) [48] | 1342 ± 743 | 251 ± 125 | µg/24h; mean ± SD |
| Yan (2010) [49] | 2442.0 ± 993.6 | 358.9 ± 143.1 | µg/24h; mean ± SD |
| Yan (2010) [49] | 2069.7 ± 925.5 | 310.0 ± 167.2 | ng/mg creat; mean ± SD |
| Eckert (2011) [34] | 884 (104-4423) | 146 (39.3-1562) | µg/g creat or ng/mg creat; median (range) |
| Schettgen (2008) [50] | 1630 (213–4242) | 112.8 (57–1090) | µg/g creat [ng/mg creat]; median (range) |
| Mascher (2001) [51] | 2809 ± 385 | 812 ± 123 | µg/24h; mean ± SD |
| Minet (2011) [52] | 810.7 ± 559.7 (680.4) ISO tar yield 1mg | 228.4 ± 70.4 (231.6) | µg/24h; mean ± SD |
| Minet (2011) [52] | 1231.0 ± 687.0 (1129.0) ISO tar yield 4mg | 228.4 ± 70.4 (231.6) | µg/24h; mean ± SD |
| Minet (2011) [52] | 1945.0 ± 968.0 (1129.0) ISO tar yield 10mg | 228.4 ± 70.4 (231.6) | µg/24h; mean ± SD |
| Morin (2011) [53] | 2494 (2252-2735) 14 cig/day | 983 (879-1088) | µg/24h; mean (95%CI) |
| Ding (2009) [30] | 75-3678 | ND-245 | µg/g creat [ng/mg creat]; (range) |
| Scherer (2007) [54] | 1297 ± 1164 | 337 ± 383 | µg/24h; mean ± SD |
| Roethig (2009) [29] | 2,030 (24) | 458 (17) | µg/24h; weighted mean ± SE) |
| Alwis (2015) [55] | 1089 (469, 2012) | 219 (140, 353) | µg/g creat [ng/mg creat]; sample-weighted median ($25^{th}$, $75^{th}$ percentile) |
| Bergen (2015) [56] | 1941.9 ± 1326 | 416.7 ± 532 | µg/24h; mean ± SD |
| Pluym (2015) [57] | 372 (157–606) | 62.5 (39.1-284) | µg/g creat; median (min-max), >10 cig/d |
| Jain (2015) [58] | 529.7 (455.5-615.9) | 399.4 (351.6-453.6) | ng/mL; adjusted geometric mean (95% CI) |

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**Table 2 Representative urinary 3-HPMA levels after smoking abstinence or switching to e-cigarettes**

| Author (year) | Levels in ex-smokers or switchers | Levels in smokers | Units; variability |
|---|---|---|---|
| Carmella (2007) [31] | 719 ± 272 [159 ± 60] [a] | 3725 ± 2417 [824 ± 535] | pmol/mg creat [ng/mg creat]; mean ± SD |
| Frost-Pineda (2008) [59] | 302.3 ± 94.7 [b] | 1834.3 ± 598.8 | µg/24h; mean ± SD |
| Carmella (2009) [40] | 1336 ± 923 [c] [296 ± 204] | 10020 ± 5150 [2217 ± 1140] | nmol/24h [µg/24h]; mean ± SD |
| Tricker (2012) [43] | 0.50 ± 0.20 [d] [500 ± 200] | 1.37 ± 0.74 [1370 ± 740] | mg/24h [µg/24h]; mean ± SD |
| Tricker (2012) [44] | 0.40 ± 0.23 [b] [400 ± 230] | 1.91 ± 0.74 [1910 ± 740] | mg/24h [µg/24h]; mean ± SD |
| Tricker (2012) [41] | 1.68 ± 0.37 [b] [1680 ± 370] | 2.61 ± 0.73 [2610 ± 730] | mg/24h [µg/24h]; mean ± SD |
| Tricker (2012) [42] | 0.48 ± 0.15 [b] [480 ± 150] | 1.68 ± 0.97 [1680 ± 970] | mg/24h [µg/24h]; mean ± SD |
| Theophilus (2015) [45] | 0.5 ± 0.2 [d] [500 ± 200] | 2.4 ± 0.8 [2400 ± 800] | mg/24h [µg/24h]; mean ± SD |
| McRobbie (2015) [46] | 343 ± 178 [f] | 1,623 ± 850 | ng/mg creat; mean ± SD |

[a] After 4 weeks of smoking cessation; means and SDs were calculated from tabulated data

[b] After 8 days of smoking cessation

[c] After 3 days of smoking cessation

[d] After 5 days of smoking cessation

[e] After 7 days of smoking cessation

[f] After 4 weeks of switching to e-cigarettes

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## List of abbreviations

| | |
|---|---|
| 3R4F | Kentucky 3R4F reference research cigarette [60] |
| ACToR | Aggregated Computational Toxicology Resource |
| AD | Addictive |
| ADME | Absorption and Distribution, Metabolism, and Excretion |
| CA | Carcinogen |
| CAS number | Chemical Abstracts Service number |
| Creat | Creatinine |
| CT | Cardiovascular toxicant |
| FDA | Food and Drug Administration |
| HC | Health Canada |
| HCI | Health Canada Intense regime [61] |
| HPHC | Harmful and Potentially Harmful Constituents |
| IARC | International Agency for Research on Cancer |
| ISO | International Organization for Standardization smoking regime [62] |
| IUPAC | International Union of Pure and Applied Chemistry |
| MW | Molecular weight |
| ND | Not detectable |
| PMI | Philip Morris International |
| RDT | Reproductive or developmental toxicant |
| RT | Respiratory toxicant |
| SE | Standard error |
| SD | Standard deviation |
| WHO | World Health Organization |

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