APPENDIX A6.1.3.1 CARBON MONOXIDE - FACTSHEET

	HPHC	BoExp assessed by PMI
IUPAC name	Carbon monoxide	1) Carboxyhemoglobin 2) Exhaled carbon monoxide
Usual abbreviation	CO	1) COHb 2) COex
Other names	Carbon monoxide, carbonous oxide, carbon(II) oxide, carbonyl	
CAS number	630-08-0	9061-29-4
Formula and molecular weight	CO MW: 28.01	NA

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Main characteristics

Chemical class	Gas
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): 11.2 mg (ISO); 32.7 mg (HCI) [7] (up to 5% of MSS) [8] Smoke phase: gas/vapor phase [9] Presence in tobacco / pyrosynthesis (T°): not present in uncombusted tobacco [10] / Two major temperature areas for CO formation. Small (2.5%) but steady increase of CO formation from 250-500°C, where organic material decomposes to, among other compounds, carbon oxides. More than 73% of CO is formed at temperature higher than 650°C, where carbonized tobacco is oxidized [11, 12]. It is generally accepted that three mechanisms play a role in CO formation during smoking [13]: 1. Combustion (with any carbon source as precursor in tobacco), 2. Pyrolysis, 3. Boudouard equilibirium between CO, CO_2 and carbonized coal at high temperature.
Other sources [14]	The general population is exposed to varying levels of CO through inhalation of air. CO is a temporary atmospheric pollutant in some urban areas, chiefly from the exhaust of internal combustion engines (including vehicles, portable and back-up generators, lawn mowers, power washers, etc.), but also from incomplete combustion of various other fuels (including wood, coal, charcoal, oil, paraffin, propane, natural gas, and trash). Exposure may be greater during times of the day when vehicular traffic is heavy; however, the most dangerous levels of carbon monoxide occur in indoor air. In the home, exposure may occur as a result of improperly adjusted or installed appliances that burn natural gasoline, kerosene or other fuels. These include stoves, furnaces, heaters, and generators. Occupational exposure to CO occurs in petroleum refineries, gas and coal burning power plants, petrochemical plants and coke oven plants, and cigarette smoke-filled environments. Exposure may also occur in taxi cab drivers and toll booth workers as a result of vehicular exhaust. CO is produced from the enzymatic degradation of endogenous and exogenous precursors. The major endogenous source of CO

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	production is the oxidative metabolism of heme by the enzyme heme oxygenase (HO). Endogenous CO production contributes to a blood carboxyhemoglobin (COHb) concentration of approximately 0.4–0.7%.
Toxicity class (FDA) [1]	RDT
Toxicity – CA	-
Toxicity – RT [14]	Results of controlled clinical studies in healthy subjects indicate that the respiratory tract does not appear *per se* to be a primary target organ for carbon monoxide toxicity. However, CO exerts effects on cell metabolism through hypoxic and non-hypoxic modes of action. CO readily combines with hemoglobin (Hb) to produce COHb, and occupies the space in hemoglobin that normally carries oxygen, making hemoglobin ineffective in delivering oxygen to bodily tissues. Both hypoxic and non-hypoxic modes of action are thought to be largely (if not entirely) the result of the ability of CO to bind to heme and alter function and/or metabolism of heme proteins. The binding affinity of CO for hemoglobin is over 200 times greater than that of O_2 for hemoglobin. Through similar mechanisms, CO decreases O_2 storage in muscle cells by binding to, and displacing O_2 from, myoglobin. Although all tissues are vulnerable to carbon monoxide-induced hypoxic injury, those having the highest O_2 demand are particularly vulnerable, including the brain and heart.
Toxicity – CT [15]	Cardiovascular effects of inhalation exposures to CO have been evaluated in controlled human clinical studies, epidemiology studies, and various animal models. In general, these studies provide convincing evidence for adverse cardiovascular effects in association with carbon monoxide exposures that result in blood COHb levels ≥ 2.4%, with effects occurring at the lowest levels in subjects with compromised cardiovascular function (e.g., coronary artery disease). WHO recommends for people with compromised cardiovascular functions to not exceed levels of 2% [16], while the Centers for Disease Control and Prevention (CDC) suggest a threshold of ≥ 2.4% [14] .
Toxicity – RDT [14]	In the maternal system, carbon monoxide distributes to fetal tissues and binds to fetal hemoglobin and other heme proteins. The binding affinity of fetal hemoglobin is approximately twice that of maternal hemoglobin. Fetal death in humans has been reported in cases of maternal carbon monoxide poisoning

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	during pregnancy. Possible, but not clearly demonstrated, associations between ambient air CO concentrations and risk of pre-term birth, low birth weight, heart anomalies, neonatal and infant mortality. Results of animal studies show adverse developmental effects of gestational and early postnatal CO exposure, including decreased fetal weight, adverse central nervous system development, altered peripheral nervous system development, cardiac effects, altered sexual behavior, immunological effects, and hematological effects. The heart and cardiovascular system and the brain and developing nervous system are particularly sensitive to CO.
Toxicity – AD	-
Toxicity - others	All available toxicity data can be found on the Website Aggregated Computational Toxicology Resource (ACToR). Reports of effects of longer-duration exposure of humans on central nervous system are not available [17]. CO exerts effects on cell metabolism through both hypoxic and non-hypoxic mechanisms. Both types of effects are thought to be largely (if not entirely) the result of the ability of CO to bind to heme and alter function and/or metabolism of heme proteins. CO may also act through mechanisms unrelated to heme binding, although evidence for this is currently more contentious [14]. Diagnosis and characterization of CO toxicity include both the measurement of blood COHb and assessment of signs. In order of increasing severity, these include: (1) headache, nausea, dilation of cutaneous vasculature, vomiting, dizziness, and blurred vision; (2) confusion, syncope, chest pain, dyspnea, weakness, tachycardia, and tachynea rhabdomyolysis; and (3) palpitations, cardiac dysrhythmias, hypotension, myocardial ischemia, cardiac arrest, respiratory arrest, pulmonary edema, seizures, and coma [18]. Neuropsychological testing and neuroimaging have been considered for evaluating central nervous system effects [14].

ADME properties

Absorption [14] - Humans are exposed to CO from breathing and/or skin contact with carbon monoxide in air. No information is available on the dermal absorption of carbon monoxide resulting from exposures to gaseous carbon monoxide. However, as is the case for other gases that are avidly absorbed from the lung (e.g., O_2), dermal absorption of carbon monoxide through intact skin would be expected to make a minor contribution to absorbed carbon monoxide, relative to inhalation. Inhaled carbon monoxide is absorbed through the lung alveoli but not through the upper respiratory tract [12]. Major factors that control absorption of carbon monoxide include those that affect delivery of inhaled

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air to the alveolar region of the lung and those that affect lung carbon monoxide diffusion capacity. Lung diffusion capacity refers to the driving force for diffusion of carbon monoxide across the alveolar air- blood interface and is reflected in the carbon monoxide partial pressure difference between inspired and exhaled air. In general, factors that increase alveolar ventilation, cardiac output, and gas diffusing capacity of the lung, while maintaining alveolar ventilation perfusion matching (i.e., proportional increases in alveolar ventilation rate and cardiac output) tend to increase absorption of inhaled carbon monoxide. Factors that may increase net carbon monoxide absorption include exercise, supine position, age (increased in infancy and childhood and declines in adults with age), altitude, increasing blood hemoglobin concentration, and decreasing partial pressure of O_2 in inhaled air or blood.

Distribution [14] - Following inhalation of contaminated air, carbon monoxide rapidly enters all parts the body including the blood, brain, heart, and muscles. In blood, carbon monoxide quickly distributes into erythrocytes where it exists primarily as a complex with hemoglobin to form COHb. In muscle, carbon monoxide exists as a complex with myoglobin forming carboxymyoglobin (COMb).

Binding of carbon monoxide to heme proteins (e.g., blood hemoglobin, muscle myoglobin) has profound effects on the kinetics of distribution and elimination kinetics of both carbon monoxide and O_2. Affinities of heme for carbon monoxide in hemoglobin and myoglobin are sufficiently high to result in concentrations of dissolved carbon monoxide being 103–106 times lower than the concentrations of COHb or COMb.

Binding of carbon monoxide by erythrocyte hemoglobin (to form COHb) contributes to maintaining relatively low concentrations of dissolved carbon monoxide in erythrocyte cytosol and a partial pressure gradient to drive carbon monoxide transfer from alveolar air to blood. Diffusion of carbon monoxide into erythrocytes and binding of carbon monoxide to hemoglobin is sufficiently rapid that near equilibrium is achieved between carbon monoxide partial pressures in alveolar air and alveolar end capillary (arterial) blood. Seconds after initiating inhalation exposures to carbon monoxide, COHb is detected in arterial blood. During 2–6 minutes of exposure, arterial COHb concentrations were approximately twice venous COHb concentrations and approached unity within 3–5 minutes of cessation of exposure). With continued exposure to a constant air carbon monoxide concentration, a steady-state would be achieved within 200–500 minutes.

Metabolism [14] - Metabolism of carbon monoxide consists of three major processes: (1) metabolic production of carbon monoxide from endogenous and exogenous precursors; (2) binding of carbon monoxide to heme proteins (e.g., hemoglobin, myoglobin, cytochromes); and (3) oxidative metabolism of carbon monoxide to CO_2. The rates of dissociation of carbon monoxide from hemoglobin and myoglobin are substantially slower than that of O_2 and sufficiently slow, relative to erythrocyte capillary transit times, to limit rates of exchange of carbon monoxide between tissues and capillary blood. Binding of carbon monoxide to hemoglobin also increases the affinity of hemoglobin for O_2, decreasing the availability of O_2 to tissues.

Excretion [12, 14] - The primary elimination processes include diffusive transfer of carbon monoxide to extravascular tissues and binding of carbon monoxide to intracellular

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heme proteins (e.g., muscle myoglobin), and oxidative metabolism of carbon monoxide to CO_2. Oxidative metabolism of carbon monoxide has been estimated to be a relatively small fraction (<10%) of endogenous carbon monoxide production.

The alveolar CO pressure is proportional to the concentration of COHb in blood [19]. Therefore, the exhaled CO (COex) accurately reflects blood COHb. COex can easily be measured by commercially available instruments, which measure the CO concentration either electrochemically, or by infrared spectrometry and provides a direct estimate of COHb blood levels (the two almost perfectly correlate: r = .98) [20].

COex half-life differs between men and women (9.5 and 7.2 hrs, respectively) within a time interval of 0–18 hrs (after one cigarette use) [21]. CO in breath can be confounded by many factors such as diet, physical exercise, inflammatory diseases and time of the day [21]. Sleep for example slows down the elimination of CO [22].

The decline in blood COHb following cessation of an inhalation exposure to carbon monoxide exhibits at least two kinetic phases. The fast phase is thought to reflect a combination of exhalation of carbon monoxide along with slower distribution of blood carbon monoxide to tissues that continues after cessation of exposure. The elimination half-times for both phases appear to increase with exposure intensity and/or duration; a contributing factor to this effect may be the amount of carbon monoxide accumulated in extravascular stores. It is only after approximately 5 to 6 h that the blood COHb concentration reaches a plateau after exposure to carbon monoxide [23, 24]. So if the exposure time is less than several hours, carbon monoxide distribution in the body does not reach equilibrium and the elimination process would not follow the single exponential decay. In human subjects exposed to 2% carbon monoxide for 1–3 minutes (peak COHb = 30–40%), values for elimination half-lives were 5.7 ± 1.5 and 103 ± 20.5 minutes for the fast and slow phases, respectively. In subjects whose peak COHb level ranged from 2 to 15%, the half-life was 320 minutes (range: 128–409 minutes). Globally, COHb half-life is estimated to range from 1 to 4 hours, depending on the ventilation rate and the physical activity [12]. For a person with a sedentary working activity, the half-life of COHb is about 3 hours, and about 2 hours for a person with light physical activity [12].

Clinical studies conducted on healthy individuals exposed to ambient air carbon monoxide concentrations have found that elimination half-time increases with age, with the most pronounced increase occurring from age 2 to 20 years, and is approximately 6% longer in males compared to females. The gender effect may be related to relative differences in body size and/or muscle mass. Exercise decreases the carbon monoxide elimination half-time, whereas sleep increases it [22]. The time after smoking cessation to reach the cut-off concentration also changes accordingly.

The advantage of measuring COex is that it is not invasive, however, its measurement is less sensitive and specific than COHb, and has shown to not have a linear correlation with COHb shortly after a cigarette consumption [12]. Also, depending on the analytical methods used, the comparison between COex and COHb may show very good correlation, but poor agreement [25]. Furthermore, COex measurement is reasonably specific for detecting heavy cigarette smoking but is of lesser utility for detecting light smoking because CO levels become similar to environmental sources of CO [22]. In a

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clinical setting, when blood is in any case being drawn, and when exposure to CO is low, COHb thus remains a valuable marker of exposure.

Exposure levels in smokers and non-smokers

Because of the endogenous production of CO and environmental exposure, as well as the influence of physical activity, levels of COex can be quite variable, including for non-smokers (Table 1). Thus, cut-off values have been established to distinguish smokers from non-smokers. This cut-off is 8-10 ppm, irrespective of the physical activity, meaning that any result above 10 ppm is considered as a consequence of cigarette smoking [22]. For COHb (Table 2), Scherer has suggested that to differentiate smokers from non-smokers with acceptable sensitivity and specificity, cut-off levels should be 1.6-2.0% COHb [12]. This cut-off seems indeed to be able to make the distinction, as shown in a European study on exposure markers for smoking [26], as well as in a US study [27]. In the report by the Institute of Medicine on the "Scientific standards for studies on modified risk tobacco products", the values considered both for COex and COHb are within these cut-off criteria [28].

The relation between cigarette consumption expressed either as the average number of cigarettes smoked per day or as the number of cigarettes smoked before taking the sample has been investigated in many studies (reviewed in [12]). The coefficients of correlation are in the range of 0.3–0.8. Thus, the number of cigarettes smoked explains about 10–60% of the variability in the COHb or COex concentrations [12]. The coefficients of correlation are usually slightly higher when the number of cigarettes smoked prior to sampling was assessed rather than using the average daily cigarette consumption figures [12].

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 S-PMA (i.e. 22.9, 16.7, 12.6 and 2.0 ppm, respectively, for COex and 4.3, 3.3, 2.7 and 1.0 %, for COHb) [29].

For COex, a cut-point of 4 ppm is both appropriately sensitive and specific, identifying most smokers, especially those who have smoked 5+ cigarettes in the last 24 h [20]. Very few non-smokers are falsely identified as smokers, and all had other risk factors for a positive test [20]. By using individual cut-off values, it is possible to separate smokers who had refrained from smoking for at least 8 hours from those who had smoked within this time frame with a specificity of 95% and a sensitivity of 98% [21]. However, as calculation of individual values is impractical in a clinical setting, and requires prior collection of at least two COex time points from the subject, a generic cut-off value was also evaluated. Through a model based on 38 smokers, a cut-off value of 12 ppm could discriminate between recent smokers and smokers that have refrained from smoking for >8 hrs with a specificity of 94% and a sensitivity of 90%.

COex is reasonably specific for detecting heavy cigarette smoking but is of marginal utility for detecting light smoking because CO levels from smoking are low, and there are

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environmental sources of CO of similar magnitude [22]. Also, CO may have too short a half-life such that it is highly dependent on the time at which smoking last occurred.

The mean COHb concentrations for smokers were reported to be about 4–7% and those for non-smokers about 1–2% [12]. In heavy smokers, COHb concentrations can reach more than 12%. No differences in COHb concentrations between nonsmokers and passive smokers exposed to environmental tobacco smoke under real-life conditions have been reported [12].

In smoking abstinence studies (Table 3), the reduction of COHb levels were of 4- to 10-fold after 5 to 8 days. COHb levels were shown to change towards values of non-smokers after 12 hours of smoking abstinence [30]. For an average smoker, 24 hours should be sufficient to reach the cut-off level for COex [22]. However, it would be possible for someone to smoke a few cigarettes within 24 hours and still be below cut-off, as a result of a variable half-life. In a recent smoking abstinence study of 5 days, levels of COex and COHb decreased by about 11-fold and 8-fold, respectively [29]. The average elimination half-time of absorbed carbon monoxide has been estimated to be approximately 300 minutes, therefore, a value for COHb measured at a given time following exposure will reflect both the exposure and elimination rate of carbon monoxide.

A small but significant increase in COex levels of real-life ETS exposed nonsmokers was reported in two studies. Non-smokers exposed to ETS under extreme (experimental) conditions showed significantly elevated COHb and COex concentrations. Acceptable cut-off levels for COHb and COex would be 1.6% and 8–10 ppm, respectively [12, 22]. Frederiksen and Martin [31] listed 4 groups of smoking related variables affecting the CO exposure levels: ‘1) Substance smoked’ (type of tobacco product, tobacco moisture, paper, filter, size/shape of cigarette, packing of tobacco, brand of tobacco), 2) smoking occurrence’ (number smoked, temporal distribution), 3) smoking topography’ (puff volume, number of puffs, puff duration, puff interval, cigarette duration, amount of tobacco used, puff intensity, puff distribution), 4) passive (involuntary) smoking’ (ambient CO level, type of ETS, proximity to a smoker, room ventilation). Regarding the fourth point, it should however be noted that no differences in COex concentrations between nonsmokers and passive smokers exposed to ETS under real-life conditions have been reported [12].

COHb can be determined by various methods, such as photometry, infrared spectroscopy or gas chromatography [12]. Nearly all carbon monoxide in the body (60–70%) exists as complexes with Hb and other heme proteins. Therefore, measurement of COHb provides a useful indicator of the elevated carbon monoxide body burden. The relationship between COHb levels and exposure is, however, complex because of the numerous physiological factors that influence carbon monoxide uptake and elimination.

Limitations of COex, but also of COHb, are the unspecificity of CO for tobacco smoke and the relatively short half-lifes of 2–3 h, for COex, and of 1-4 h for COHb. The latter property makes these biomarkers very dependent on the time interval to the last cigarette smoked. Since there is a nonsteady-state exposure pattern for COHb in smokers, COex measurements taken directly after smoking a cigarette may not accurately reflect COHb concentrations.

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COex

In non-smokers Ranges: 2.6-6.5 ppm [28]

In smokers Ranges: 17.4-34.5 ppm [28]

Table 1 Representative values of COex in smokers, non-smokers and quitters

Author (year)	Levels in smokers	Levels in non-smokers (or ex-smokers)	Units; variability
Scherer (2006) [12]	20–40	3–7	ppm; range
Scherer (2007) [32]	17.4 ± 10.6	2.6 ± 2.2	ppm; mean ± SD
Jarvis (1984) [33]	20.8	5.6	ppm
Frederiksen (1979) [31]	34.38	4.93	ppm
Fortmann (1984) [34]	27.3 ± 12.8	4.64 ± 1.97	ppm; mean ± SD
Heinemann (1984) [35]	29.5 (15.5, 44.5)	6.5 (4.5, 9.5)	ppm; median (P_{10}, P_{90})
Laranjeira (2000) [36]	5 (3-8)	2.5 (1-4)	ppm; median (interquartile range)
Theophilus (2015) [29]	22.9 ± 7.2	2.0 ± 0.7 [a]	ppm ± SD

[a] After 5 days of smoking cessation

COHb

In non-smokers Ranges: 0.25-1.45% [28]

In smokers Ranges: 3.4-7.1% [28]

Table 2 Representative COHb levels in smokers and non-smokers

Author (year)	Levels in smokers	Levels in non-smokers	Units; variability
Cole (1975) [8]	5.5 (2.2-13)	0.97 (0.2-2.5)	%; mean (range)
Szadkowski (1976) [37]	3.37 ± 1.76	0.72 ± 0.25	%; mean ± SD
Frederiksen (1979) [31]	up to 15%	0.5-2	%; range
Saloojee (1982) [38]	7.09 ± 2.9	0.73 ± 0.41	%; mean ± SD
Heinemann (1984) [35]	6.9 (4-10.2)	2.2 (1.7-2.7)	%; P_{10}-P_{90}
Jarvis (1984) [33]	4.36	0.93	%
Pojer (1984) [39]	4.36 ± 2.09	0.93 ± 0.52	%; mean ± SD
Scherer (2006) [12]	4.0–8.0	0.8–1.5	%; range
Lowe (2009) [40]	5.69 ± 1.82	0.35 ± 0.38	%; mean ± SD
Roethig (2009) [27]	5.26 ± 0.04	1.45 ± 0.02	%; weighed mean ± SE
Lindner (2011) [26]	3.63 ± 2.21	0.23 ± 0.4	%; mean ± SD
Bergen (2015) [41]	5.26 ± 2.3	1.46 ± 0.5	%; mean ± SD

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Table 3 Representative COHb levels after smoking cessation or switching to a reduced toxicant prototype

Author (year)	Levels in smokers	Levels in quitters or switchers	Units; variability
Frost-Pineda (2008) [42]	6.8 ± 1.7	1.1 ± 0.2 [a]	%; mean ± SD
Ramos (2011) [43]	1.52 ± 1.0	0.552 ± 0.30 [b]	%; mean ± SD
Tricker (2012) [44]	3.54 ± 1.5	0.48 ± 0.23 [a]	%; mean ± SD
Tricker (2012) [45]	5.53 ± 1.63	1.17 ± 0.32 [a]	%; mean ± SD
Polonia (2009) [47]	5.9 ± 1.67	1.7± 0.56 [c]	%; mean ± SD
Tricker (2012) [48]	4.41 ± 1.38	0.45 ± 0.25 [a]	%; mean ± SD
Pezzuto (2013) [50]	3.6 ± 1.5	2.1 ± 0.9 [d]	%; mean ± SD
Theophilus (2015) [29]	4.3 ± 1.2	1 ± 0.1 [e]	%; mean ± SD

[a] After 8 days of smoking cessation
[b] After 180 days of smoking cessation
[c] After 6 months of smoking cessation
[d]After 3 months of smoking cessation
[e] After 5 days of smoking cessation

List of abbreviations

3R4F	Kentucky 3R4F reference research cigarette [51]
ACToR	Aggregated Computational Toxicology Resource
AD	Addictive
ADME	Absorption and Distribution, Metabolism, and Excretion
CA	Carcinogen
CAS number	Chemical Abstracts Service number
COex	Exhaled Carbon monoxide
COHb	Carboxyhemoglobin
COMb	carboxymyoglobin
CT	Cardiovascular toxicant
ETS	Environmental tobacco smoke
FDA	Food and Drug Administration
HC	Health Canada
HCI	Health Canada Intense regime [52]
HO	heme oxygenase

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IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization smoking regime [53]
IUPAC	International Union of Pure and Applied Chemistry
MSS	Main stream smoke
MW	Molecular weight
NA	Not applicable
P_{10}, P_{90}	10^{th}-90^{th} percentiles
PMI	Philip Morris International
RDT	Reproductive or developmental toxicant
RT	Respiratory toxicant
SD	Standard deviation
WHO	World Health Organization

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