APPENDIX A6.1.3.12 *o*-TOLUIDINE - FACTSHEET

	HPHC	BoExp assessed by PMI (matrix)
IUPAC name	*o*-toluidine	Parent compound, i.e. *o*-toluidine (in urine)
Usual abbreviation	*o*-tol	
Other names	*o*-aminotoluene, 2-aminotoluene, 1-methyl-2-aminobenzene, *o*-methylaniline, 2-methylaniline, *ortho*-toluidine, *o*-tolylamine	
CAS number	95-53-4	
Formula and molecular weight	C_7H_9N MW; 107.17	

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Main characteristics

Chemical class	Aromatic amine, arylamines; *ortho*-toluidine (*o*-toluidine) is one of three isomers of toluidine, the others being meta-toluidine (m-toluidine), and para-toluidine (*p*-toluidine). All three compounds are arylamines whose chemical structures are similar to aniline except that a methyl group is substituted onto the benzene ring. The difference between these three isomers is the position where the methyl group ($-CH_3$) is bonded to the ring relative to the amino functional group ($-NH_2$).
HPHC lists	FDA-93 [1], PMI-58
Source in cigarettes	Amount in MSS (3R4F): 54.1 ng (ISO); 99.3 ng (HCI) [2] Smoke phase: particulate phase (3% gas/vapor phase) [3] Presence in tobacco / pyrosynthesis (T°): can be found in uncombusted tobacco [4] / *o*-Toluidine is formed during tobacco pyrolysis; temperature of formation not reported [4-6]. Precursors in tobacco are presumably the same as for other aromatic amines, namely proteins, amino acids, tobacco pigments. [4, 7].
Other sources [8-10]	The general population is known to be exposed ubiquitously to *o*-toluidine. *o*-Toluidine is detected in µg/kg amounts in meat and dairy products and in upper ng/kg amounts in salads, vegetables, eggs, alcoholic beverages, cereals and fish. Even higher concentrations in the upper µg/kg range were reported for ice cream powders, food colors and soft drink concentrates. Particles from rubber tires in road dust could be another significant source of exposure to *o*-toluidine since extracts from shredded used tires contains on average 58.2 mg/kg (range 0.07-130) of *o*-toluidine. *o*-Toluidine also occurs in tobacco leaves without pyrolysis (urinary *o*-toluidine is detected in users of smokeless tobacco, even though at lower levels than in smokers of conventional cigarettes [6]) and in the aroma of black tea. It is an intermediate in the biodegradation of *o*-nitrotoluene. The principal use of *o*-toluidine worldwide is in the manufacture of dyestuffs (including hair dyes), although it is also used in the production of rubber,plastic, chemicals, herbicides, and pesticides [5] and as a curing agent for epoxy resin systems. *o*-Toluidine is also used as a corrosion inhibitor in paint formulations and has limited use in analytical laboratory procedures. There are no known domestic or household uses for

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	o-toluidine. Toluidine (isomers not specified, but *o*-toluidine likely to be present) was detected as a component of coal oil. *o*-Toluidine is a metabolite of the local anesthetic prilocaine [11].
Toxicity class (FDA) [1]	CA
Toxicity – CA [12, 13]	IARC: group 1: carcinogenic to humans [12]. Several cohort studies have been conducted among workers potentially exposed to *o*-toluidine [12]. Overall, the epidemiological studies show consistent associations between exposure to *o*-toluidine and bladder cancer. Risks are greatest for workers with the strongest likelihood of exposure and for those with long-term exposure (> 10 years). There is sufficient evidence in experimental animals for the carcinogenicity of *o*-toluidine [8].
Toxicity – RT	-
Toxicity – CT	-
Toxicity – RDT	-
Toxicity – AD	-
Toxicity - others	Acute effects include cyanosis, increased methemoglobin levels, and related effects in the spleen, such as increase of organ weight [9]. Repeated dose studies show that *o*-toluidine is toxic to erythrocytes and a methemoglobin forming chemical, as shown in rats, cats and humans [5]. Minimal skin irritation and ill-defined eye irritation have been observed in *o*-toluidine-exposed rabbits. All available toxicity data can be found on the Website Aggregated Computational Toxicology Resource (ACToR) [14] .

ADME properties

Absorption [5] - Biological monitoring to assess human exposure to *o*-toluidine indicates that absorption may occur through inhalation and dermal contact; however, quantitative information was not identified.

Distribution [15] – 48 hr following subcutaneous injection of radiolabeled *o*-[methyl-^{14}C]-toluidine hydrochloride into rats, radioactivity was detected in decreasing range as follows: liver > kidney > spleen, colon > lung and bladder [16]. In another study, 72 hr following oral application to rats, radioactivity was detected in decreasing range as follows: whole blood > spleen > kidney > liver > subcutaneous abdominal fat > lung > heart > abdominal skin> bladder >gastrointestinal tract > bone marrow > brain > muscle > testes [17].

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Metabolism [9] - The results from metabolism study in rats show that N-acetylation and hydroxylation at position 4 of the aromatic ring of *o*-toluidine are the major metabolic pathways in rats (Figure 1) [16]. Minor pathways include hydroxylation at position 6, oxidation of the methyl-group and oxidation of the amino-group. Sulphate conjugates predominate over glucuronides by a ratio of 6:1. The metabolites which were found in the urine of rats included: azoxytoluene, *o*-nitrosotoluene, N-acetyl-*o*-toluidine, N-acetyl-*o*-aminobenzyl alcohol, 4-amino-*m*-cresol, N-acetyl-4-amino-*m*-cresol, anthranilic acid, N-acetyl-anthranilic acid, 2-amino-*m*-cresol, other unidentified substances and unchanged *o*-toluidine (Table 1). The major metabolites being 4-amino-*m*-cresol and, to a lesser extent, N-acetyl-4-amino-*m*-cresol. Sulfate conjugates of *o*-toluidine predominate over glucuronide conjugates.

Binding of *o*-toluidine metabolites to hemoglobin has also been observed in rats [16], and in subjects treated with prilocaine, independently of their smoking status [8]. Hemoglobin adducts are formed after activation of arylamines through N-hydroxylation and do not undergo any significant repair. Fundamental studies of chemical carcinogenesis have revealed a central role for DNA adducts in the genesis of cancer [18]. This has been especially well documented for arylamines, including *o*-toluidine [19].

o-Toluidine is a metabolite of prilocaine [11]. Prilocaine, a local anesthetic, is metabolized to ortho-toluidine, and increased *o*-toluidine hemoglobin adducts have been found in patients treated subcutaneously with this anesthetic [8]. In humans, plasma half-life was evaluated from the application of an anesthetic gel containing lidocaine and prilocaine and was found to be around 4 hours [20]. Because *o*-toluidine can oxidize hemoglobin into methemoglobin, it has been suspected as being a potential cause of methemoglobinemia sometimes observed with the use of prilocaine, however, the therapeutic doses used display a large safety margin with respect to plasma levels normally associated with systemic toxicity [20].

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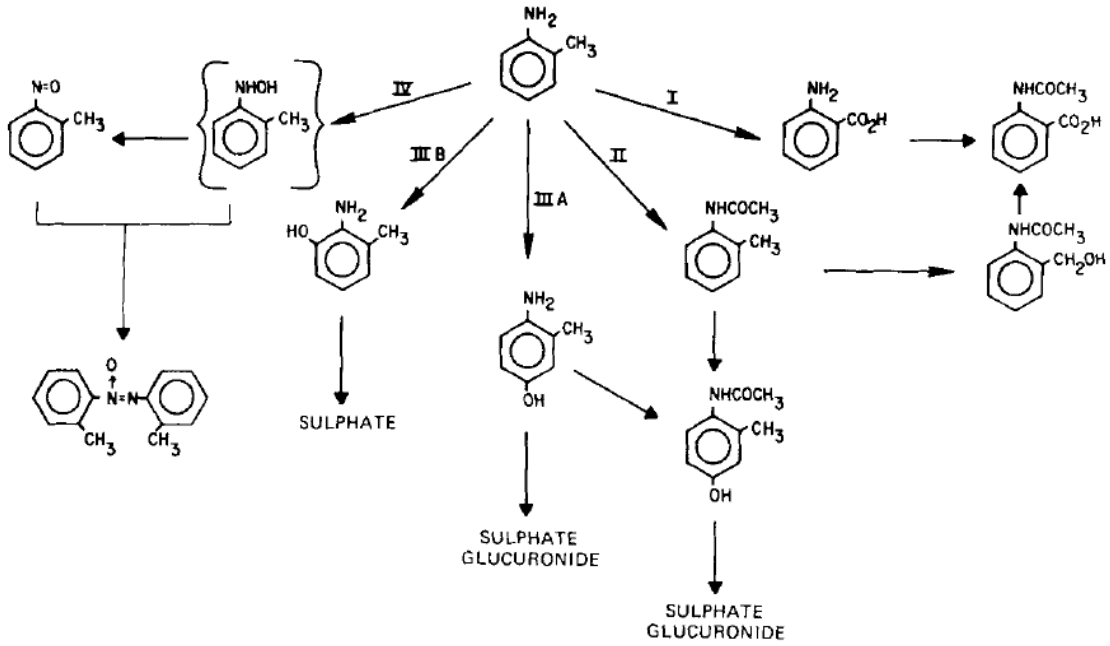


Figure 1 Summary of the metabolism of *o*-[methyl-^{14}C]toluidine hydrochloride in rats [16].

Brackets indicate metabolite, not actually identified, for which indirect evidence exists.

Figure by Son OS, Everett DW, Fiala ES. Metabolism of o-[methyl-14C]toluidine in the F344 rat. Xenobiotica. 1980; 10(7-8):457-68. Reprinted with permission of the publisher Informa Healthcare. Copyright © Informa Healthcare USA, Inc

Excretion - In rats, unchanged *o*-toluidine and its metabolites (Table 1) are largely excreted in the urine: up to 83 % of the dose 48 hr after s.c. injection, and up to 94.7 % of the dose 72 hr after oral application. Fecal excretion and exhalation of *o*-toluidine is minimal with up to 3.5 % and 1.46 % of the dose [21]. Free *o*-toluidine in urine only accounts for a minor percentage of *o*-toluidine uptake. This has been clearly demonstrated in human studies with prilocaine. Prilocaine is the only amide ester used as a local anesthetic that is hydrolyzed to release *o*-toluidine. Within 24 h after subcutaneous administration of 20 mg prilocaine/kg body weight to human volunteers, only a minor amount of the initial prilocaine dose was excreted in urine as *o*-toluidine (0.75%), whereas 4- and 6-hydroxy-*o*-toluidine accounted for 34% and 2.7% of the dose, respectively [10, 22].

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Anthranilic	
N-Acetylan	
Total	

Table 1 Metabolites present in 24-h urine of rats dosed s.c. with 400 mg/kg *o*-[^{14}C-]toluidine [16]

Table by Son OS, Everett DW, Fiala ES. Metabolism of o-[methyl-14C]toluidine in the F344 rat. Xenobiotica. 1980; 10(7-8):457-68. Reprinted with permission of the publisher Informa Healthcare. Copyright © Informa Healthcare USA, Inc.

Exposure levels in smokers and non-smokers

Because of the implication of arylamines in occupational cancer, biomonitoring of exposure started first in workers of chemical plants. For human biomonitoring of exposure to *o*-toluidine, its parent compound is noninvasively determined in urine [23]. The drawback of monitoring human urine is that arylamines undergo significant metabolism in the human body resulting in the excretion of only small amounts of the parent amines together with a large number of different metabolites. Furthermore, significant polymorphism of enzymes involved in the metabolism of arylamines, including cytochromes P450, *N*-acetyltransferases, UDP-glucuronosyltransferases, sulfotransferases, cyclooxygenases and peroxidases, can result in considerable variation in the percentage of dose excreted as the parent compound or certain individual metabolites and, therefore, provides only a crude estimate of an individual's exposure to arylamines [10].

Tobacco smoke as a source of human exposure to *o*-toluidine was first shown by El-Bayoumy et al. [24] who reported a 1.5.-fold higher excretion of free *o*-toluidine in smokers compared to nonsmokers. This was confirmed in a series of other studies. The differences between smokers and non-smokers range between 1.3- and 1.8-fold (Table 2). Clearly, other environmental sources of *o*-toluidine exposure also play an important role

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in total *o*-toluidine exposure, including nutrition. In 10 non-smokers, excretion of *o*-toluidine in urine increased 3-fold from 31 to 102 ng/24 h after one day on a controlled diet rich in eggs, fat and meat.[25]. The observed intra- and inter-individual variations in the amounts of hemoglobin adducts and urinary levels of *o*-toluidine were relatively large, suggesting that sources other than cigarette smoke contribute significantly to the exposure of the general population [24, 26, 27]. 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 *o*-toluidine (i.e. 190.0, 167.0, 129.0 and 96.ng/24h, respectively) [28].

Riedel et al. [26] measured *o*-toluidine in urine from smokers and non-smokers using gas chromatography mass spectrometry negative-ion chemical ionization (GC-MS-NICI). Excretion of *o*-toluidine was significantly higher in smokers than in non-smokers. Large variation was observed among non-smokers and attributed to one subject with *o*-toluidine values of 731 ng/24 hours, the highest of all subjects. After excluding this subject, the difference for *o*-toluidine excretion between smokers and non-smokers was still significant. Urinary arylamine excretion in smokers was associated with the extent of smoking as assessed by daily cigarette consumption ($P < 0.177$), urinary excretion of nicotine equivalents ($P < 0.021$), cotinine in saliva ($P < 0.118$), and carbon monoxide in exhaled breath ($P < 0.373$). All non-smokers had quantifiable amounts of *o*-toluidine in their urine, confirming that other environmental sources of exposure also occur. This study was compared to three other studies reporting *o*-toluidine excretion in smokers and non-smokers [24, 29, 30]. While only one of those studies reported significant differences between smokers and nonsmokers [29], higher absolute values were reported for non-occupationally exposed non-smokers in all three studies. Possible reasons for this were suggested to be alkaline rather than acid hydrolysis of urine leading to cleavage of N-acetylated metabolites; the method of detection, i.e., electrochemical or electron capture versus GC-MS-NICI; and non-occupational exposure to *o*-toluidine.

In five confined 8-days studies, urinary levels of *o*-toluidine decreased from 2- to 3.6-fold in abstinent smokers [6, 31-34] (Table 3). In a 5-day study, this decrease was by 2.7 fold [35] (Table 3). The method of detection i.e. electrochemical or electron capture versus gas chromatography - mass spectrometry - negative ion chemical ionization (GC-MS-NICI), preparation of the samples and non-occupational exposure to *o*-toluidine can all contribute to the relatively high variability within and between groups [8].

For biomonitoring of the metabolically activated moiety of arylamines, hemoglobin and serum albumin adducts can be determined as well as DNA adducts in leukocytes and target tissue [36, 37]. In man, hemoglobin adducts persist as long as the lifespan of hemoglobin, which is ~120 days. Therefore, adduct levels reflect a 4 month record of the time-weighted average exposure. However, only that part of the arylamine that has undergone *N*-hydroxylation is recorded. Because of its shorter turnover (half-life of 20-25 days) serum albumin adducts are regarded as more suitable for tracking exposure in the workplace [38], but are not as suitable in short-term exposure studies.

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Table 2 Representative urinary *o*-toluidine levels in smokers and non-smokers

Author (year)	Levels in smokers	Levels in non-smokers (or ex-smokers)	Units; variability
El-Bayoumi (1986) [24]	6357 ± 4041	4222 ± 3798	ng/24 h; mean ± SD
Seidel (1994) [39]	NM	61.8 (ND‑401)	ng/24 h; median (range)
Riffelman (1995) [29]	1.7 ± 1.6	0.0 ± 0.0	µg/L; mean ± SD
Ward (1996) [30]	0.9 ± 0.7	1.3 ± 1.3	µg/L; mean ± SD
Weiss (2002) [40]	NM	0.12 (0.05–3.1)	µg/L; median (range)
Weiss (2005) [41]	206 (ND‑838)	85 (ND‑1660)	ng/L; median (range)
Riedel (2006) [26]	204.2 ± 59.1	105.2 ± 25.6[a]	ng/24h; mean ± SD
Kutting (2009) [42]	130 (ND‑173)	100 (ND‑34)	ng/L; mean (range)
Lindner (2011) [27]	179.2 ± 491.4	63.5 ± 128.3	ng/24h; mean ± SD
Shepperd (2013) [43]	149 ± 62.3 (40-277)	53.9 ± 29.6 (19.3–137.2)	ng/24h; LS Means ± SD (ranges)

[a] One non-smoker with 731 ng/24 h excluded

Table 3 Representative urinary *o*-toluidine levels after smoking cessation

Author (year)	Levels in smokers	Levels in ex-smokers or switchers	Units; variability
Frost-Pineda (2008) [31]	82.2 ± 54.8	40.9 ± 29.2 [b]	ng/24 h; mean ± SD
Sarkar (2010) [6]	192 ± 75	54 ± 16 [b]	ng/24 h; mean ± SD
Tricker (2012) [32]	169.8 ± 79.2	47.6 ± 24.4 [b]	ng/24h; mean ± SD
Tricker (2012) [33]	70.53 ± 29.64	30.2 ± 8.35 [b]	ng/24h; mean ± SD
Tricker (2012) [34]	111.5 ± 49.7	33.8 ± 23.6 [b]	ng/24h; mean ± SD
Tricker (2012) [35]	91.1 ± 95.8	33.8 ± 29.0 [c]	ng/24h; mean ± SD
Theophilus (2015) [28]	210 ± 63.5	96.6 ± 38.9 [c]	ng/24h; mean ± SD

[b] Smoking cessation for 8 days

[c] Smoking cessation for 5 days

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List of abbreviations

3R4F	Kentucky 3R4F reference research cigarette [44]
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
GC-MS-NICI	Gas chromatography-negative-ion chemical ionization mass spectrometry
HC	Health Canada
HCI	Health Canada Intense regime [45]
HPHC	Harmful and Potentially Harmful Constituents
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization smoking regime [46]
IUPAC	International Union of Pure and Applied Chemistry
MSS	Main stream smoke
MW	Molecular weight
ND	Not detectable
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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