Nicotine

(CAS No: 54-11-5)

Health-based Reassessment of Administrative Occupational Exposure Limits
(Revised version)

Committee on Updating of Occupational Exposure Limits,
a committee of the Health Council of the Netherlands

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1 Introduction

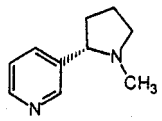
The present document contains the assessment of the health hazard of nicotine by the Committee on Updating of Occupational Exposure Limits, a committee of the Health Council of the Netherlands. The first draft of this document was prepared by MA Maclaine Pont, M.Sc. (Wageningen University and Research Centre, Wageningen, the Netherlands).

The evaluation of the toxicity of nicotine has been based on reviews published in the monograph 'Patty's industrial hygiene and toxicology' (Tro94) and by the American Conference of Governmental Industrial Hygienists (ACGIH) (ACG99). Where relevant, the original publications were reviewed and evaluated as will be indicated in the text. In addition, in September 2000, literature was searched in the databases Toxline, Medline, and Chemical Abstracts, starting from 1985, 1966, and 1937, respectively, and using the following key words: nicotine, green tobacco disease, green tobacco sickness, 3-(1-methyl-2-pyrrolidinyl)-(S)- pyridine, and 54-11-5. Care has been taken to select only those studies dealing with nicotine as the only substance. For example, publications on nicotine in tobacco smoke have not been considered. The final literature search was carried out in Toxline and Medline in March 2003.

In October 2003, the President of the Health Council released a draft of the document for public review. No comments were received.

After the final report was published in March 2004, the Health Council received comments from T van Messel (Vereniging Nederlandse Kerftabakindustrie, The Hague, the Netherlands) and JCP Wilhelmus (Philip Morris Holland BV, Amstelveen, the Netherlands). These comments were taken into account in deciding on this revised version.

2 Identity

name	:	nicotine
synonyms	:	(*S*)-3-(1-methylpyrrolidin-2-yl)pyridine; (*S*)-3-(1-methyl-2-pyrrolidinyl)pyridine; 1-methyl-2-(3-pyridyl)pyrrolidine; β-pyridyl-α-*N*-methylpyrrolidine
molecular formula	:	$C_{10}H_{14}N_2$
structural formula	:	
CAS number	:	54-11-5

3 Physical and chemical properties

molecular weight	:	162.23
boiling point	:	247ºC
melting point	:	-79ºC
flash point	:	95ºC (closed cup)
vapour pressure	:	at 20ºC: 6 Pa
solubility in water	:	miscible (below 60°C)
log $P_{octanol/water}$	:	1.17 (experimental); 1.00 (estimated)
conversion factors	:	at 20ºC, 101.3 kPa: 1 mg/m^3 = 0.15 ppm 1 ppm = 6.7 mg/m^3

Data from: ACG99, NLM03, Tro94, http://syrres.com/esc/est_kowdemo.htm.

Pure nicotine is a colourless oily viscous liquid that darkens readily and develops pyridine-like odour on exposure to air. The compound decomposes upon heating and combustion, forming corrosive vapours (nitrogen oxides). It reacts vigorously with oxidants (ACG99, Tom94).

Nicotine is a naturally occurring alkaloid found primarily in members of the solanaceous plant family, but widely distributed in the plant kingdom through 12 families and 24 genera (Dav91, Doo95). It is mainly isolated from the leaves of *Nicotiana tabacum* and *Nicotiana rustica* where it occurs at concentrations up to 8% (ACG99, Tro94).

4 Uses

The most widespread use of nicotine is encountered in tobacco (Tro94). Nicotine and its salts are used in medicine for the therapy of ulcerative colitis,

Alzheimer's disease, Parkinson's disease, Tourette's syndrome, sleep apnoea, and attention deficit orders (Ben96). Nicotine has therapeutic utility to aid smoking cessation.

Nicotine is also used as a non-systemic insecticide, mostly as a 40% solution of the sulphate. This use has declined considerably over the past 3 decades, primarily because of its high toxicity (Tro94). According to the database of the Dutch Pesticide Authorisation Board (CTB)*, nicotine is at present not permitted for its use as an active ingredient in insecticides in the Netherlands.

The general population might be exposed to nicotine present in foods and beverages (Dav91, Sie99). The mean estimated daily dietary intake in the USA is approximately 1.4 μg (Sie99).

5 Biotransformation and kinetics

Human data

In a human volunteer study, non-smoking women (n=17) were exposed to nicotine concentrations in air ranging from 40 to 200 $\mu g/m^3$ for 1 hour. Nicotine concentrations were generated by burning 5 cigarettes every 10 minutes (30 cigarettes/hour). The respiratory absorption of nicotine was 60-80% (mean 71.3±10.2%) and was independent of the nicotine air concentration (Iwa91).

In another human volunteer study, 12 males with a smoking history of at least 1 pack of cigarettes per day were treated after 24 hours of smoking abstinence with Nicoderm, a commercial nicotine transdermal system. With 24-hour time intervals, one transdermal system containing 78 mg nicotine was applied to 3 different skin sites (the chest, the arm, or the back), and remained on place for 24 hours. Nicotine plasma concentrations increased rapidly after application, reaching peak levels after 3.1 to 6.4 hours, and then declined slowly until removal of the transdermal system after 24 hours. After removal, the mean half-life of nicotine elimination from the plasma ranged between 3.1 and 3.5 hours. The mean plasma concentrations of the metabolite cotinine increased steadily during the 24-hour treatment period and then declined also. There were no significant differences in any of the kinetic parameters after application to the arm, back, or chest. The average dermal nicotine absorption from Nicoderm was 14% (Gor92).

Male human volunteers (n=10), who smoked 15 to 50 cigarettes/day, were given a capsule containing 3 mg (7 subjects), 4 mg (2 subjects), or 6 mg

* At: http://www.ctb-wageningen.nl.

(1 subject) nicotine base as the bitartrate salt, after overnight abstinence from tobacco. Nicotine was absorbed quickly, with a peak level in the plasma occurring at about 90 minutes. The oral bioavailability averaged 44% (range: 24-59%) (Ben91a).

Benowitz and Jacob also conducted a kinetic study with deuterium-labelled nicotine, which was administered by intravenous injection to 11 male smokers on 2 occasions at doses of 0.015 and 0.060 mg/kg bw or to 11 non-smokers on 1 occasion at a dose of 0.015 mg/kg bw. The half-lives of elimination from the plasma were 153, 157, or 122 minutes for nicotine and 29.5, 23.9, or 21.0 hours for cotinine in the low-dose smokers, high-dose smokers, and non-smokers, respectively (Ben93).

The metabolism of nicotine was studied in 12 male individuals following inhalation or dermal exposure. Inhalation exposure took place by smoking of cigarettes for 2 days, and dermal exposure by treatment with a nicotine transdermal system for 5 days, without smoking. Most of the absorbed doses of nicotine (98% and 88%, in case of inhalation or dermal treatment, respectively) was excreted as unchanged nicotine and 8 of its metabolites in 24-hour urine, collected during day 2 of inhalation exposure, or day 5 of dermal application. Following inhalation or dermal exposure, unchanged nicotine accounted for 10.4% and 11.1% of total urinary excretion products, respectively. The major metabolites were *trans*-3'-hydroxycotinine (39.1% and 37.0% of total urinary excretion products, respectively), followed by cotinine glucuronide (15.8% and 15.4%, respectively), and unconjugated cotinine (13.3 and 14.9%, respectively). Minor metabolites (less than 10% of total urinary excretion products) were *trans*-3'-hydroxycotinine glucuronide, nicotine glucuronide, cotinine-*N*-oxide, nicotine-1'-*N*-oxide, and nornicotine (see Annex I). Benowitz and Henningfield concluded that the metabolic profile in man is generally similar when nicotine is inhaled or absorbed dermally (Ben94).

In another metabolism study, 6 non-smoking men received a single intravenous dose of 0.190 mg of ^{14}C-labelled racemic nicotine. The half-lives of elimination from the plasma were 1.4 and 14.3 hours for nicotine and cotinine, respectively. Unchanged nicotine (29% of the dose) and 8 of its metabolites were detected in urine collected over a 120-hour period after administration. Metabolites were nicotine-1'-*N*-oxide (0.9% of the dose administered), nornicotine (2.4%), cotinine (16.3%), 3-hydroxycotinine glucuronide (11.9%), 3-hydroxycotinine (1.1%), cotinine-*N*-oxide (4.9%), norcotinine (5.8%), and demethylcotinine $\Delta^{2',3'}$-enamine (8.4%) (Kye90, Kye91).

A case of a female with deficient oxidation of nicotine into cotinine has been reported. This woman converted only 8% of nicotine into cotinine, resulting in an unusually long half-life of nicotine (Ben96).

The maximum nicotine level in urine of female workers in the tobacco industry was reached at the end of the shift. The half-life of nicotine elimination from the urine was 8.4 hours (Hu94).

Animal data

In a dermal absorption study, a single dose of ^{14}C-labelled nicotine in acetone was applied under semi-occluded conditions to the clipped backs of a group of young and a group of adult female Fischer 344 rats (n=3/group) at doses of 0.016, 0.54, or 2.68 µmol/cm^2 (2.6, 87, or 435 µg/cm^2). Three days after application, the mean absorption percentages were 49, 84, or 88%, respectively, in young rats and 75, 83, or 86%, respectively, in adult rats (Hal88, Sha87).

Pulmonary nicotine absorption was studied in mongrel dogs (n=8) after installation of 0.5 mg of nicotine at 3 levels of the tracheobronchial tree, i.e., the trachea, a subsegmental bronchus site, or a distal site. Each of the 3 applications was carried out on a different day with a minimum time interval of 48 hours between applications. On a fourth day, the dogs were treated with a single intravenous dose of 0.5 mg of nicotine. The absorption of nicotine from the subsegmetal bronchus and distal sites was rapid, time-to-reach-peak concentrations (t_{max}) being comparable to that from the intravenous dose. Absorption from the tracheal region was slower, t_{max} being significantly longer than those from intravenous and subsegmental administration. Compared to intravenous injection, peak plasma concentrations of nicotine (C_{max}) following application at the distal site were comparable while those following application at the subsegmental site and the trachea were significantly lower than those from intravenous injection. Total amounts of nicotine absorption as well as the half-life of nicotine elimination from the plasma (84-91 minutes) were the same for all routes of administration (Her92).

Female Swiss-Webster mice were given ^{14}C-nicotine in drinking water at an average daily dose of 17 mg/kg bw for 10 days. The amount of radioactivity retained in tissues was about 10-fold higher in the adrenal gland and the uterus than in the brain or the blood. Other tissues (liver, salivary, ovaries, kidney, thymus, and lung) contained about 6 times more radioactivity than blood (Row83).

Male Sprague-Dawley rats were given a dose of 1 mg of ^{3}H-nicotine, either by a bolus intravenous injection or a 1-hour constant infusion. One hour after the

last dose, the highest concentration of radioactivity was found in the kidneys (about 50% of the dose) via either route of administration, followed by the salivary gland (14 and 16%), the spleen (11 and 13%), and the adrenal gland (11 and 8%). The lowest concentrations of radioactivity were found in the blood (1.2%) after bolus injection and in the caecum (4.7%) after constant infusion. Comparing the 2 routes of administration, the retention of radioactivity following constant infusion was greater in all tissues, except the adrenal gland. The greatest difference was found in the blood, where the level of radioactivity after constant infusion was 6-fold higher than after bolus injection (Cho93).

In another study, male Sprague-Dawley rats were given an intravenous dose of 5 mg/kg bw ^{14}C-nicotine. Within 5 to 10 minutes after administration, peak concentrations of nicotine were found in plasma, liver, kidney, heart, and brain. Concentrations were highest in kidneys, followed by liver, blood, heart, and brain. The primary metabolite, cotinine, accumulated in plasma, and by about 30 minutes, the concentrations of nicotine and cotinine were about equal. While the plasma cotinine concentration remained constant at 20 to 60 minutes after administration, nicotine was eliminated by the kidneys. The half-life of disappearance of nicotine from the blood was approximately 10 minutes and less than 20 minutes for the other tissues (Ram95).

The metabolism of nicotine has been studied in male Sprague-Dawley rats, following a single intra-arterial injection of 0.1 mg ^{14}C-labelled nicotine/kg bw. The half-lives of elimination from the plasma were 1.0 hour and 5.2 hours for nicotine and cotinine, respectively. The total amount of radioactivity excreted in the urine over 96 hours was 70% of the administered dose. Nicotine was excreted unchanged in amounts of 11.3% of the dose. Nicotine metabolites excreted in the urine were cotinine (7.2% of the dose), nicotine-1'-*N*-oxide (11.6 % of the dose), nornicotine (8.9% of the dose), and isomethylnicotinium ion (2.7% of the dose). Glucuronidation of nicotine was not reported. Most of the cotinine formed from nicotine was further metabolised, and urinary metabolites detected were cotinine *N*-oxide (9.3% of the dose), 3-hydroxycotinine (4.5% of the dose), 3-hydroxycotinineglucuronide ('metabolite A': 3.1% of the dose), norcotinine (1.0% of the dose), γ-(3-pyridyl)-γ-methylaminobutyric acid (4.4% of the dose), γ-(3-pyridyl)-γ-oxo-*N*-methylbutyramide (2.0% of the dose), γ-(3-pyridyl)-γ-oxobutyric acid (1.8% of the dose), and 3-pyridylacetic acid (2.2% of the dose) (see Annex I). Glucuronidation of 3-hydroxycotinine was not reported (Kye91, Kye87).

In an *in vitro* study in isolated perfused dog lung, cotinine and nicotine-1'-*N*-oxide were detected in the venous blood and the lung, respectively, following administration of ^{14}C-labelled nicotine via the pulmonary artery (Tur75).

Physiologically based pharmacokinetic (PB-PK) models have been described for tissue and plasma kinetics of nicotine and cotinine in man (Rob92) or the Sprague-Dawley rat (Plo92). The committee concludes that the rat data do not accurately predict the kinetics of nicotine or cotinine in humans.

6 Effects and mechanism of action

Human data

Mechanism of action

The *S*-isomer of nicotine binds stereoselectively to central and peripheral nicotinic cholinergic receptors, while the *R*-isomer is a weak agonist at cholinergic receptors. These receptors are found in the brain, autonomic ganglia, and the neuromuscular junction. Nicotine acts by direct stimulation of the nicotinic cholinergic receptors, which causes a release of neurotransmitters, including acetylcholine, noradrenaline, dopamine, and may result in myriad of symptoms, i.e., modulation of neurological, neuromuscular, cardiovascular, respiratory, glandular, or gastro-intestinal function. The major effects of nicotine are an initial stimulatory effect on these organs due to parasympathetic ganglionic stimulation at nicotinic receptor sites. At larger doses, the initial stimulatory effect is followed by prolonged ganglionic and neuromuscular blockade, which may result in depression and paralysis of the central nervous system, all peripheral autonomic ganglia, and motor end-plates in skeletal muscles. Fatalities are believed to result from respiratory arrest secondary to muscle paralysis (Ben96, Mon94, Lav91).

Irritation and sensitisation

Several studies have been reported on local and systemic skin reactions to nicotine patches. In one study, 35-54% of users showed a localised erythematous, pruritic skin reaction at the patch site, sometimes associated with local oedema. According to Fiore et al., a reaction to the adhesive was the most likely cause (Fio92). A case of systemic skin reactions has been described concerning a 31-year-old female patient, who developed swelling of the feet, hands, face, and

throat, and a blisterlike rash under the patch, after using of a first set of 21-mg nicotine patches (Fra93). Another report describes 11 cases of local and 5 cases of systemic skin reactions to 35 to 52.5-mg nicotine patches. Skin reactions usually occur after about 15 days' use (Kla94). In a number of placebo-controlled trials, skin reactions in groups of people treated with nicotine or placebo patches were compared. In all studies, a statistically significant increase in the incidence of local skin reactions was found in the group treated with nicotine patches (Dau91, Hur90, Imp93, Rus93, Ton91). Cases of occupational dermatitis have been reported in persons processing tobacco or employed in nicotine production (Tro94).

Sensitisation responses to pure nicotine were studied in 10 males and 4 females, who had previously experienced adverse skin reactions from the use of nicotine patches. Tests were conducted with aqueous solutions of 1%, 10%, and 50% nicotine base and 5% nicotine sulphate, applied under occlusion to the backs of the subjects for 2 to 3 days. The incidences of positive allergic patch test reactions to nicotine base were 1/14, 4/14, and 5/14 at concentrations of 1%, 10%, or 50%, respectively, and 1/14 when exposed to nicotine sulphate (5%). At the high concentration, irritant reactions due to occlusion were present in the remaining 9 subjects (Bir91). The committee concludes that nicotine is a skin irritant and a skin sensitiser.

Respiratory effects of nicotine were investigated in 13 non-smoking subjects, after single-breath inhalation by each subject of 0.01 mL nebulised nicotine solution (1, 2, 4, 8, 16, 32, or 64 mg/mL; 15-minute time interval between inhalations) on 1 day or on 3 separate days. A concentration-dependent cough response and airway obstruction was produced, which was reproducible over 3 different days. According to Hansson et al., these effects were due to stimulation of afferent nerve endings in the bronchial mucosa and mediated by parasympathetic cholinergic pathways (Han94). In 12 male non-smoking volunteers, airway irritation in the substernal region was induced after inhalation of a single puff of cigarette smoke of 3 different types of cigarettes with high nicotine content during 3 consecutive days. No or very mild irritation was recorded after inhaling cigarette smoke with a low nicotine content or gas-phase cigarette smoke without nicotine (Lee91).

Acute toxicity

In the older literature, many cases of nicotine poisoning have been recorded, the majority from ingestion or skin absorption. Most fatalities occurred in the 1920s and 1930s, when concentrated nicotine solutions were commonly used as insecticides, and reports of fatal poisoning frequently involved the mistaking of nicotine solutions for other medications (Had83). There were 288 fatalities reported in the United States between 1930 and 1935. Occupational intoxications have also been described in persons engaged in nicotine extraction and in spraying insecticides, mainly from percutaneous nicotine absorption. Fatal occupational poisoning has been relatively uncommon (Mon94, Tro94). Symptoms of nicotine toxicity are manifested by effects on the gastrointestinal, central nervous, neuromuscular, cardiovascular, respiratory, and glandular systems. The common symptoms of moderate intoxication include nausea, vomiting, abdominal pain, diarrhoea, headache, sweating, fatigue, and palpitations. More severe symptoms include faintness, dizziness, weakness, and confusing, progressing to muscular weakness, collapse, and respiratory arrest. Following large doses of nicotine, symptoms develop quickly and death results from paralysis of respiratory muscles, produced by peripheral neuromusular blockade, and cardiovascular collapse (Lav91, Tro94). Dermal exposure to nicotine produced the same range of symptoms that were produced by ingestion (ACG99, Tro94).

A more recent case of fatal nicotine ingestion was reported of a 17-year-old boy, who deliberately ingested a solution containing an estimated dose of 5000 mg nicotine alkaloid. Approximately 1 to 2 minutes after ingestion, he vomited and developed cardiopulmonary arrest. During the first few hours following admission, the patient had multiple grand mal seizures, cerebral oedema, and no cortical function. The boy died at 64 hours after the ingestion (Lav91).

A 45-year-old male developed atrial fibrillation and seizures following inhalation of nicotine, used as a substitute of tobacco smoking (Nun01).

When a male subject was given a capsule containing 6 mg of nicotine (as the bitartrate salt), he complained of nausea and abdominal cramping that began about 30 minutes after ingestion and lasted for 1 hour. Subjects receiving doses of 3 or 4 mg had no signs or symptoms of toxicity (Ben91a).

In another study, a nicotine patch (Nicoderm), containing 78 mg nicotine, was applied with 24-hour time intervals to 3 different skin sites (the chest, the arm, or the back) of 12 male smokers, and remained on place for 24 hours (see Section 5). At 4 hours after application, mean heart rate and systolic blood pressure had significantly increased compared to baseline values (Gor92).

When 5 non-smoking subjects (3 males, 2 females) took a single breath inhalation of 0.01 mL nebulised nicotine solution at times 0 and 10 minutes, no cardiovascular effects were found at concentrations up to 64 mg/mL. Cadiovascular effects were also evaluated in 8 non-smoking volunteers (4 men, 4 women) inhaling nicotine solutions of 0, 2, 4, or 8 mg/mL on 4 different days. A single breath was taken every 15 seconds up to 5 minutes (total 21 inhalations), giving a dose of 0, 0.4, 0.8, or 1.7 mg nicotine/5 minutes. Heart rate and systolic blood pressure were significantly increased in a dose-related fashion compared with the vehicle controls. Maximal responses were seen within 3 minutes after inhalation, and the responses lasted between 6 and 10 minutes. No significant changes were found in diastolic blood pressure. Nicotine caused a decrease in skin temperature, with maximal responses at 5 minutes. Seven of the subjects complained of headache that had a maximum at 5-6 minutes and lasted for 20 minutes. None of the subjects noticed a tremor or nausea (Han94).

Several reports have been published on the so-called 'green-tobacco sickness'. This sickness is acute nicotine poisoning caused by the dermal absorption of nicotine from mature tobacco plants during cultivation and harvesting. Green-tobacco sickness is normally a self-limiting condition from which workers recover in 2 to 3 days and is characterised by nausea, vomiting, weakness, dizziness, headache, and occasional fluctuations in blood pressure or heart rate (Arc01a, Bal95, Geh74). In an epidemiological study among Latino farm workers in North Carolina, USA, the workers experienced green-tobacco sickness 2 days for every 100 days worked. Factors at risk were lack of work experience, picking tobacco leaves late in the season, and working in wet clothing (Arc01a, Arc01b). Salivary cotinine levels increased across the season, independent of smoking status (Qua01). In a case-control study, crude annual incidences in the USA of 10 to 14 per 1000 tobacco workers were reported. The study demonstrated that ill workers were younger and were more likely to have worked in wet conditions, compared with healthy workers (Bal95).

In a previous study on tobacco workers in India, the frequency of green-tobacco-sickness symptoms was 53%. Mean 24-hour urinary nicotine and cotinine concentrations in workers handling tobacco were 4.1 and 3.8 mg/L, respectively, and below detection limit in control subjects (Gho86). In a study among 100 workers in 2 tobacco-processing factories in India, the main route of exposure during tobacco processing was inhalation. The average nicotine air concentration in the breathing zone of the workers (10-minute samples) was 1.18 mg/m^3. Urinary nicotine and cotinine concentrations were 3.3 and 2.8 mg/L for non-smoking females and males, respectively. Corresponding urinary cotinine levels were 3.3 and 4.1 mg/L, respectively. No abnormalities were found in

pulmonary function tests in workers. However, 69 subjects exhibited one or more of symptoms, such as vomiting, giddiness, headache, weakness, or loss of appetite (Gho85).

In a 1-day biological monitoring study on 10 female Italian tobacco harvesters, the mean blood nicotine level rose to a peak value of 3.45 µg/L at 2 hours after the end of the working day. The mean urinary nicotine reached a peak value of 158 µg/L in samples collected at the end of the working day. No significant trend in mean plasma or urinary cotinine levels was observed at different time points during the working day. Plasma cotinine levels were approximately 15 µg/L, indicating a daily intake of 1.2 mg of nicotine, and urinary cotinine levels were approximately 100 µg/L. No symptoms of nicotine poisoning were reported among the workers (Ale01).

Long-term toxicity

In a case-control study among farmers in the USA, the risk for multiple myeloma for farmers who personally mixed, handled, or applied nicotine as an insecticide for more than 1 year was studied. Included in the analysis were 6 cases and 22 controls. Of the total farmer population (111 cases and 378 controls), 84% of the cases and 78% of the controls received an in-person interview on the use of pesticides. No statistically significant association between multiple myeloma and the use of nicotine as an insecticide was found. The Odds Ratio (OR), relative to non-farmers (62 cases and 272 controls) was 1.4 (95% confidence interval (CI): 0.5-3.6) (Bro93). In another case-control study, a statistically significantly increased risk for leukaemia was reported for farmers engaged in spraying of nicotine. Included in the analysis were 30 cases and 47 controls. The OR was 1.6 (95% CI: 1.0-2.6). For the group handling nicotine at least 20 years ago (28 cases and 36 controls), the OR was 2.0 (95% CI: 1.2-3.4). This result must be viewed with caution since of the total farmer population (578 cases and 1245 controls), only 25% of the cases and 75% of the controls were interviewed. Furthermore, accurate recall of pesticide use probably declines with the passage of time (Bro90). The committee is of the opinion that the number of nicotine applicators (both cases and controls) was too small to draw a reliable conclusion on the association between the handling of nicotine and the emergence of leukaemia or multiple myeloma.

The possible association between long-term exposure to pure nicotine and cardiovascular disease has been investigated in 2 studies among smokeless tobacco users. During smokeless tobacco use, nicotine is absorbed through the buccal mucosa, which results in a larger overall exposure to nicotine than by

cigarette smoking owing to prolonged absorption. In a cross-sectional study, middle-aged and older smokeless tobacco users had a significantly higher prevalence of hypertension and cardiovascular symptoms than non-users of tobacco (Bol92). A cohort study was conducted among 6297 male smokeless tobacco users, employed in the Swedish construction industry, who attended a health examination between 1971 and 1974 and were followed regarding cause-specific mortality during the period 1974 through 1985. The age-adjusted relative risk (RR) of dying from cardiovascular disease compared with non-users (n=32,546) was 1.4 (95% CI: 1.2-1.6). Among men aged between 35 and 54 years at the beginning of the follow-up (n=1672), the RR was 2.1 (95% CI: 1.5-2.9). The RR for cardiovascular disease for smokeless tobacco users was lower than that for current cigarette smokers, but higher than for ex-smokers. Cancer mortality was not raised in smokeless tobacco users compared with non-users (Bol94).

Addiction

Addiction can be defined as the compulsive use of a drug that has psychoactivity and that may be associated with tolerance and physical dependence, i.e., may be associated with withdrawal symptoms after cessation of drug use (DHH88). For smokers, addiction is assumed to involve daily smoking of cigarettes, difficulty in not smoking every day, and a high likelihood of withdrawal symptoms after cessation of smoking. Smokers who regularly smoke 5 or fewer cigarettes per day appear not to be addicted (Shi89). In their report, Benowitz and Henningfield stated that the consumption of 5 cigarettes per day corresponds with an average serum cotinine level of 70 μg/L. Therefore, they consider a serum cotinine level of 50 to 70 μg/L as a cut-off point for the addictive threshold. In a separate study, Benowitz and Henningfield estimate the daily intake of nicotine in smokers as 0.08 times the blood cotinine concentration. Thus, a level of 50 to 70 μg cotinine/L corresponds to a daily nicotine intake of 4 to 6 mg. The authors, therefore, propose that a daily intake of 5 mg of nicotine is a threshold level, below which no addiction occurs (Ben94).

No data of nicotine addiction have been reported in workers who cultivate and harvest tobacco or in workers processing tobacco, where the exposures to nicotine were high enough to cause green-tobacco sickness (Arc01a, Arc01b, Bal95, Geh74, Gho85, Gho86). From urinary cotinine levels, the committee concludes that the daily nicotine intake was 1.2 mg for Italian workers, but substantially higher than 5 mg for Indian workers.

Non-smoking patients with ulcerative colitis did not develop symptoms of addiction when treated with nicotine patches that released 15 to 25 mg of nicotine daily for 6 weeks (n=35) or 15 mg daily for 6 months (n=40). Mean steady-state plasma cotinine levels varied from 102 to 150 µg/L in the 6-week study and from 62 to 76 µg/L in the 6-month study (Pul94, Tho95). In another study, patients with primary sclerosing cholangitis (n=8) were treated with oral doses up to 24 mg/day for 1 year. No patient experienced withdrawal symptoms when nicotine administration was discontinued. Due to signs of nicotine toxicity, 3 out of the 8 patients completed only 1 to 4 months of treatment (Ang99).

From the above studies, the committee concludes that the addictive properties of nicotine were exclusively found in cigarette-smoking subjects.

Developmental toxicity

Studies of the effects of cigarette smoking and nicotine in humans suggest that nicotine may contribute to adverse reproductive outcomes. Mechanisms of particular concern include reduction of utero-placental blood flow, leading to hypoxia-induced brain damage, peri-natal mortality, and sudden infant death. Direct effects on neurotransmitter receptors in the developing fetal brain may lead to cognitive and learning defects in childhood or adolescence (Ben91b, Slo98).

Animal data

Irritation and sensitisation

Nicotine is moderately to severely irritating to the rabbit eye (Sug90). The committee did not find data on skin irritation or skin sensitisation properties of nicotine

Acute toxicity

Results of acute lethal toxicity tests with nicotine are summarised in Table 1.

Table 1 Summary of acute lethal toxicity studies in mammals.

exposure route	species	strain (sex)	LD_{50} (mg/kg bw)	reference
intratracheal	rat	Long-Evans	19.3	Kim84
dermal	rat		140	Tro94
	rabbit		50	Tro94
oral	rat		53	ACG99
	rat		50-60	Tro94
	rat	Sprague-Dawley (male, female)	71	Yam90
	rat		188	Ray91
	mouse		3.3	ACG99
	mouse		24	Ray91, Tro94
	mouse		50-60	Tro94
intraperitoneal	rat		30	Ray91
	rat		14.6	Tro94
	mouse		5.9	Tro94
	rabbit		14	Tro94
intravenous	rat		7	Tro94
	mouse		7.1	Tro94
	rabbit		9.4	Tro94
	dog		5	Tro94

Signs of intoxication in dogs treated with a lethal intravenous dose were vomiting, initial stimulation, followed by depression, seizures, and paralysis of the central nervous system, peripheral autonomic nervous ganglia, and skeletal muscle endings (Ray91).

To investigate the role of nicotine in the development of pulmonary emphysema, groups of Long-Evans rats (n=13-16) received single intratracheally administered doses of 3 or 7.5 mg nicotine/kg bw. Four weeks after treatment, ventilatory, mechanical, and gas exchange functions were not significantly different compared with control rats. It was concluded that intratracheal installation of a single, relatively high dose of nicotine does not induce the development of pulmonary emphysema in the rat (Kim84).

Short-term toxicity

In order to investigate a possible role of nicotine in cigarette smoke, which, through the mediation of catecholamines, was thought to 'waste' a portion of the oxygen received by the heart, in being one of the potential causes of increased morbidity and mortality from 'coronary heart disease', a number of physiological and biochemical parameters likely to be affected by repeatedly administered

nicotine were measured in male Sprague-Dawley rats. Groups of 100 animals were given nicotine as the alkaloid in their drinking water at doses of 1.14 or 4.56 mg/kg bw/day for 34 weeks, and mortalities, gross cardiac lesions, haematocrits, and the activities of several heart enzymes, selected as potential indices of early cellular injury, were examined. After the end of treatment, half of each group was exposed to 6% oxygen for 12 hours, after which aforementioned parameters were measured in separate sets of animals at several 'post-hypoxia' intervals The only effects reported in nicotine-only-treated animals were a statistically significant increase in the activity of myocardial enzymes isocitric dehydrogenase and acid phosphatase and a statistically significant decrease in the activity of β-glucuronidase at the high dose (Wen70).

To assess the effects of long-term treatment with nicotine on several behavioural measures, including locomotor activity, exploratory efficiency, habituation, short-term and long-term memory, groups of 5-month-old ('young') and 22-month-old ('old') female Sprague-Dawley rats (n=15/group) were given nicotine (as its acid tartrate) via the drinking water at concentrations of 0, 20, or 50 mg/L* for 131 days. Mean nicotine plasma concentrations in low- and high-dose rats (n=12) were 16.6 and 56.2 µg/L, respectively. Mean terminal body weights were statistically significantly decreased (by 5-10%) at both dose levels in 'young' rats and at the high dose in 'old' rats. Water intake was reduced during the first half hour of the daily 4.5-hour access to drinking water. Locomotor activity was increased throughout the experiment, but dropped immediately to control values when treatment was discontinued during a 7-day withdrawal period. In 'young' rats, exploratory efficiency was attenuated. Nicotine did not affect habituation and memory tasks (Wel88).

Five-week-old male NMRI mice were given increasing concentrations of nicotine in drinking water for 50 days, equivalent to 60-65 mg/kg bw/day from the 3rd week up to the end of the dosing. Locomotor activity was significantly increased on the 50th day of nicotine administration compared to control animals. However, no difference was observed 12-14 hours after cessation of exposure. Concentrations of several brain monoamines were elevated on the 50th day, but at 23-25 hours after withdrawal, only hypothalamic dopamine concentration was still significantly increased (Gad00).

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* Taking a mean body weight of 300 and 400 mg for the 'young' and 'old' rats, respectively (estimated from data presented by Welzl et al.) and assuming the dose amounts were as nicotine (and not as nicotine hydrogen tartrate) and a mean water consumption of 22.5 mL/day for both groups, these dose levels could be 1.5 and 3.8 mg/kg bw and 1.1 and 2.8 mg/kg bw in 'young' and 'old' rats, respectively.

When nicotine was administered to Charles River LEW rats by subcutaneously implanted miniosmotic pumps at the rate of 1 mg/kg bw/day for 3 weeks, Con A-induced proliferation of peripheral blood cells and of spleen cells was significantly inhibited. Effects persisted for at least 2 weeks after termination of exposure. Single intraperitoneal injection of 1 mg/kg bw inhibited Con A-induced proliferation of only the peripheral blood cells, but not of spleen cells. This was observed at 2 hours, but not at 24 hours after treatment. It was demonstrated that short-term nicotine treatment regulate T cell proliferation via nicotine acetylcholine receptors, but unlike acute treatment, the effects were independent of the hypothalamus-pituitary-adrenal axis (Sin00).

Long-term toxicity and carcinogenicity

Female Sprague-Dawley rats (n=68) were exposed to an average nicotine concentration of about 0.5 mg/m^3 (range: 0.40-0.65 mg/m^3), 20 hours/day, 5 days/week, for 103 weeks. The purity of nicotine was >99%. Non-exposed rats (n=34) served as a control group. Mean nicotine concentrations in plasma, measured after 5 days and at the end of the study, were 108 µg/L and 130 µg/L, respectively, giving the plasma concentration found in heavy smokers. Interim kills (5-10 nicotine-exposed and 5-6 controls) were performed at 6, 12, and 18 months after the beginning of exposure. After 24 months, 7 control rats (21%) and 22 nicotine-treated rats (32%) remained. The mean body weight of exposed rats was slightly lower (ca. 5%) than those of the controls throughout the study, but no statistical data were given. The proportion of animals withdrawn from the study because of general misthriving at observation was 16% in nicotine-exposed rats and 22% in controls. Macroscopic and microscopic examination revealed not statistically significant increases in incidences of fibroadenomas of the mammary gland, of adenomas of the pituitary gland, and of adenocarcinomas of the ovary, compared with the control group. Neither lung tumours nor any increase in pulmonary neuroendocrine cells were detected. The median absolute heart weights of exposed and control animals were not statistically significantly different, and no increase in atherosclerotic lesions was found. Macroscopic or microscopic examination of other tissues (brain, gastrointestinal tract, liver, kidneys) did not reveal treatment-related abnormalities (Wal96).

Mutagenicity and genotoxicity

- *In vitro* tests:
  - Gene mutation assays. Tests for reverse mutations in 5 strains of *S. typhimurium* (TA97, TA98, TA 100, TA 1535, and TA1537) were negative at concentrations up to 5000 μg nicotine/plate both with and without metabolic activation by a rat liver microsomal S9 preparation (Bra87, Doo95, Flo84, McC75, Rie82). Nicotine metabolites, i.e., nicotine-1'-*N*-oxide, cotinine, cotinine-*N*-oxide, and *trans*-3'-hydroxycotinine did not induce reverse mutations in these strains up to 1000 μg/plate, in the absence or presence of S9 (Doo95). Neither nicotine, nor its metabolites, showed missense back mutations in *S. typhimurium* strains TA100, TA7004, TA7005, or TA7006, at concentrations up to 2000 μg/plate, in the presence or absence of rat liver S9 (Yim01).
    When female Sprague-Dawley rats received a single intraperitoneal injection of 0.8 mg nicotine/kg bw (the maximum tolerated dose), 24-hour urine samples, either neat or extracts, did not induce reverse mutations in *S. typhimurium* TA98, with and without metabolic activation (Doo91).
  - Cytogenicity assays. Neither nicotine, nor its metabolites nicotine-1'-*N*-oxide, cotinine, cotinine-*N*-oxide, or *trans*-3'-hydroxycotinine induced sister-chromatid exchanges (SCE) in cultured Chinese hamster ovary (CHO) cells at concentrations up to 1000 μg/mL, with and without metabolic activation (Doo95). The frequency of SCEs in cultured CHO cells was increased in a dose-dependent manner at nicotine or nornicotine concentrations in the range of 1250- 5000 μg/mL, in the absence of S9. The increase was statistically significant at the highest dose only. In the presence of S9, no increase in the SCE frequency was found at any of the concentrations (Rie83). In another study, a dose-related increase in the frequency of SCEs in cultured CHO cells was found at nicotine concentrations in the range of 150 to 1000 μg/mL in the absence of metabolic activation. Dose-related increases in the frequency of chromosome aberrations (excluding gaps) were found at concentrations of 375 μg/mL and above (Tri90, Tri93).
  - Other genotoxicity assays. In a DNA repair assay with *E. coli pol* A^+/*pol* A^-, nicotine at an amount of 40 μg induced repairable DNA damage while its metabolites nicotine 1'-*N*-oxide (400 μg) or cotinine (800 μg) were negative (Rie82). In the SOS-chromotest with *E. coli* strain PQ 37, nicotine was negative at concentrations up to 1.62 mg/mL, either without or with activation by S9 (Bra87). In a bacterial luminescence test with

Vibrion fischeri strain NRRL-B-11177, nicotine did not induce significant light emission at concentrations of 1250 or 2500 µg/mL, in the presence or absence of S9. Cotinine, however, was positive at both concentrations in the absence of S9, but not in its presence (Yim95).

- *In vivo* tests:
  Groups of 5 young male Swiss albino mice (n=5/dose/sampling time) were given single doses of nicotine of 0.77 and 1.10 mg/kg bw by gavage. Mice (n=5) treated with isotonic saline served as a control group. The nicotine-dosed animals were sacrificed at 6, 12, 18, and 24 hours after treatment and the controls only after 24 hours, and bone marrow chromosome preparations were made. Statistically significant, dose-dependent increases in the frequency of chromosomal aberrations (excluding gaps) were found for both doses at all sampling times, compared with the controls (Sen91).
  When male mice were treated with intraperitoneal injections of doses of nicotine of 5 mg/kg bw/day on 2 consecutive days, the percentage of non-viable implants was statistically significantly increased in females inseminated in weeks 1 and 2 after treatment. This indicates that epididymal sperm and spermatids were susceptible to the induction of dominant lethal mutations by nicotine (Hem78).

In conclusion, nicotine has shown clear clastogenic activity (increased frequency of SCEs and chromosome aberrations in one *in vitro* test, and an increased frequency of chromosome aberrations and of dominant lethal mutations *in vivo*). Nicotine did not induce gene mutations in bacterial systems.

Reproduction toxicity

Numerous studies have been conducted on reproductive or developmental effects of nicotine by multiple routes of exposure and in several species of animals (Tro94). However, the committee could not find a standard 1-, or 2-generation reproduction study, or a standard developmental toxicity study.

Below, a number of studies are reported in which reproductive or developmental effects were examined following relevant routes of exposure, i.e., dermal or oral. In addition, some studies using less relevant routes of dosing, i.e., intraperitoneally or subcutaneously are reported. The committee did not find any study in which animals were exposed by inhalation.

Effects on fertility
Groups of male Swiss albino mice (n=12/group) received nicotine via the drinking water at doses equivalent to 0 or 2.7 mg/kg bw/day for 7 weeks or to 0 or 2.3 mg/kg bw/day for 20 weeks. Thereafter, each male was mated weekly with untreated virgin females for 4 and 6 consecutive weeks, respectively. In females that were mated with males treated for 7 weeks and were allowed to go to term, the numbers of litters and offspring were similar to those of controls. In females that were mated with males for 20 weeks and were killed during week 3 of pregnancy for examination of uterine contents, a statistically significant increase in the incidence of limb abnormalities was observed in fetuses resulting from mating in post-treatment week 1 and 2 , but not in fetuses from the other matings (Hem81).
Groups male albino mice (n= 8/group) received daily intraperitoneal injections of 0, 2, 4, or 6 mg nicotine/kg bw for 15 days. There were statistically significant, dose-related decreases in relative testicular, epididymis, seminal vesicle, prostate, or vas deferens weights and in spermatocyte and spermatid counts and not statistically significant, dose-related increases in spermatogonia counts. According to Reddy et al., these effects were caused by interference of nicotine with the release of pituitary gonadotrophins. A NOAEL was not established (Red98).

Effects on development
Commercial nicotine transdermal patches, which would deliver 1.75 or 3.5 mg of nicotine per day, were applied to the backs of groups of pregnant Sprague-Dawley rats (n=2-13/group) either during gestational days 2 through 19 or 2 through 7. Control animals received either no treatment or were handled daily by application of a placebo patch. Mean plasma nicotine levels in animals that received 1.75 or 3.5 mg/day during gestational days 2 through 19 were 70 or 241 µg/L, respectively. Corresponding plasma cotinine concentrations were 231 or 302 µg/L, respectively. None of the animals (n=2) exposed to 3.5 mg/day, 6/13 animals exposed to 1.75 mg/day, and 11/12 control animals exposed to a placebo patch over the full duration of gestation remained pregnant. When exposed during gestational days 2-7, 4/8 high-dose animals and 3/4 low-dose animals remained pregnant. The average litter size of animals that were exposed to nicotine and carried their pregnancy successfully to term was not significantly different in any of the groups compared with the control group. Neither was any significant difference observed in average pup weight per litter between animals exposed to nicotine and the corresponding controls. The offspring was not examined. Witschi et al. concluded that nicotine has a pre-implantation effect

and that continuous exposure to nicotine early during pregnancy may adversely affect pregnancy outcome in rats. No data on maternal toxicity were presented (Wit94). The committee concludes that the LOAEL for pregnancy failure was 1.75 mg/day.

Female Sprague-Dawley rats (n=20) received nicotine in drinking water, starting 6 weeks before mating and continuing throughout pregnancy. During the first 3 weeks of treatment, the nicotine concentration in the drinking water was gradually raised until the daily nicotine intake was 6 mg/kg bw/day. No data on meternal toxicity were presented. Small, but statistically significant decreases in the number of male rats born and in male birth weight were found in the exposed group compared with the controls. No changes were found in female offspring. After birth, the litters were cross-fostered to control dams and behavioural tests were conducted at post-natal days 25, 45, 60, and 85. Statistically significant decreases in locomotor rearing activity were found at post-natal days 60 and 85 in male but not in female offspring. No data on maternal toxicity were presented (Pet82).

In another study, female Sprague-Dawley rats (numbers not given) received nicotine via the drinking water at doses equivalent to 0, 2.4, or 4.5 mg/kg bw/day for 1 week before mating and continuing throughout pregnancy and lactation. After birth, litters from females treated with nicotine were cross-fostered with litters from control mothers to compare the effects of pre-natal and post-natal exposure to nicotine. Maternal mean plasma nicotine levels in low- and high-dose animals at the time of weaning were 1.0 and 18.5 μg/L, respectively. Maternal effects were decreases in water consumption and in body weights during lactation in high-dose animals. No significant effects were found in average birth weight of offspring. However, the litter size was significantly reduced at the high dose. Offspring exposure to the low and the high dose during either gestation or lactation caused no significant change in body weight gain in both males and females at post-natal day 10, but statistically significant reductions were found at post-natal days 20, 30, and 40. Post-natal exposure appeared to have a greater effect. The LOAEL was 2.4 mg/kg bw/day (Car85).

When pregnant Sprague-Dawley rats (n=8-9/group) were given nicotine at doses of 0 or 3 mg/kg bw/day by gavage from day 1 to 21 of gestation, maternal effects were reductions in average daily food intake and in body weight in the exposed animals compared with the controls. Body weight gain throughout pregnancy was similar in both groups. Mean litter size and fetal body weight at birth were lower in the exposed group, compared with the controls, but the differences were not statistically significant. No further data were presented (Lei91).

Nicotine was administered to female Swiss-Webster mice (n=19-26/group for controls, mid and high dose; low dose: not given) by addition to the drinking water at daily doses equivalent to approximately 0, 5.7, 17.2, or 28.6 mg/kg bw/day for at least 2 weeks before breeding and throughout gestation. The fetuses and placentas of all animals were examined on the 17th day of gestation. No data on maternal toxicity were presented. There was a dose-related decrease in fetal weight, which was statistically significant at the high and the mid doses. No significant changes were observed in average number of pups per pregnancy between any of the groups. The mean placental weight and the placental accumulation of the amino acid α-aminoisobutyric acid were significantly decreased in high-dose animals compared with controls. The NOAEL for embryotoxic effects was 5.7 mg/kg bw/day (Row82).

In a developmental toxicity study, pregnant ICR/SIM mice (n=26/group) were given nicotine at oral (gavage) doses of 0 or 35 mg/kg bw/day on days 8 through 12 of gestation. Maternal mortality was observed in 10/26 treated animals. Other maternal effects were body weight reduction and overt signs of toxicity. The number of live-born litters was not significantly different in the exposed animals compared with the controls, and no resorbed litters were found. The number of pups per litter, the number of live animals on post-natal day 3, the percentage of pup survival on post-natal days 1-3, and the pup weight on post-natal days 1 and 3 were higher than in the control group, but the difference was not statistically significant. The NOAEL for embryotoxic effects was 35 mg/kg bw/day (Sei86).

In another study, pregnant Swiss-Webster rats (n=10/group) were given daily subcutaneous injections with either vehicle (0.9% saline) or 0.5 mg nicotine/kg bw on days 10 to 20 of gestation, and offspring was subjected to developmental and behavioural test on post-natal days 1 to 22. Statistically significant effects in nicotine-exposed offspring compared with controls were reduced body weights, delayed eye opening and appearance of body hairs, and decreased sensory motor reflexes. However, motor activity was significantly stimulated in early adulthood of mouse pups (Aja98).

In a teratogenicity study, pregnant Sprague-Dawley rats (n=10/group) received nicotine, administered subcutaneously by a miniosmotic pump, at a daily dose of 3.6 mg from day 6 through 12 of gestation. Pair-fed and untreated control animals were given physiological saline in a similar manner. Evaluation of the fetal skeletal system on gestation day 20 revealed no statistically significant differences in the number of complete sternal ossification centres or in the ossification of the skull and facial bones in nicotine-exposed rats, when

compared with the untreated controls. However, when compared with the pair-fed control group, a statistically significant increased incidence of ossification in sternae and skull was observed. A lower incidence of wavy ribs was observed in the nicotine-exposed animals, but the difference with the controls was not statistically significant. No data on maternal toxicity were presented (Nas89). In the same laboratory, another developmental toxicity study was conducted, in which groups of pregnant Sprague-Dawley rats (n=10) received nicotine subcutaneously by a miniosmotic pump at daily doses of 1.8 or 3.6 mg from gestational days 6 through 12. Pair-fed control rats (n=10) received physiological saline in a similar manner. Fetuses were examined for developmental effects on day 12 of gestation. The embryos treated with the higher dose of nicotine were significantly different from control values for crown-rump and head length, and the development of the embryos was significantly delayed for 12 out of 17 developmental endpoints, e.g., heart, brain, otic and optic systems, hind limb, and somites. However, the maxillary and mandibular processes and the olfactory system were observed to have accelerated development. No hind limb development was observed. Embryos treated with the lower dose of nicotine showed significant differences from controls with respect to yolk sac diameter, crown-rump and head length, and development of the olfactory system (delayed). However, the optic and otic systems showed accelerated development. No data on maternal toxicity were presented (Dae91).

In a study with Rhesus monkeys (n= 3/group), pregnant animals were given 0 or 1 mg/kg bw/day of nicotine from days 26 to 134 of gestation by subcutaneous implantation with miniosmotic pumps. Fetal monkeys were obtained by Caesarean section. Nicotine treatment did not affect maternal weight gain or food intake compared with controls. Nicotine administration reduced fetal body weight by 8% compared with controls. Similar reductions were also seen in body length, biparietal, and weights of fetal heart, pancreas, adrenals, kidneys, and brain. Fetal lung weight and volume were reduced by 13% and 12%, respectively (not significant). The lungs of offspring had hypoplasia and a reduced surface complexity of developing alveoli. The findings demonstrate that nicotine can alter fetal monkey lung development by crossing the placenta to interact directly with nicotine receptors on non-neuronal cells in the developing lung (Sek99).

7 Existing guidelines

The current administrative occupational exposure limit (MAC) for nicotine in the Netherlands is 0.5 mg/m^3 (0.07 ppm), 8-hour TWA, with a skin notation.

Existing occupational exposure limits for nicotine in some European countries and in the USA are summarised in Annex II.

8 Assessment of health hazard

Workers can be occupationally exposed to nicotine through inhalation of dust or aerosols or by direct skin contact with tobacco leaves or a formulation of the compound. Nicotine is absorbed through the lungs, the skin, the gastrointestinal tract, and the buccal and nasal mucosa. In humans, the percentage of uptake of the compound through the lungs is 60 to 80%. The average dermal absorption of nicotine, measured in human volunteers who were treated with nicotine patches, was 18% over 24 hours. In the rat, the mean percentage of dermal absorption under semi-occluded conditions varied between 49 and 88%. The bioavailability of nicotine in humans following oral intake ranged from 24 to 59%. Following absorption, peak concentrations of nicotine in human plasma were found at 3-6 hours after dermal application and at 90 minutes after oral administration. Nicotine disappears rapidly from the blood, with a half-life of 2-3 hours. In the rat, at 1 hour after an intravenous injection, the highest concentrations of nicotine residues or metabolites were found in the kidneys and the lowest in blood. In humans, the majority of absorbed nicotine (about 80%) is biotransformed into cotinine, which has a much longer half-life in blood (20-30 hours) than nicotine. Cotinine and the remaining nicotine are further biotransformed into a range of products that are mainly excreted in the urine. The major metabolite identified in human urine is *trans*-3'-hydroxycotinine. The metabolic profile of nicotine is generally similar following inhalation or dermal exposure.

Case studies in humans show that nicotine induces both local and systemic skin reactions and skin sensitisation following the application of nicotine patches. Occupational dermatitis has also been reported in workers processing tobacco or employed in nicotine production. In the older literature, many cases of fatal acute nicotine poisoning have been reported. More recent cases of non-fatal acute toxicity have been reported in tobacco workers in the field who had direct skin contact with tobacco plants especially under wet circumstances or in tobacco-factory workers who inhaled nicotine-containing dust. This so-called 'green-tobacco sickness' produces mild symptoms of intoxication, such as nausea, vomiting, weakness, and dizziness. In case-control studies, there was no association between spraying nicotine and the incidence of multiple myeloma in farmers, neither was there convincing association between spraying nicotine and the incidence of leukaemia. The committee, however, is of the opinion that the number of subjects (both cases and controls) using nicotine was too small to

draw a reliable conclusion. In none of the occupational studies, there were reliable exposure data available. Experience on the long-term toxic effects of nicotine has also been obtained from users of smokeless tobacco. Among this group of tobacco users, there was an increased risk of dying from cardiovascular disease, but not from cancer. In cigarette smokers, a daily intake of 5 mg of nicotine has been suggested as a threshold level, below which no addiction occurs. However, no signs of addiction were reported in patients treated with oral doses of up to 25 mg nicotine/day for 6 weeks to 1 year. In humans, nicotine may contribute to adverse reproductive outcomes. Mechanisms include reduction of uteroplacental blood flow and direct effects on the developing fetal brain.

Based on the results of acute lethal toxicity studies in experimental animals, the committee concludes that the compound is toxic after dermal and oral exposure. No data from acute inhalation toxicity studies were found.

In a rat study, it was demonstrated that both acute and short-term parenteral administration of nicotine had an effect on the immune system. In a 2-year inhalation toxicity study, in which female rats were exposed to a nicotine concentration of 0.5 mg/m^3, the only concentration tested, 20 hours/day, 5 days/week, for 2 years, there were slightly decreased body weights (about 5%; no data on statistical analysis presented) but no increases in mortality, atherosclerosis, or in the incidences of neoplastic or non-neoplastic lesions were found. The committee does not regard the slightly lower body weights as the expression of an adverse effect of exposure to nicotine. Therefore, the committee considers 0.5 mg/m^3 a NOAEL, implying that this long-term inhalation study does not provide information on the lowest exposure level at which adverse effects are becoming manifest. Therefore, this study is inappropriate for deriving a health-based occupational exposure limit.

In a drinking water study, focussing on potential behavioural effects, slightly decreased body weights, increased locomotor activity, and attenuated exploratory efficiency were observed in female rats (males not tested), being 5-month old at the start of the experiment, following administration of daily doses of 20 and 50 mg/L, which could be equivalent to 1.5 and 3.8 mg/kg bw/day (for 131 days). In another drinking water study, only investigating myocardial biochemical parameters, changes in the activities of certain myocardial enzymes were seen in male rats (females not tested) at doses of 4.56 mg/kg bw/day (for 34 weeks), but not at 1.14 mg/kg bw/day. Because of the limited scope, the committee considers these studies inappropriate for deriving a health-based occupational exposure limit.

Nicotine did not induce gene mutations in *in vitro* tests, but positive results were obtained in *in vitro* and *in vivo* cytogenicity tests. According to the committee, nicotine has clear clastogenic activity.

The committee did not find data from standard reproduction toxicity tests. In most of the studies available, no data on maternal toxicity were presented and no NOAELs were found. Nicotine has been shown to affect fertility and embryo and fetal development by multiple routes of exposure in several species of animals. In male mice, intraperitoneal injection of daily doses of 2, 4, or 6 mg/kg bw for 15 days caused decreases in the weight of reproductive organ weights and histopathological changes in these organs. When male mice were given oral (drinking water) doses of 2.7 mg/kg bw/day for 7 weeks and mated weekly with untreated females for 4 weeks, there was no effect on number of litters or offspring; when treated with 2.3 mg/kg bw/day for 20 weeks and mated for 6 weeks, there was an increase in limb abnormalities in fetuses resulting from mating in week 1 and 2.

In rats, nicotine caused pregnancy failure after dermal application of 1.75 mg/day throughout gestation. Drinking water doses of 2.4 mg/kg bw/day given 1 week before mating and during gestation and lactation did not affect litter size or pup weight, but induced statistically significantly reduced body weights from post-natal day 20 onwards. This dose did not affect the body weights of the pregnant and lactating animals. Following continuous subcutaneous administration by miniosmotic pump of doses of nicotine of 1.8 mg/kg bw/day during organogenesis, developmental end points, such as yolk sac diameter, head and crown length (all decreased), olfactory system (delayed), otic and optic system (both accelerated), were affected. A NOAEL was not established in any of the rat studies.

In mice, no embryotoxicity was observed at clearly maternally toxic oral (drinking water) doses of 35 mg/kg bw/day administered on gestational days 8 through 12. Given 2 weeks before breeding and during gestation, decreased fetal weights were observed at oral (drinking water) doses of 17.2 mg/kg bw/day but not at 5.7 mg/kg bw/day.

In monkeys, continuous subcutaneous administration by osmotic minipump of doses of 1 mg/kg bw/day on gestational days 26 through 134 did not affect maternal body weight gain or food intake, but caused decreased fetal body and organ weights and altered fetal lung development.

Despite a rather rich toxicological database, the committee considers the toxicological database on nicotine too poor to justify recommendation of a health-based occupational exposure limit.

The committee concludes that there is insufficient information to comment on the level of the present MAC-value.

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Annex I

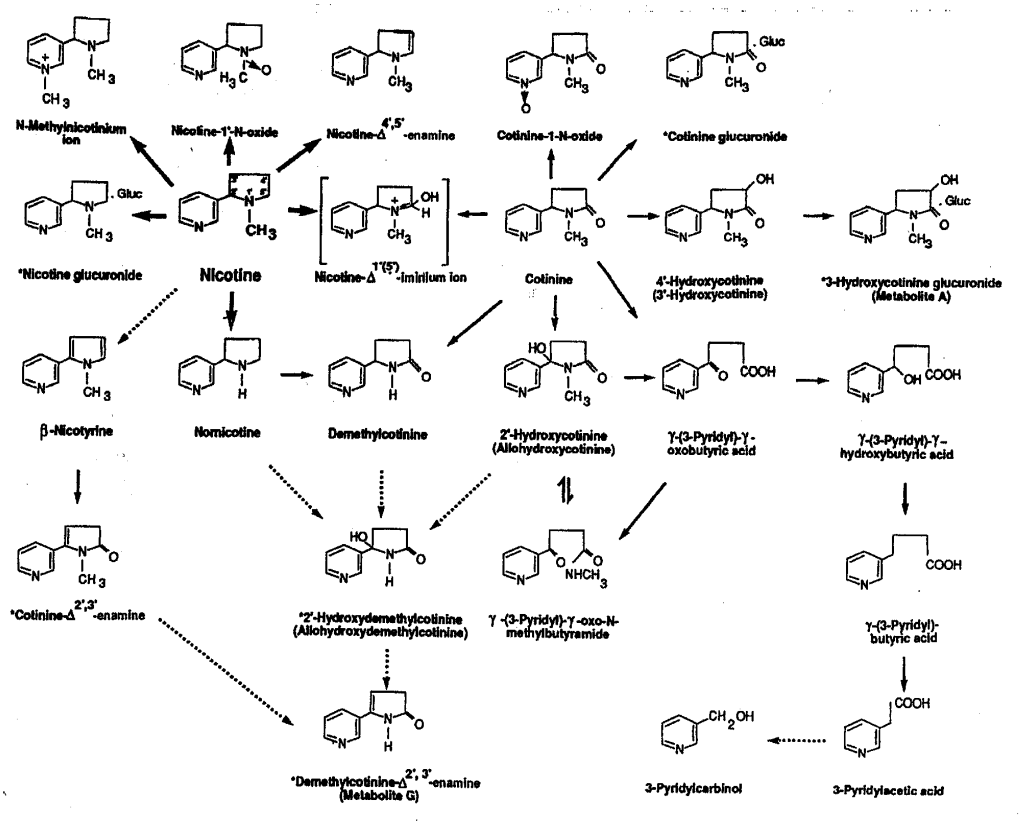

Figure 1 Proposed metabolism for nicotine (from Kye91)

Annex II

Occupational exposure limits for nicotine in various countries.

country - organisation	occupational exposure limit		time-weighted average	type of exposure limit	note[a]	reference[b]
	ppm	mg/m^3				
the Netherlands						
- Ministry of Social Affairs and Employment	0.07	0.5	8 h	administrative	S	SZW03
Germany						
- AGS	0.07	0.5			S	TRG00
	0.28	2.0				
- DFG MAK-Kommission	-	-[c]			S	DFG03
Great Britain						
- HSE	-	0.5	8 h	OES	S	HSE02
		1.5	15 min	STEL		
Sweden	-	-				Swe00
Denmark	-	0.5	8 h		S	Arb02
USA						
- ACGIH	-	0.5	8 h	TLV	S	ACG03b
- OSHA	-	0.5	8 h	PEL	S	ACG03a
- NIOSH	-	0.5	10h	REL	S	ACG03b
European Union						
- SCOEL	-	0.5	8 h	ILV[d]		EC04

[a] S = skin notation, which means that skin absorption may contribute considerably to body burden; sens = substance can cause sensitisation.

[b] Reference to the most recent official publication of occupational exposure limits.

[c] Listed among compounds for which studies of the effects in man or experimental animals have yielded insufficient information for the establishment of MAK values.

[d] Listed among compounds for which OELs are already included in Commission Directives.