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The contribution of cocoa additive to cigarette smoking addiction

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Abstract

In this report the effect of these compounds on the addiction to cigarette smoking was assessed, using currently available information in the literature on psychoactive compounds of cocoa. The investigated psychoactive cocoa compounds were theobromine, caffeine, serotonin, histamine, tryptophan, tryptamine, tyramine, phenylethylamine, octopamine and anandamide. The general conclusion is that the level of these compounds in added cocoa in cigarettes is not sufficient to increase the addiction to cigarette smoking.

Contents

Samenvatting

In dit rapport wordt de mogelijke bijdrage van cacao aan rookverslaving beschreven. Cacao wordt aan tabak toegevoegd om de smaak te verbeteren. Daarnaast bevat cacao tal van psychoactieve stoffen die mogelijk bijdragen aan rookverslaving

Dit literatuuronderzoek beschrijft de blootstelling, farmacologie, farmacokinetiek, toxicologie, interacties en verslavende eigenschappen van de tien meest bekende stoffen in cacao. De onderzochte stoffen zijn theobromine, caffeïne, serotonine, histamine, tryptofaan, tryptamine, tyramine, fenylethylamine, octopamine en anandamide. Deze stoffen komen ook via dranken en voedsel het lichaam binnen of worden door het lichaam zelf aangemaakt. Dit rapport laat zien dat de aan roken gerelateerde blootstelling aan de psychoactieve stoffen uit cacao gering is ten opzichte van de inname via voeding en dranken en/of de lichaamseigen productie van deze stoffen. Een systemisch effect lijkt derhalve onwaarschijnlijk ook al omdat lichaamseigen stoffen snel worden afgebroken.

Daarnaast kunnen deze stoffen, omdat ze geïnhaleerd worden, een direct effect op de luchtwegen hebben. Daarmee zou de opname van nicotine beïnvloed kunnen worden. De nicotineopname zou bijvoorbeeld kunnen toenemen via luchtwegverwijding door theobromine en caffeïne, of kunnen afnemen door luchtwegvernauwing door histamine. Dit rapport laat zien dat de aan roken gerelateerde blootstelling aan deze stoffen waarschijnlijk te gering is voor een direct effect op de luchtwegen.

Verder dient te worden opgemerkt dat de hoeveelheid tryptamine, tyramine en fenylethylamine die via cacao wordt toegevoegd verwaarloosbaar is ten opzichte van de hoeveelheid die in tabak zelf aanwezig is. Tot slot is aandacht besteed aan de verbrandingsproducten van cacao. Amineverbindingen als serotonin, tryptofaan, tyramine, tryptamine en fenylethylamine vormen tijdens het roken stoffen die het enzym mono amine oxidase (MAO) remmen. MAO-remmers hebben een anti-depressieve werking en kunnen op die manier bijdragen aan rookverslaving.

De conclusie van dit literatuuronderzoek is dat de afzonderlijke psychoactieve stoffen in tabak als gevolg van toevoeging van cacao niet direct bijdragen aan rookverslaving. De verbrandingsproducten van cacao doen dit, via remming van het enzym mono amine oxidase, mogelijk wel. Ook de smaak van cacao wordt geassocieerd met verslaving. De literatuur biedt geen inzicht in het effect op gezondheid en verslaving van het inhaleren van de combinatie van de 10 onderzochte stoffen uit cacao.

Summary

This report discusses the cocoa additive in relation to cigarette smoking addiction. Cocoa is added to cigarettes for flavour enhancement. Cocoa contains also various psychoactive compounds that can affect the addiction to cigarette smoking. This literature survey describes the exposure, pharmacology, pharmacokinetics, toxicology, interactions and dependency of ten best-known psychoactive compounds in cocoa. The ten psychoactive cocoa compounds were theobromine, caffeine, serotonin, histamine, tryptophan, tryptamine, tyramine, phenylethylamine, octopamine and anandamide. The body is exposed to these compounds via food and drinks or is synthesized by the body itself. This report showed that the exposure to the psychoactive compounds originated from cocoa via cigarette smoking is negligible compared with intake via food and drinks or compared with the endogenous production of those compounds. A systemic effect of the psychoactive compounds via cigarette smoking seems unlikely, also because some compounds (the biogenic amines) are degraded rapidly.

The fact that the exposure to these compounds via inhalation implies that they could have local effect on the respiratory system. The local effects might influence the level of nicotine absorption. For example, the level of nicotine absorption may increase through bronchodilatation by theobromine and caffeine or may decrease through bronchoconstriction by histamine. However, this report indicates that the level of the psychoactive compounds of cocoa in cigarettes is probably too low to exert any local bronchoactive effects.

Furthermore, the quantities of tyramine, tryptamine and phenylethylamine in cigarettes originating from cocoa is negligible compared with the quantities originating from tobacco itself.

The combustion products of the compounds are also discussed. The combustion products of the amine psychoactive compounds, such as serotonin, tryptophan, tryptamine, tyramine and phenylethylamine, inhibit the enzyme mono amine oxidase (MAO). These MAO-inhibitors have anti-depressive properties and may thus increase the addiction to cigarette smoking.

This report concludes that the individual level of the psychoactive compounds in cigarettes originating from cocoa does not increase the addiction to cigarette smoking by itself. The combustion products of the compounds may increase the addiction to cigarette smoking via MAO-inhibition. Furthermore, the flavour of cocoa may act as a conditioned stimulus and the organoleptic properties of cocoa may be associated with dependency. There is no information available in the literature about the effects on health and addiction of the inhalation of the combination of the ten investigated compounds.

1.   Introduction

Cigarette smoking is an easy way to administer multiple doses of the psychoactive drug nicotine. However, it leads to nicotine addiction and it is the most important cause of preventable death (1). Hence, prevention and quitting smoking are major public health goals. It has been suggested that cigarette smoking is more addictive than nicotine alone due to the fact that tobacco or smoke seems to contain compounds which increase the addictive potency of nicotine (e.g. ammonium compounds) (2) or may be addictive in their own right (e.g. cocoa) (3, 4).

Craving for chocolate, which contains cocoa, is a well-known phenomenon and to emphasize its addictive properties the term "chocoholics" is used for individuals who report overeating chocolate. However, whether cocoa has addictive properties, remains debatable. There has been speculation that chocolate craving is related to organoleptic properties of chocolate and probably to rewarding effects of psychoactive compounds in chocolate. The organoleptic properties of chocolate improve the mood, leading to an increase in pleasant feeling and a reduction of tension. Chocolate is generally rated as highly palatable, which is attributed by high levels of carbohydrate and fat. The sensory characteristics and the palatability of chocolate attribute the organoleptic properties. However, there are other foods, which have similar palatable properties as chocolate, but are less craving (4). Michener and Rozin (1994) (5) suggested that sensory experience at one hand and palatability at the other hand satisfy chocolate cravings. There is no convincing evidence that eating chocolate leads to physical dependence to one or more of the psychoactive compounds it contains. The recent discovery of endocannabinoids in cocoa (6) suggested that psychoactive compounds in chocolate might attribute to chocolate craving. However, it seems that the level of the psychoactive compounds in chocolate is too small to elicit chocolate dependency (7).

Cocoa is used at a level between 1 % (w/w) and 3 % (w/w) in the casing of tobacco products as a flavour enhancer (8, 9). The suggestion that chocolate may have addictive properties was extrapolated to the addictive properties of cocoa as an additive in tobacco products (9, 10). It is speculated (9, 10) that cocoa added to tobacco increases the addictive properties of cigarettes by the action of psychoactive compounds in cocoa. Although there is no indication that eating chocolate leads to dependency on the psychoactive compounds, some distinction has to be made to the addictive qualities between oral exposure to cocoa by eating chocolate and pulmonary exposure to cocoa by smoking cigarettes. Firstly, the different exposure route of cocoa may have different pharmacological effects on the body. The psychoactive compounds may exert a local pulmonary effect, thereby affecting the nicotine availability. In this case, it is argued (9, 10) that cocoa compounds, such as theobromine, may have bronchodilating effects, thereby increasing the level of nicotine absorption. Furthermore, by exposing through the pulmonary system, the rapid degradation of the psychoactive compounds by the liver is avoided. Secondly, the psychoactive compounds are combusted during smoking and reaction products of these compounds with other compounds are formed. These reaction products may affect the addictive properties of cigarettes.

So far, the effect of cocoa on the addictive properties of tobacco products has not been investigated. In this study ten psychoactive compounds of cocoa are reviewed: theobromine, caffeine, serotonin, histamine, phenylethylamine, tryptamine, tyramine, tryptophan, octopamine and anandamide. These compounds are reviewed by their chemical, environmental and smoking exposure, pharmacological, pharmacokinetic, toxicological, interaction and dependency properties. These properties are discussed in relation to the pulmonary exposure by smoking cigarettes.

The purpose of this study is to evaluate whether the psychoactive compounds of cocoa or their combustion products increase the addictive properties of cigarettes. The data on compounds used for this report were drawn from currently available literature.

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2.   Method

Publications on cocoa and its psychoactive compounds were identified through Medline, Toxline and Current Contents and from electronic citations in the Merck Index, DOSE (1), RTECS (2), HSDB (3) , BIG (4), Martindale, SAX Dangerous Properties of Industrial Materials and Comprehensive Toxicology. Further information not obtained from the above mentioned search engines was derived from the references cited in these publications and from publications on Internet.

References

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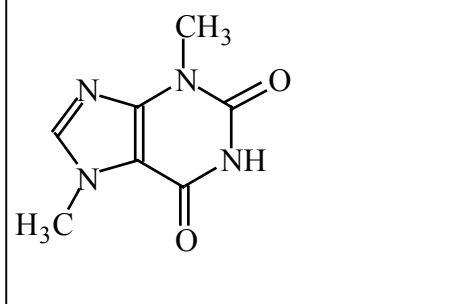
3.   Results

3.1   Theobromine

GENERAL
IUPAC systematic name: 3,7-dihydro-3,7-dimethyl-1H-purine-2,6-dione (1, 2)
Synonyms: 3,7-dimethylxanthine, diurobromine, santheose, SC 15090, theosalvose, theostene, thesal, thesodate (1, 2)
Molecular formula: $C_7H_8N_4O_2$ (3)

Molecular structure

Molecular weight: 180.17 (2-4), 180.19 (1)
Alifatic: no
Aromatic: yes
N containing:yes
Halogen containing: no
CAS registry no.: 83-67-0 (3)
Storage:
R/S classification: no data available.
dangercode (transport): no data available.
Properties:
➢ melting point: 357 °C (3, 4)
➢ boiling point: 290 –295 °C (sublimes) (2, 3)
➢ density: no data available.
➢ refractive index: no data available.
➢ solubility: H_2O, ethanol (3, 4), ether (3), moderately in ammonia, slightly soluble in chloroform (4), almost insoluble in benzene, (diethyl)ether, carbon tetrachloride (1, 2, 4)
➢ substance description:
    •   white (1)
    •   powder or monoclinic needles (1, 2)
    •   bitter taste (1)
➢ volatility: no data available.
➢ pK_a: 7.89 (18 °C) (3)
        $K_b = 1.3 \times 10^{-14}$ (18 °C) (2, 4)
        $K_a = 0.9 \times 10^{-10}$ (2, 4) $^{\triangledown}$
        $^{\triangledown}$ NB: The pK_a-value of ref (3) is not in accordance with the K_a -value given by refs. (2, 4). Refs (2, 4) mention both a K_a- **and** a K_b-value, indicating that theobromine can act both as a proton acceptor (a base) as well as a proton donor (an acid). Ref (3) only indicates a pK_a-value: most probably this value reflects a netto result in water of both the acid and base properties.
➢ PA: no data available.

➢ flammability:
  • FP = no data available.
  • FL Limits = no data available.
  • IT = no data available.
➢ decomposition temperature: no data available.
➢ stability: no data available
➢ vapour pressure/ vapour tension (20 °C): no data available.
➢ vapour pressure (50 °C): no data available.
➢ relative density: no data available.
➢ octanol water partition coefficient, log P, log K_{OW}: log P = -0.8 (4).
➢ conversion factor: not applicable.

Critical assessment
Theobromine is a heterocyclic natural product, occurring in the cacao bean, and it is classified as an alkaloid. It is a white, bitter tasting, crystalline powder, which readily sublimates upon heating (direct change from solid into gas). In its structure it is closely related to caffeine. Its solubility properties indicate a polar compound.
The presence of four nitrogen atoms distributed over two aromatic heterocyclic rings forms a characteristic feature for purine-derived compounds. In theobromine two of the nitrogen atoms are methylated, while the remaining two nitrogen atoms have a quite different character. One of the remaining nitrogen atoms has a pyridine-like configuration, i.e. it contains a free, unshared pair of electrons, which is known as a strong feature for interaction possibilities, e.g. the ability to bind a proton, causing the compound to have basic properties. Complexation interactions are likely to occur as well (5). The other nitrogen atom is bound to a hydrogen atom that can be released as a proton, so causing the compound to have acidic properties. Both basic and acidic properties are weak, resulting in almost neutral solutions when dissolved in water. Compounds formed upon reaction with bases are more stable than salts obtained with acids (decompose in water). More general: it has the ability to form complexes with several compounds.
Except for the purine-like nitrogen, the unshared pairs of electrons of all other nitrogens participate in the formation of the π-clouds, so adding to the aromatic, stable character of the purine ring system.
Little is known about combustion products. Preliminary pyrolysis data indicate as products: methane, ethene, ethane, propene, propane, trimethylamine.

Conclusion
Theobromine is a natural product, nitrogen containing, water soluble, with an amfoteric and complexating character.

FUNCTION IN TOBACCO
No data available.

AMOUNT IN TOBACCO PRODUCTS
Typical concentration of cocoa powder for cigarettes is 1 % (6). Assuming 1.9 % (w/w) theobromine concentration in cocoa powder (7), a cigarette weighing 1 g, contains ± 0.19 mg theobromine.

AMOUNT IN SMOKE
- **main stream**
  No data available.
- **side stream**
  No data available.

SOURCE
A source of theobromine in cigarettes is cocoa powder, which occurs in the casing of cigarette (6).

ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE
Theobromine is the principal alkaloid of the cacao bean which contains 1.5 – 3 % of the base. Cacao husks contain 0.7 – 1.2 %. It is also present in cola nuts and in tea. (2, 4, 8) Levels have been reported to be 20 mg/kg in green coffee beans, 0.15 – 0.20 % in manufactured tea and 0.3 % in dried mate (4). Theobroma oil may contain up to 2% theobromine (8).
Theobromine is a component of the cocoa solids, or nonlipid portion of chocolate liquor (4).
Cacao is the major natural source of theobromine; the concentration in whole cacao beans and nibs (cotyledon) increases during the first day of fermentation and that in the shells increases subsequently. Hot chocolate beverages have average levels of of 65 mg/180 mL serving; chocolate milk samples prepared from instant, cold, sweetened cocoa powders have an average level of 58 mg theobromine per serving, and hot cocoa prepared from nine commercial instant mixes had an average of 62 mg theobromine per serving. Dark chocolate contains the largest amount of theobromine per serving of any type of eating chocolate; concentrations vary widely, but 1 bar of approximately 30 g dark chocolate contained 130 mg theobromine, and 1 bar of approximately 30 g milk chocolate contained 44 mg theobromine. In the USA in 1980, the daily per-caput intake of theobromine from food and beverages was estimated to be 39.05 mg; daily per-caput consumption of theobromine from cocoa was calculated to be 38.3 mg on the basis of the 276 million kg of cocoa imported. The daily per caput intake is 16.7 % of the total intake of methylxanthines (4). Theobromine is also one of the primary metabolites of caffeine (9). In rats the mean fraction of caffeine converted to theobromine was 16 % (10).

COMBUSTION PRODUCTS
When heated to decomposition it emits toxic fumes of NO_x (1).

CONSENSUS REPORTS
Reported in EPA TSCA Inventory. EPA Genetic Toxicology Program (1).
There is inadequate evidence for the carcinogenicity in humans of theobromine.
There are no data on the carcinogenicity of theobromine in experimental animals (4).

STANDARDS AND RECOMMENDATIONS
ADI: no data available.
TWA$_{NL}$ = MAC: no data available.
TWA$_D$ =MAK: no data available.
TWA$_{USA}$: no data available.
STEL$_{NL}$: no data available.

STEL$_{USA}$: no data available.
LTEL: no data available.
TLV-C: no data available.
TLV-CARCINOGENICITY: no data available.
MAK-REPRODUCTION: no data available.

Others:
The levels of theobromine in the plasma of humans might be quite high following the combined exposure of man to theobromine directly in cocoa diets and indirectly through biotransformation of ingested caffeine in vivo to form theobromine (9).

Reference value:
Six nursing mothers ingested 113 g of Hershey's milk chocolate containing 240 mg of theobromine. Samples of plasma, saliva, and breast milk were assayed for theobromine. Peak theobromine concentrations of 3.7 to 8.2 µg/ml were found in all fluids at 2 to 3 hour after ingestion of chocolate (11).
Theobromine disposition was measured twice in 12 normal men, once after 14 days of abstention from all methylxanthines and once after 1 week of theobromine (6 mg/kg/day) in the form of dark chocolate. The serum theobromine ranged from 5 – 15 µg/ml (12).

CLASS
EG Carc. Cat.: No data available.
IARC-category: 3 (4)
CEC: No data available.

Critical assessment
Comparison of smoking related daily consumption with daily consumption of theobromine (mg) from other sources:

	SMOKING	DRINKING OR EATING			
	25 cig. (1 % cocoa)	3 tea drinks	3 chocolate drinks	3 chocolate bars of 60 g	3 cocoa drinks
THEOBROMINE	4.75[6]	138[13]	195[13]	864[12] 810[14] 360[15, 16]	750[4] 765[9]

Little is known about pyrolysis/combustion products.

Conclusion
The daily intake of theobromine from cigarettes is marginal compared with the intake of theobromine from other sources, like teas, chocolate drinks and sweets. So, the plasma concentration reached after ingestion of theobromine from tea or chocolate sources is expected to be significantly higher, than after intake from cigarettes. However, the different route of application via smoking as compared to other sources should be taken into account. Therefore, local effects of theobromine on the respiratory system might be a point of concern.

PHARMACODYNAMICS

Mechanism of action

The methylxanthines affect many physiological systems of the body through the mediation of the central nervous system. The probable biochemical basis being the ability of methylxanthines to inhibit phosphodiesterase breakdown of cAMP leading to the accumulation of the latter. Theobromine produces central stimulation because of its effect on the brain cortex. Theobromine has stimulatory effects on the brain, heart, gastric secretion and urine flow (9).

The action of theobromine on the smooth muscle may depend on the balance between effects of cAMP and cGMP accumulation rather than cAMP alone. Two adenosine receptor sites (A_1 and A_2) are affected by methylxanthines and therefore these components antagonized the effect of adenosine. Adenosine acts like an inhibitor to neurotransmitter release and this could explain the mechanism of the methylxanthines on the CNS. Theobromine, was tested in mice, to determine whether it could function in vivo as an adenosine receptor antagonist, in keeping with its reported in vitro effects as a blocker of agonist binding to the adenosine A-1 receptor. Theobromine doses, which themselves had no direct effects on spontaneous locomotor activity, completely blocked N6-cyclohexyladenosine (CHA) induced suppression of locomotor activity but were without effect on ethylcarboxyamido adenosine (NECA) induced decreases in motor activity. In contrast to the specific antagonism, theobromine blocked the hypothermia induced by both of these adenosine analogs. These results demonstrate that theobromine is an active in vivo adenosine receptor antagonist and that the antagonism of CHA-sensitive systems occurs even though theobromine does not stimulate spontaneous locomotor activity. Thus, the behavioral stimulant effects of methylxanthines may be more related to effects on NECA-sensitive systems, which are not blocked by theobromine (17).

Theobromine is also an inhibitor of cholinesterase.

Theobromine protected sensitized guinea pig against anaphylactic shock induced by aerosolized antigen by inhibition of the release of a slow reacting substance (SRS) of anaphylaxis and some reduction in histamine release. The methylxanthines have an active vasodilator action on the coronary vessels and on the vessels of the lungs and the legs. The protrombin time and plasma coagulation time in humans were considerably shortened by theobromine. Theobromine also inhibited and reversed platelet aggregation induced by ADP in vitro. The hepatic drug metabolizing microsomal enzymes were stimulated in the rat. Theobromine is less effective than other methylxanthines like caffeine and theophylline on different organs (18).

Pulmonary system

- **breathing frequency**: 1-Substituted theobromine is a respiratory stimulant in mice and stimulates respiration of the isolated diaphragm of the rat (18).
- **Tidal volume**: No data available.
- **Lung compliance**: No data available.
- **Airway resistance**: Theobromine has a vasodilation effect in the lungs (18) and a bronchodilatory effect (19). The airway resistance by inhalation of theophylline aerosol, a theobromine derivate, was investigated. A dose of 15 mg theophylline aerosol showed significant decrease of the airway resistance after 60 min. of administration. The airway resistance decrease was not significant immediately or after 30 min of theophylline administration (20). Theobromine is significantly less active as a bronchodilator than theophylline. (7, 18)

Cardiovascular system
- **Blood pressure**: Due to peripheral vasodilation the blood pressure could become slightly decreased (18).
- **Heart rate**: Theobromine, at a dose of 500 mg, increased pulse rate slightly, but not significantly more than placebo (18). Theobromine is a cardiotonic (2).

Renal system
- **Diuresis**: An increased diuresis is observed with theobromine (2, 9, 18)
- **Saluresis**: The excretion of uric acid was not increased by theobromine (18).

Nervous system
- **Central nervous system**: Theobromine has the general properties of the other xanthines. However, it has a much weaker activity than theophylline or caffeine on the CNS. Large doses can cause nausea and vomiting (8).
- **Autonomic system**: Theobromine (50 mg/kg dose) increased catecholamine concentration in the rat myocardium 1 hr after intraperitonial injection (18).

Other
The gastric juice acidity and volume increased following intravenously or orally administered xanthine vasodilators (18).

Critical assessment
Theobromine has various effects in the body, but is effects are weaker compared with other methylxanthines. It has a relaxation effect on the smooth muscles, thereby exerting a weak bronchodilating and a vasodilating effect. It increases the heart rate at high doses. It has also a stimulating effect on the CNS. Theobromine is significantly less active as a bronchodilator than theophylline. As compared to the bronchodilatory effects of a theophylline dosis of 15 mg applied as an aerosol in humans (20), it is questionable whether the theobromine dose of 0.19 mg per cigarette is high enough to have a bronchodilatory effect.

Conclusion
Although theobromine exerts a broad active spectrum in the body, at least 100 fold higher doses than obtained from the daily consumption of cigarettes are needed to be clinical active. Whether theobromine has bronchodilatory effects is questionable, due to the low pharmacological effects compared with other methylxanthines. As other methylxanthines (caffeine) also occur in cigarettes, the combined effects of these methylxanthines on the pulmonary system are not known.

PHARMACOKINETICS
Absorption
In humans, theobromine is readily absorbed from food (4).

Bioavailability
No data available.

Distribution
Human

After oral intake, theobromine is evenly distributed in body fluids and has been reported to pass into the breast milk of nursing mothers. The apparent volumes of distribution and clearance were estimated to be 0.76 l/kg bw and 0.88 ml/min/kg body weight, respectively (4). The plasma clearance of theobromine is known to be enhanced in cigarette smokers (21).
Theobromine has a low protein binding capacity in both serum (15 – 21 %) and breast milk (12 %) (4).
Animal
Transport across the placental membrane into the fetus has been identified for theobromine in rats (9). Furthermore, the disposition of theobromine in the fetal rat brain is reported at single doses of 5 or 25 mg/kg caffeine. Unlike the adult, the fetal rat brain accumulates theobromine when exposed to caffeine doses comparable to those attainable by normal human consumption (22).
Theobromine was identified in the brain of mice after chronic ingestion of caffeine (23).

Metabolism
The major metabolite of theobromine in human urine is 7-methylxanthine (34 – 48 %), followed by 3-methylxanthine (20 %) and 7-methyluric acid (7 – 12 %), 6-amino-5-[N-methylformylamino]-1-methyluracil (6 – 9 %) and 3,7-dimethuluric acid (1 %) (4). Cytochrome P450 monoxygenase is an enzyme involved in the metabolism of theobromine (9).
A week of daily theobromine consumption in the form of dark chocolate did not alter the elimination kinetics or metabolic pattern of theobromine (12).

Excretion
Of the dose in humans, 1 – 18 % is recovered in the urine as unchanged theobromine (4).

Kinetic parameters
The half-times in plasma and saliva are highly correlated. The mean half-time of theobromine in human serum ranged from 6.1 to 10 h (4).
In man the disposition of theobromine follows first order kinetics (9).

Critical assessment
Orally, theobromine is readily absorbed and widely distributed in tissues, including brain. Transplacental transport in rats and human was reported and theobromine was identified in fetal rat brain. There are no data on pharmacokinetics in animals and humans from respiratory studies.

Conclusion
Conclusions on potential differences in kinetics between respiratory and oral administration can neither be drawn based on the pharmacogical and toxicological data.

TOXICOLOGY
Acute toxicity
Human
Oral human dose of 26 mg/kg bw (TDLo, LOAEL) showed central nervous system

and gastrointestinal tract effects (1).
It has been stated that 'in large doses' theobromine may cause nausea and anorexia. In a study of 13 volunteers who consumed 200 mg theobromine orally three times during a 24 h period, no clinical symptom or other pharmacological activity was observed. Ingestion of theobromine in sweet chocolate at a dose of 6 mg/kg bw per day had no effect on clinical parameters in 12 human subjects (4).

Animal
Oral: LD_{50} rat = 950 mg/kg bw (for the sodium acetate) (4)
LD_{50} rat = 1265 mg/kg (1)
LD_{50} mice = 1356 mg/kg bw (for the sodium acetate) (4)
LD_{50} mice = 837 mg/kg (1)
LD_{50} dog = 300 mg/kg bw. (1, 4)

Local tolerance
Human
No data available.

Animal
High doses – 250 – 300 mg/kg bw (mature animals) and 500 mg/kg bw (immature animals) – have been shown to cause complete thymic athrophy in male and female rats. This effect was seen in hamsters only at a level of 850 mg/kg bw and in mice at levels of 1840 – 1880 mg/kg bw (4).

Repeated dose toxicity
Subacute
In a study where male dogs were fed 100 – 150 mg theobromine per kg bw for 21 – 28 days, a degenerative and fibriotic lesion in the right atrial appendage of the heart was reported. (4)

Semichronic
Theobromine fed to male and female Sprague-Dawley rats at levels of 0, 0.02, 0.1 and 0.2 % of a chow diet for 90 days (corresponding to 25, 125 and 250 mg/kg bw/day), revealed only a reduction in body weight gain and testicular weight in males at the high dose. There were no pathological lesions and no haematological changes observed (4).

Chronic
Daily intake by humans of 50 – 100 g cocoa (0.8 – 1.5 g theobromine) has been associated with sweating, trembling and severe headache (4).

Carcinogenicity
Human
There is inadequate evidence for the carcinogenicity of theobromine in humans (4). It has been suggested that older men (>67) consuming 11 to 20 and over 20 mg of theobromine per day are at increased risk of prostate cancer (odds ratio (OR) for all tumors = 2.06 and 1.47, respectively; OR for aggressive tumors (defined as undifferentiated localized tumors and well-differentiated to undifferentiated regional or distant tumors) = 1.90 and 1.74, respectively) (24). It should be noted that these data are based on a small number of cases (<50) from a population (Mormons) which

is not representative for the common population.

Animal
No data on the carcinogenicity of theobromine were available (4).

Reproduction toxicology
Human
No data were available to evaluate the carcinogenicity of theobromine *per se* (4).

Animal
Oral administration of high doses (90 – 600 mg/kg bw per day) theobromine to rats for 28 days or 64 weeks caused severe testicular atrophy, which was largely irreversible. Administration of lower levels for prolonged periods had no significant adverse effect on the testis. In mice, (doses 300 – 1850 mg/kg bw per day) testicular changes were seen only at concentrations that caused considerable mortality (4). No adverse reproductive effect was observed in a three generation study in rats given cocoa powder containing 2.50 – 2.58 % theobromine in their diet at concentrations of 0, 1.5, 3.5 and 5.0 % (4).
Theobromine is used as an experimental teratogen. Intraperitoneal-Mouse TDLo (LOAEL): 500 mg/kg (female 13d post): teratogenic effects (1).
Teratogenic effects (decreased fetal body weight at doses of 125 or 200 mg/kg bw, and increased skeletal variations at 75 mg/kg and over) were observed in rabbits after gavage but not after dietary administration of theobromine. No teratogenic effect was seen in rats (4).
Sertoli cells are the target cells of theobromine toxicity on rat testes and reproductive toxicity (25). Theobromine caused vacuolation within the Sertoli cell, abnormally shaped spermatids, and failed release of late spermatids in treated rats. The ability of theobromine to alter testis structure after oral exposure has been demonstrated (26).

Mutagenicity
Human
According to the IARC concensus report of 1991 no data were available (4).
According to the SAX Dangerous properties and environmental fate Handbook of 1999 human mutation data are reported (1).

Animal
Mutation in Microorganisms-Euglena gracilis 600 mg/L (1).
Sister Chromatid Exchange-Human:lymphocyte 100 mg/L (1).
In vivo, theobromine did not induce dominant lethal effects in mice or rats. It induced sister chromatid exchange and micronuclei but not chromosomal aberrations in the bone marrow of Chinese hamsters. In human cells *in vitro*, theobromine induced sister chromatid exchange and chromosomal breaks. In cultured mammalian cells, it induced gene mutations and sister chromatid exchange but not chromosomal aberrations or cell transformation. In plants, theobromine did not induce chromosomal aberrations. It induced gene mutations in lower eukaryotes and bacteria but gave negative results in the *Salmonella*/mammalian microsome assay (4).

Other

Critical assessment
The acute toxicity of theobromine is low. In humans clinical signs such as sweating, trembling and severe headache are observed at high daily doses. After semichronic treatment of rats with high doses of theobromine a reduction in body weight and testicular atrophy is observed. Theobromine may have mutagenic properties. There is no evidence that theobromine is carcinogenic. No data on the effects of theobromine administered through inhalation are available.

Conclusion
Toxic effects of theobromine appear to be found at high doses. It is unlikely that exposure to theobromine through smoking leads to systemic theobromine levels that exert toxicologically relevant effects. Since no data on the toxicological effects of theobromine exposure through inhalation are available, the influence of exposure to theobromine through smoking on the respiratory system cannot be established.

INTERACTIONS
Chemical
Forms salts which are decomposed by water, and compounds with bases which are more stable. (2, 4) Theobromine formed 1:1 complexes with the local anesthetic lidocaine (lignocaine) (27).

In vivo
Theobromine plasma clearance (Cl-TB) was increased in smokers after pretreatment with cimetidine (1 g/day) and sulfinpyrazone (800 mg/day) due to induction of all metabolic pathways (3-demethylation, 7-demethylation, and formation of 6-amino-5-(N-methylformylamino)-1-methyluracil (AMMU)). Cimetidine pretreatment inhibited theobromine 3-demethylation and AMMU formation resulting in a 27 % decrease in Cl-TB in the combined smoker/nonsmoker group. Sulfinpyrazone pretreatment increased Cl-TB by 50 % in the whole group by approximately equal induction of each metabolic pathway. In addition, since AMMU formation was inhibited by cimetidine and induced by cigarette smoking and sulfinpyrazone, it would appear that the conversion of theobromine to AMMU is also mediated by cytochrome P-450 (28).

The four primary metabolites of caffeine , 1,3-dimethylxanthine (theophylline), 3,7-dimethylxanthine (theobromine), 1,7-dimethylxanthine (paraxanthine), and 1,3,7-trimethyluric acid were effective and virtually complete antagonists of acetaminophen (ACM)-induced hepatotoxicity when given immediately after ACM, as were the secondary metabolites, 1-methylxanthine and 1,3-dimethyluric acid. It is suggested that caffeine and its primary metabolites compete with ACM for biotransformation by the cytochrome P-450 mixed function oxidase system, thereby reducing the rate of formation of the hepatotoxic ACM metabolite (29).

The ingestion of theobromine in combination with ephedrine improves cold tolerance by increasing heat production, mainly from a greater lipid utilization (30).

Adaptation of the human tongue to methylxanthines at concentrations ranging from 10^{-5} mol/L to 10^{-2} mol/L was found to potentiate taste. Theobromine could potentiate the artificial sweetener acesulfam (31).

The in vivo effects of methylxanthines on 2',5'-oligoadenylate (2,5An) synthetase activity, an interferon-inducible enzyme, were investigated in rat liver nuclei. Theobromine given at 80 mg/kg sc twice daily for 5 d resulted in a 60 % reduction of 2,5An synthetase activity in liver nuclei. Nuclear 2'-phosphodiesterase activity, which catalyzes the degradation of 2,5An, remained low and unchanged following the drug treatments. These results suggest that methylxanthines may interact with interferon-mediated actions. The reason for the inhibitory effect of methylxanthines on the basal but not on the induced 2,5An synthetase is unclear (32).

The renal effects of xanthines were studied in vitro in the isolated perfused rat kidney (IPRK) and cultured opossum kidney (OK) cells, a continuous cell line that resembles proximal tubule and responds to parathyroid hormone (PTH). A 1 –nmol/L bolus of PTH elevated urinary and perfusate cAMP 50- and 10-fold, respectively OK cells produced a 2-fold cAMP response to 10 nmol/L PTH alone. The rank order of potency at 50 μmol/L to augment OK cell cAMP with 10 nmol/L PTH was (DPX)1,3-Diethyl-8-phenylxanthine> 1,3-dipropyl-8-cyclopentylxanthine (DPC) > 1-methyl-3-isobutylxanthine > theobromine > theophylline > caffeine. These studies demonstrate a direct tubular effect of the xanthines. Inhibiton of renal proximal tubular cell phosphodiesterase may explain some effects (e.g., diuresis) of xanthines on renal function (33).

The effect of acutely administered adenosine and adenosine analogs on methylxanthine-induced hypercalciuria was concurrently investigated. When rats were fed theobromine urinary Ca^{2+} excretion increased; on day 7 values were increased over controls by 54 %. On day 20, an injection of adenosine reduced Ca^{2+} excretion in methylxanthine-treated rats to levels not different from control values (34).

Theobromine (25-100 mg/kg) significantly reduced the duration of the ethanol-induced behavioral sleep, although not in a dose dependent manner. The most effective reduction of ethanol-induced behavioral sleep was in experimental groups which received 100 mg/kg theobromine (35 %) (35).

The antitumor activity of adriamycin (ADR) was enhanced by combination with theobromine or pentoxifylline, without enhancing the side effects of this drug (36).

Critical assessment

Chemical
Theobromine has the potential for complexation and salt formation.

In vivo
Theobromine shows interaction effects with agonists/antagonists of the adenosine receptors, the liver enzym system and phosphodiesterase. Taking into account the low theobromine dose in cigarettes it is unlikely that significant interactions will occur.

Conclusion
Chemical
Theobromine is able to form compounds with several chemicals.

In vivo
Theobromine has several systemic interaction effects in the body. Based on the low theobromine dose in cigarettes, it is unlikely that these interactions play a role in the health effects of smoking.

DEPENDENCY
Mechanism of addiction
The pharmacology of theobromine in cocoa products has been thoroughly reviewed and the conclusion seems to be that this agent is not responsible for the craving qualities of chocolate (14, 37).

Effects of smoking cessation
No data available.

Critical assessment
In the literature, theobromine is not considered as an addictive compound, however it could increase the nicotine availability through bronchodilatation, which subsequently could increase the addictive property of tobacco.

Conclusion
Theobromine does not seem to play a major role in smoking addiction.

COMMERCIAL USE
Theobromine is used principally to make caffeine (4).
Formerly, theobromine and its derivatives (salts of calcium salicylate (theosalicin), sodium acetate (themisalum) and sodium salicylate (theobromsal)), were used in diuretics, myocardial stimulants, vasodilators and smooth muscle relaxants in both veterinary and human medicine. (1, 2, 4, 8) Now, these applications of theobromine are rather limited. (4, 9)

BENEFICIAL EFFECTS
Aberrant angiogenesis, the new vessels formation, is a crucial event in the process of tumor growth and expansion. Theobromine significantly suppressed cutaneous neovascular reaction induced in mice by human lung cancer cells (38) and human blood leucocytes and ovarian cancer cells (39). Theobromine also diminished vascular endothelial growth factor (VEGF) (40). These findings suggest that theobromine might be a potent inhibitor of angiogenesis and that its mechanism of action is related to inhibition of VEGF production (40).
The antitumor activity of adriamycin was enhanced by combination with theobromine. Theobromine increased the concentration of adriamycin in the tumor without any effects on that in the heart and the liver. The combination of theobromine with adriamycin also significantly increased the inhibition of DNA biosynthesis in the tumor. These findings indicate that the combination of theobromine with adriamycin have no effect on the side effects of adriamycin in the liver and the heart (36).

Critical assessment
The use of theobromine in human and veterinary medicine for its diuretic, myocardic

and vasodilatory effects is limited. As an inhibitor of angiogenesis and enhancer of antitumor activity of adriamycin it might be of more value in the future.

Conclusion
In view of cigarette smoking no relevant beneficial effects can be expected.

SUMMARY AND FINAL CONCLUSION
A source of theobromine in tobacco is cocoa powder, which is used as a flavouring agent. There are no data available on the pyrolysis products of theobromine. Assuming similar systemic and potential effects after oral and inhalation exposure, the additional risk of theobromine by cigarette smoking will be low comparing the low daily intake via cigarettes smoke (estimated to be 4.75 mg/day) with the oral intake via tea drinks, chocolate and cocoa drinks (estimated 138 mg – 864 mg/day). Although oral intake is significantly larger from other sources than from cigarettes, the local effects of theobromine via inhalation on the respiratory system are not studied and might be a point of concern.

Theobromine affects the adenosine receptor sites (A_1 and A_2) and antagonizes the effect of adenosine. Theobromine exerts various pharmacological effects in the body, but these effects are much weaker than those of other methylxanthines, like caffeine and theophylline, and therefore its bronchodilatory capacity is questionable. As other methylxanthines (caffeine) also occur in cigarettes, the combined effect of these methylxanthines on the pulmonary system is not known.

After oral intake, theobromine is readily absorbed and widely distributed in tissues, including the brain. Transplacental transport in rats and human was reported and theobromine was identified in fetal rat brain. CYP450 is involved in the metabolism of theobromine. The half-times in serum ranged from 6.1 to 10 h. There are no data on pharmacokinetics in animals and humans from respiratory studies.

The acute toxicity of theobromine is low. In humans clinical signs such as sweating, trembling and severe headache are observed at high daily doses (0.8 – 1.5 g theobromine). Animal lethal dose (LD_{50}) for animals range from 300 mg/kg for dogs to 1356 mg/kg for mice. After semichronic treatment of rats with high doses of theobromine (25 – 250 mg/kg) a reduction in body weight and testicular atrophy is observed. Theobromine may have mutagenic properties. There is no evidence that theobromine is carcinogenic. No data on the toxic effects of theobromine administered through inhalation are available. Toxic effects are observed at high oral theobromine doses. It is unlikely that exposure to theobromine through smoking leads to systemic theobromine levels that exert toxicologically relevant effects. Since no data on the toxicological effects of theobromine exposure through inhalation are available, the influence of exposure to theobromine on the respiratory system through smoking cannot be established.

Theobromine is able to form stable compounds with bases and unstable compounds with salts. Furthermore it can form complexes. Theobromine shows interaction effects with agonists/antagonists of the adenosine receptors, the liver enzym system and phosphodiesterase. All these in vivo interaction effects are described for other than inhalation route. Whether these interaction effects also occur by intake through

inhalation need to be studied.

Although, chocolate craving qualities are well known, it is generally accepted that theobromine does not seem to play a role in this addiction process. Due to the weak pharmacological effects of theobromine on the pulmonary system, it seems unlikely whether theobromine plays a role in the tobacco addiction process.

Some beneficial effects of theobromine are reported: it inhibited the angiogenesis in lung cancer cells and enhanced the antitumor activity of adriamycin. In view of cigarette smoking these reported beneficial efects are not known.

It can be concluded that theobromine exerts various pharmacological and toxicological effects in the body. For smoking the bronchodilatory effect seems to be most relevant, but the doses occurring in cigarettes seem not sufficient to evoke such an effect. However, there are no data available on the pharmacodynamics, pharmacokinetics and toxicology after inhalation exposure.
More studies are needed on:
- the determination of pyrolysis and combustion products of theobromine in cigarette smoke;
- the local (respiratory system) and the systemic effects of longterm use of theobromine alone and in combination with other xanthines via inhalation.

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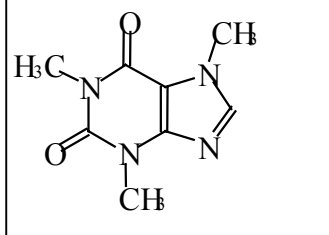   reports 1998; 5(2): 517-520.

3.2 Caffeine

GENERAL
IUPAC systematic name:
Synonyms:3,7-Dihydro-1,3,7-trimethyl-1H-purine-2,6-dione, 1,3,7-trimethylxanthine, 1,3,7-trimethyl-2,6-dioxopurine, coffeine, thein, guaranine, methyltheobromine, No-Doz, anhydrous caffeine, methyltheophylline (1, 2).
Molecular formula: $C_8H_{10}N_4O_2$ (1, 2)

Molecular structure

Molecular weight: 194.19 g/mol (1, 2)
Alifatic: no
Aromatic: yes
N containing: 4
Halogen containing: no
CAS registry no.: 58-08-2 (1)
Storage:
R/S classification: R22, S(02) (3)
dangercode (transport): free (3)
Properties:
➢ melting point: 234-239 ºC (1, 2)
➢ boiling point: sublimation point is 178 - 180 ºC (1, 2)
➢ density: d_4^{18}= 1.23 g/ml (1, 2).
➢ refractive index: no data available
➢ solubility: in water (1.0 g/46 ml at 20ºC, 1.0 g/5.5ml at 80 ºC, 1.0 g/1.5 ml at 100 ºC), ethanol (1.0 g/130ml, 1.0 g/22 ml at 60 ºC), acetone (1.0 g/50 ml), chloroform (1.0 g/5.5 ml), diethylether (1.0 g/530 ml), benzene (1.0 g/100 ml at 20 ºC, 1.0 g/22 ml in boiling benzene), slightly soluble in petroleum ether (1). One gram dissolves in 46 ml water, 5.5 ml water at 80 deg, 1.5 ml boiling water, 66 ml alcohol, 22 ml alcohol at 60 deg, 50 ml acetone, 5.5 ml chloroform, 530 ml ether, 100 ml benzene, 22 ml boiling benzene. Freely soluble in pyrrole; in tetrahydrofuran containing about 4% water; also soluble in ethyl acetate; slightly in petroleum ether. Solubility in water is increased by alkali benzoates, cinnamates, citrates or salicylates (2).
➢ Substance description:
  • white (1, 2, 3)
  • crystalline powder (1)
  • odorless, slightly bitter taste (1)
➢ volatility: sublimizes at 178 – 180 °C (2).
➢ pK$_a$: K_a = < 1.0 x 10^{-14} at 25 °C, K_b = 0.7 x 10^{-14} at 19 ºC (1)
➢ PA: kcal/mol: No data available
➢ flammability: poorly flammable; increased flammability by heating (3)
  • FP = no data available

- FL Limits = no data available
- IT = no data available
➢ decomposition temperature: no data available
➢ stability: no data available
➢ vapour pressure/ vapour tension (20 °C): No data available
➢ vapour pressure (50 °C): No data available
➢ relative density: E 1.23 (3)
➢ octanol water partition coefficient, log P, log K_{OW}: log K_{OW} = 0.0 at pH 7.4 (1)
➢ conversion factor: not relevant

Critical assessment
Caffeine is a heterocyclic natural product, occurring in more than 60 plant species througout the world. Belonging to the methylxanthine-group it is an alkaloid. Its structure is closely related to theobromine and it exhibits similar chemical properties: it is a purine derivative and contains as such aromatic properties. Polarity forms the main factor for its good solubility in water.
Its acid/base-properties are extremely weak. Salt forms exist but these salts decompose readily in water.
No data were found concerning identification of pyrolysis products of caffeine.

Conclusion
Caffeine is a natural product, nitrogen containing, soluble in many solvents especially in water. It is a light sensible solid, readily sublimizing.

FUNCTION IN TOBACCO
No data available.

AMOUNT IN TOBACCO PRODUCTS
A typical casing concentration of cocoa powder for cigarette tobacco is 1% (4).
The average amount of caffeine in cocoa powder is 0.2 % (5, 6).
Assuming one cigarette weights approximately 1 g, the caffeine amount in one cigarette is ± 0.02 mg = 20 µg.
Caffeine determined in cigarettes ranged from 0.031 – 16 µg/g cigarette (7).

AMOUNT IN SMOKE
- **main stream:** no data available
- **side stream:** no data available

SOURCE
A source of caffeine is cocoa powder, which is added to tobacco products as a flavour enhancer (4, 5, 6).

ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE
Caffeine is widely consumed in beverages such as coffee and tea and as softdrinks and (as content of) over the counter drugs to which caffeine is added as well. The average daily consumption of caffeine in the US is estimated at 200-300 mg/day and marked higher amounts are consumed in Western Europe (1, 8, 9).

COMBUSTION PRODUCTS
By heating/combustion of caffeine, toxic and corrosive gas/vapour are formed, such as nitrous gasses, carbon monoxide, carbon dioxide. Caffeine reacts with (strong) oxidants (3).

CONSENSUS REPORTS
There is inadequate evidence for the carcinogenicity of caffeine in humans. There is inadequate evidence for the carcinogenicity of caffeine in experimental animals. Caffeine is not classifiable as to its carcinogenicity to humans (group 3) (1).

STANDARDS AND RECOMMENDATIONS
ADI: Most obstetricians recommend that caffeine intake be limited to less than 400 mg/day during pregnancy (9). No ADI data are available for normal caffeine consumption.

TWA$_{NL}$ = MAC: no data available
TWA$_D$ =MAK: no data available
TWA$_{USA}$: no data available
STEL$_{NL}$: no data available
STEL$_{USA}$: no data available
LTEL: no data available
TLV-C: no data available
TLV-CARCINOGENICITY: no data available
MAK-REPRODUCTION: no data available

Others:

Reference value:
The median plasma caffeine concentration of a population over a wide age, was 1.71 μg/ml (range 0.10-6.74 μg/l) (10). Although the caffeine intake was not increased during pregnancy by women, the mean caffeine plasma concentration increased from 2.35 μg/l at beginning to 4.12 μg/l at 36 weeks of pregnancy, due to decreased clearance of caffeine during pregnancy (11).

CLASS
EG Carc. Cat.: No data available
IARC-category: group 3 (1).
CEC: No data available

Critical assessment

Comparison of smoking related daily consumption with daily consumption of caffeine (mg) from other sources:

	SMOKING		DRINKING OR EATING			
	25 cig. (1% cocoa)	3 Coffee	3 Tea drinks	3 Chocolate drinks	3 Chocolate bars of 60 g	3 Cocoa drinks
Caffeine (mg)	0.5	186[12] 240-405[9]	95[12]	12[12]	40 (milk)[13] 36 (milk)[14] 12 (sweet)[15]	15[1]

Little is known about the profile of the pyrolysis/combustion products of caffeine.

Conclusion

The daily intake of caffeine from cigarettes through inhalation is marginal compared with the oral intake of caffeine from other sources, like coffee, tea, chocolate drink and sweets. So, the plasma concentration reached after ingestion of caffeine from coffee, tea or chocolate sources is expected to be significantly higher, than after intake from cigarettes. However, the different route of application via smoking as compared to other sources should be taken into account. Therefore, local effects of caffeine on the respiratory system might be a point of concern.

PHARMACODYNAMICS

Mechanism of action

It was initially thought that caffeine and other methylxanthines acted primarily as phosphosdiesterase inhibitors. However, the inhibition is minimal at typical serum levels. At present it appears that the most important mechanism of action of caffeine is the antagonism of adenosine receptors. Adenosine is a locally released purine hormone that acts on two different receptors, A1 and A2. Receptors mediate either an increase or a decrease in cellular concentrations of cyclic adenosine monophosphate. High affinity (A1) receptors inhibit adenylate cyclase; low affinity (A2) receptors stimulate adenylate cyclase. Adenosine receptors are found throughout the body, including the brain, the heart and bloodvessels, the respiratory tract, kidneys, adipose tissue and the gastrointestinal tract. Adenosine acts locally as a vasodilator. It also reduces platelet aggregretion in vitro, inhibits catecholamine and renin release and inhibits lipolysis. Caffeine nonselectively inhibits the action of adenosine (9).

Pulmonary system

- **breathing frequency**: The respiratory rate correlates closely with the plasma caffeine level (250 mg oral intake) (9). The major respiratory effect of caffeine (ingested from coffee) is an increased output of the respiratory centre. In healthy subjects caffeine (650 mg ingestion) significantly increases ventilation at rest, accompanied by a fall in an end tidal carbon dioxide tension (16).
- **tidal volume**: Caffeine increased the tidal volume during exercise after ingestion of 3.3 mg/kg body weight (17) or after 650 mg ingestion (18).
- **lung compliance**: The expired ventilation volume increased significantly after caffeine ingestion (18, 19).
- **airway resistance**: Caffeine has a bronchodilatory effect in humans through oral

administration (9, 17-20). The airway resistance by inhalation of theophylline aerosol, a caffeine derivative, was investigated. A dose of 15 mg theophylline aerosol showed significant decrease of the airway resistance after 60 min. of administration. The airway resistance decrease was not significant immediately or after 30 min of theophylline exposure (21). The caffeine activity as a bronchodilator is reported to be equipotent or less than of theophylline (5, 6).

Cardiovascular system
- **blood pressure**:
  The systolic blood pressure increases abruptly about 10 mm of mercury with caffeine. However, tolerance develops quickly and longterm ingestion has little or no effect on the blood pressure and heart rate (9). The administration of 250 to 350 mg of caffeine to methylxanthine-naive individuals may produce modest increase in both systolic and diastolic blood pressure, but such doses are usually without effect on these parameters in those who consume caffeine regularly. The effects of therapeutic doses of caffeine on the peripheral blood flow or vascular resistance in man are variable. The conflicting hemodynamic patterns that are observed suggest that caffeine has little direct effect on the major resistance vessels. It is likely that caffeine affect the peripheral resistance through the brain stem (22).

- **heart rate**: After ingestion of ± 200 mg to ± 400 mg caffeine, the heart rate slows for about an hour, then increases for two to three hours thereafter; however longterm use does not have an effect on the heart rate (9, 19). At high caffeine plasma concentrations, caffeine produces tachycardia; sensitive individuals may experience other arrhythmias, such as premature ventricular contractions (22).

Renal system
- **diuresis**: Methylxanthines increase the production of urine (22, 23).
- **saluresis**: An increase in diuresis and in urinary sodium, potassium, and osmol excretion was observed within 1 h after caffeine ingestion (23). Women were given a decaffeinated beverage to which 6 mg caffeine/kg lean body mass or no caffeine were added. Total urine output of water, calcium, magnesium, sodium, chloride, potassium, and creatinine increased in the 2 h following the caffeine ingestion when compared to the control beverage (24).

Nervous system
- **central nervous system**: Caffeine acts principally as a stimulant and reduces fatigue. Caffeine has also substantial effects on sleep. It increases sleep latency, decreases total sleep time and substantially worsen subjective estimations of sleep quality (9).
- **autonomic system**: Caffeine could induce catecholamine release (9).

Other
Caffeine stimulates the secretion of gastric acid and pepsin (9).
Caffeine has a pronounced effect on the blood components and coagulation time; caffeine inhibited and reversed platelet aggregation induced by adenosine diphosphate in vitro (2).
The administration of caffeine (4 to 8 mg/kg) to normal or obese human subjects elevates the concentration of free fatty acids in plasma and increases the basal metabolic rate (22).

Critical assessment

Caffeine has various effects in the body. It has a relaxation effect on the smooth muscles, notably on the bronchial muscle, stimulates the CNS, stimulates the cardiac muscle and increases the diuresis. Caffeine has contradicting effect on the vascular system, which is explained by the central action of caffeine. Relatively large oral doses are needed (> 200 mg) to exert effects on the respiration system.

There are no data on pharmacology in animals and humans from respiratory studies of caffeine. The caffeine activity as a bronchodilator is reported to be equipotent or less than of theophylline. As compared to the bronchodilatory effects of a theophylline dosis of 15 mg applied as an aerosol in humans (21), it is questionable whether the caffeine dose of 0.02 mg per cigarette is high enough to have a bronchodilatory effect.

Conclusion

Caffeine exerts a bronchodilatory effect through oral administration, but the effects of caffeine through inhalation on the respiration system are unknown. Compared with inhalation studies of theophylline, it is unlikely that caffeine dose in cigarette have a bronchodilatory effect. As other methylxanthines (theobromine) also occur in cigarettes, the combined effects with these methylxanthines on the pulmonary system are not known.

PHARMACOKINETICS

Absorption

Caffeine absorption from gastrointestinal routes is rapid and complete (1, 8).

Bioavailability

The absorption of oral doses quickly approaches that from the intravenous route (1, 8).

Caffeine is 99 % absorbed from beverages and reaches peak serum concentrations within 30 to 60 min (9).

Distribution

The undissociated form of the molecule, which is soluble in the gastric membrane, penetrates all biologic membranes and is distributed to all body tissues. It does not accumulate in any organs and tissues. Caffeine readily crosses the blood-brain barrier and the placenta. It is also present in breast milk (1, 5, 8, 9).

The percentage plasma binding for caffeine was 10 – 30 % (1, 8).

Metabolism

Caffeine, which is a N-methylated compound is degraded by demethylation. When administrated to 20 day old fetal rats, it is demethylated to yield primary metabolites such as theobromine, theophylline and paraxanthine. Caffeine is extensively metabolised in the liver through a complex process mediated primarily by the microsomal cytochrome P450 reductase system.

The cytochrome P450 monooxygenase metabolises caffeine yielding trimethyl uric acids, paraxanthine and minor amounts of theobromine (8).

Excretion

From 2-3 % (w/w) of the ingested caffeine is excreted unchanged in the urine (9).

Kinetic parameters

The rate of caffeine metabolism varies, with half lives ranging from 2 to 12 hours and an average half-life of 4 to 6 hours (about 15 % metabolised per hour). Longer half-lives are seen in patients with chronic liver disease and in pregnancy. A shorter half-life is seen in smokers (5, 9).

Critical assessment

The oral data indicate a high bioavailabilty and extensive distribution and metabolism of caffeine.

There are no data on pharmacokinetics in animals and humans from respiratory studies.

Conclusion

Conclusions on potential differences in kinetics between respiratory and oral administration can neither be drawn based on the pharmacogical and toxicological data.

TOXICOLOGY

Acute toxicity

3.2.1.1 Human

Acute toxicity due to caffeine is not very common, although some adverse effects (e.g. gastric symptoms, insomnia, diuresis) have been observed as a result of overdoses. In volunteers who abstained from caffeine-containing products, a bolus dose of 250 mg led to a 5-10 % increase in both systolic and diastolic blood pressure for 1-3 h. At low doses (up to 2 μg/ml in blood), caffeine stimulates the CNS and many caffeine users perceived this effect as beneficial. High blood concentration (10-30 μg/ml) of caffeine may produce restlessness, excitement, tremor, tinnitus, headache and insomnia (1).

A one-year-old white female ingested approximately two to three grams of caffeine (200-300 mg/kg). The patient survived the ingestion with a maximum caffeine concentration of 385 micrograms/ml four hours postingestion. The child developed ventricular arrhythmias, seizures, metabolic disturbances, and severe pulmonary edema. She survived without apparent long-term sequelae despite having reached a serum caffeine concentration that is the second highest reported level in a survivor (25).

Only three human fatalities from caffeine have been reported and the lowest toxic dose was 2-3 g or 57 mg/kg body weight (8).

The lethal dose is about 10 g or 170 mg/kg BW, which equivalent to 75 cups of coffee, 125 cups of tea, or 200 cans of cola. In high doses caffeine causes hypotension from vasodilatation (ß-adrenergic mediated) and pronounced tachycardia with massive systemic catecholamine release (9).

Animal

Oral

LD_{50} rat is 200 mg/kg, mouse is127 mg/kg, hamster is 230 mg/kg, guinea pig is 246 mg/kg (1).

LD_{50} in mouse, hamster, rat, rabbit (mg/kg) is respectively: 127, 230, 355, 246

(males); 137, 249, 247, 224 (females) (2).
LD_{50} rat is 261 – 383 mg/kg (26).
Intraperitoneal
LDs_{50} rat is 200 mg/kg, guinea pig is 235 mg/kg (1).
I.V.
LD_{50} rat is 105 mg/kg, mouse is 100 mg/kg and dogs is 175 mg/kg (1).

Local tolerance
Human
No data available.
Animal
No data available.

Repeated dose toxicity
Subacute
No data available
Semichronic
The maximum dose for rats to produce no deaths in 100 days (100 mg/kg) corresponds to a man drinking some 60-100 cups of coffee a day. However, oral doses of 110 mg/kg for 100 days in rats exhibited a stressor reaction in the form of hypertrophy of the adrenal cortex and atrophy of the adrenal cortex and the thymus gland. Some animals manifested a psychotic-like mutilation, gastric ulcers, hypertrophy of the salivary glands, liver, heart, kidneys and lungs, inhibition of oogenesis, minor changes in organ water levels and an occasional death apparently from bronchiopneumonia. Although major changes in growth rates, eating and drinking habits were not apparent, some polydipsia and diuresis, thyroiditis, occasional dermatitis, some degree of nephritis and loss of red pulp in the spleen were seen (5).
Caffeine also induced thymic atrophy at a dietary level of 0.5 % (± 150 mg/kg BW) when fed for eight weeks in rats (27).
Chronic
The available data indicate that consumption of caffeine in moderate amounts does not cause a persistent increase in blood pressure in normotensive human subjects. Some controversy results were obtained about correlation between fibrocystic breast disease and the use of caffeine (1).

Carcinogenicity
Human
A cohort study and four case control studies of breast cancer showed no association with caffeine intake. A slight increase in risk was seen in premenopausal women in one study, but in general the relative risk was below unity. Another case control study of bladder cancer showed a weak association with caffeine consumption. The problem in these population studies is that caffeine intake is highly correlated with coffee intake, which makes it difficult to evaluate the effect of caffeine adequately (1).
Animal
Caffeine was tested for carcinogenicity in five studies in rats by oral administration, with caffeine concentration as high as 2000 mg/l drinking water. From the data evaluation of these studies it was concluded that tumour incidence was not increased significant at any site in the body in the caffeine group. Administration of caffeine in

combination with known carcinogens resulted in decreased incidences of lung tumours in mice treated with urethane, of mammary tumours in rats treated with diethylstilboestrol and of skin tumours in mice treated with either ultraviolet light or cigarette-smoke condensate (1).

Reproduction toxicology
Human
Total caffeine intake, as determined from various sources including coffee, tea, cola and drugs, was positively associated with the proportion of low-birthweight babies after controlling for smoking and other potential confounders (1).
For spontaneous abortion, five studies were evaluated; the combined odds ratio was 1.36 (95% confidence interval 1.29-1.45), indicating that mothers who consumed caffeine had a higher risk of spontaneous abortion than those who did not. The birth weight of the babies showed a statistical correlation with the caffeine consumption during pregnancy (28).
In human some conflicting results were reported about the effects of caffeine and coffee consumption on fertility. Some studies did not show any correlation between caffeine intake and fertility and other studies showed a threshold and negative dose-response correlation between caffeine intake and fertility (29).
Animal
Caffeine in a dose of 25 mg/kg body weight administered by oral gavage to pregnant rats on days 8-9 of gestation caused delayed neuraltube closure in rat embryos; also the development of the heart, eyes and limbs were reduced.
From the various recent studies on the reproductive toxicity of caffeine, it is evident that administration of caffeine during pregnancy affects the normal differentiation of foetal ovaries and testis resulting in significant foetal and post natal growth retardation and an increase in post natal mortality and impaired brain differentiation resulting in delayed closure of the neural tube (8).

Mutagenicity
Human
Cultured human lymphocytes from volunteers on a regime of 800 mg caffeine daily for four weeks, resulting in caffeine blood levels as high as 29.6 µg/ml after four weeks showed no significant increase in the frequency of chromosomal damage. Drinking coffee or tea to result in a total caffeine intake corresponding to that in five cups of coffee per day [exact amount not stated] was associated with increased micronucleated reticulocytes and micronucleated mature erythrocytes in splenectomized but otherwise healthy individuals after adjustment for smoking. Drinking decaffeinated coffee was not associated with an increase in the number of micronucleated cells.
Although it has been suggested that caffeine may induce gene mutations in mammals and man, direct evidence in vivo is limited. The indirect evidence is based largely on extrapolation from results in lower organisms, in which there is no doubt about the mutagenic action of caffeine, and from cultured mammalian cells, in which caffeine is clastogenic at high concentrations (1).
Animal
Using dominant lethal method, no significant increase in dominant-lethal mutations (embryonic deaths) were found, whether expressed as early deaths per pregnant female or as mutation index in animals consuming caffeine in drinking water at 3.6, 13.4, 49 and 122 mg/kg for 8 weeks. Although males consuming the two highest

levels of caffeine produced fewer pregnancies, litter sizes of females were not reduced (5). Caffeine can enhance the teratogenic effect of ionizing radiation in mice (30). However, intraperitoneal injections of caffeine prior to, subsequent to, and following X-rays did not enhance the mutagenicity of the radiation (5).

Other
Caffeine interacts in different ways with DNA structure and metabolism. Non-DNA targets that are important to the genotoxic and related effects of methylxanthines are (i) cytochrome P450s, (ii) cAMP metabolism, (iii) DNA metabolism, chromatin structure and function and (iv) nucleotide pools (1).
Caffeine increased the mutagenic effect of UV light on *E. Coli* (5). In mammal *in vivo* experiments, most of the experiments showed negative test in the micronucleus test and only three significant positive tests were observed; in each case the doses were in the toxic range (1).

Critical assessment
The toxicity of caffeine after acute or chronic administration to humans and animals is low. Caffeine intake has been associated with lower birth weights and increased incidence of spontaneous abortion in man. In animals relatively high doses of caffeine affected normal prenatal development.

Conclusion
Toxic effects of caffeine are observed at high doses. It is unlikely that exposure to caffeine through smoking leads to toxicologically relevant systemic caffeine levels. No data on the toxicological effects of caffeine exposure through inhalation are available. Therefore the influence of exposure to caffeine through smoking cannot be established.

INTERACTIONS
Chemical
Heating/combustion of bulk amounts may release toxic and corrosive gas/vapour: nitrous gasses, carbon monoxide, carbon dioxide; caffeine is able to react with (strong) oxidants (3).
The photo-oxidation of caffeine in presence of peroxydiphosphate (PDP) in aqueous solution at natural pH (similar to 7.5) was performed. On the basis of the experimental results and product analysis, 3 probable mechanisms have been suggested in which PDP is activated to phosphate radical anions (PO_4^{2-}) by direct photolysis of PDP and also by the sensitizing effect of caffeine. The phosphate radical anions thus produced react with caffeine by electron transfer reaction, resulting in the formation of caffeine radical cation, which deprotonates in a fast step to produce C8-OH adduct radicals. These radicals might react with PDP to give final product 1,3,7-trimethyluric acid and PO_4^{2-} radicals, the latter propagates the chain reaction (31).
Caffeine displays complex formation with hydroxylic derivatives through the hydrogen bonding at the carbonyl functions (32).

In vivo
Caffeine can enhance the teratogenic effect of ionizing radiation in mice. Also for a variety of pharmaceutical agents (acetazolamide, mitomycin C, hydroxyurea, 5-

fluorouracil), caffeine enhanced the teratogenic effect of these agents. With 5-azacytidine in rats, caffeine suppressed limb malformations. Administration of inhibitors of beta-adrenergic function reduces the teratogenic effect of caffeine in mice. The interpretation of the experimental studies in terms of human hazard is complicated by the general use of high-dose bolus exposures, which are not typical of human exposures, and the use of test systems that are not readily applicable to human (30).

The ability of caffeine to inhibit mutation repair, has been investigated thoroughly (33); it is well known that under specific conditions caffeine is able to enhance radiation risk of mammalian cells by a factor of approximately 1.5-2. It was shown that at the concentration necessary for increasing radiation risk (2 mmol/l), caffeine effectively inhibits the restitution of radiation-damaged DNA (34). Caffeine caused a moderate increase of spontaneous micronucleus frequency in human hepatoma cells at high concentration. Caffeine reduced micronucleus frequency significantly in HCA 2-amino-3-methylimidazo-[3,4-f]quinoline micronucleus at low concentration (35).

Caffeine and derivatives are compounds with pleiotropic effects on the genetic material, which are thought to originate from binding of these drugs to DNA. Using 2 different topologic methods showed, that methylated oxypurines, at biological relevant concentrations, unwind DNA in a fashion similar to that of known intercalators. The methylated oxypurines could be ranked by decreasing unwinding potency: 8-methoxycaffeine > 8-ethoxycaffeine > 8-chlorocaffeine > caffeine > theophylline. These findings confirm, with a different assay, the interaction of caffeine and its analogs with DNA and add additional support for an intercalative mode of binding of these drugs to DNA (36).

Caffeine has also protecting properties against DNA-intercalating antitumor drugs (Novantrone (mitoxantrone, doxorubicin, ellipticine, or the doxorubicin analogue AD198)). It inhibits the DNA-intercalating properties of these drugs by complex-formation (37).

Caffeine is an effective analgesic adjuvant because it increases the antinociceptive effect of NSAIDs (Non-Steriodal Anti-Inflammatory Drugs) while reducing the probability of side effects. The potentiation appears to be due to a pharmacokinetic mechanism including actions at the central and the peripheral levels (38).

The thermogenic effect of tea is generally attributed to its caffeine content. Its thermogenic properties could reside primarily in an interaction between its high content in catechin-polyphenols and caffeine with sympathetically released noradrenaline (NA). Since catechin-polyphenols are known to be capable of inhibiting catechol-O-methyl-transferase (the enzyme that degrades NA), and caffeine to inhibit transcellular phosphodiesterases (enzymes that break down NA-induced cAMP), it is proposed that the green tea extract, via its catechin-polyphenols and caffeine, is effective in stimulating thermogenesis by relieving inhibition at different control points along the NA-cAMP axis (39).

Several interactions with caffeine through the liver enzym system are described. Caffeine is an inducer of CYP1A2 in rat liver (40).
Caffeine reduced the hepatotoxicity of acetaminophen (ACM) in mice when it was

administered immediately after ACM. However, the hepatotoxicity was increased when it was given before ACM. It is proposed that caffeine interferes with the metabolism of ACM when administered concomitantly, but induces the microsomal mixed function oxidase system when used in a pre-treatment regimen, leading to a more rapid rate of formation of the hepatotoxic arylating ACM biotransformation product (41).

From the course of the plasma concentration of theophylline, a prolonged half-time of 7.4 to 10.4 h as well as a reduction of the total clearance of 0.71 to 0.37 mL/min/kg was observed if caffeine and theophylline are administered at the same time. As both methylxanthines have the same localization of metabolization in the microsomal enzyme system of the liver a competition by caffeine may be the cause of the delayed theophylline metabolization. During treatment with theophylline the daily caffeine consumption should be taken into consideration (42).

The effects of the widely consumed drugs caffeine and phenylpropanolamine are mediated through activation of the central and sympathetic nervous systems. Greater increases in both systolic and diastolic blood pressures occurred after the combination than after either drug alone. Because caffeine levels can be increased greatly when certain other drugs are co-consumed, these data indicate that phenylpropanolamine may enhance absorption or inhibit elimination of caffeine and may explain increased side effects reported after their combined use (43).

The influence of multiple doses of ciprofloxacin on the disposition of caffeine and its major metabolite, paraxanthine, was investigated in healthy volunteers. Ciprofloxacin increases the half-life of caffeine and the area under the caffeine concentration-time curve by reducing total body clearance. This interaction is due at least in part to a delay in the conversion of caffeine to paraxanthine. Also, caffeine may alter the kinetics of ciprofloxacin (44).

Grapefruit juice inhibits the biotransformation of several drugs, including caffeine (23% clearance reduction), which is metabolised by the cytochrome P450 isoform CYP1A2 (45).

Carbamazepine (CBZ) interacts with the adenosine receptor, which is related to the inhibition of release of neurotransmitter. The anticonvulsive and sedative effects of CBZ and its derivates appear due to action on adenosine receptors (A1 and partially A2) at the therapeutic level, while the methylxanthines, like caffeine have stimulant and convulsant effects due to occupation on both A1 and A2 adenosine receptors (46).

In mice chronically administered caffeine decreased the ED_{50} for morphine-induced analgesia significantly while the naloxone ED_{50} for withdrawal jumping was increased by 2-fold after both types of morphine pre-treatment. Chronic administration of caffeine increases the potency of acutely administered morphine and reduces the development of morphine-induced tolerance and dependence. These effects of caffeine may be independent of adenosine receptor interaction (47).

The interactive effects of caffeine in coffee and cigarette smoking were studied in 15 subjects. Subjective arousal showed antagonistic interaction between caffeine and

smoking; smoking blocked the subjective stimulant effects of caffeine. The only cardiovascular effect noted was an increase in heart rate after smoking. Caffeine did not influence puffing behaviour; however, the increase in end-expired CO concentration after smoking was greater in the caffeine condition, suggesting subjects inhaled more smoke after caffeinated than decaffeinated coffee (48).

In a placebo-controlled, double blind randomized design, the cardiovascular interaction between caffeine (250 mg intravenously) and nicotine (4 mg chewing gum) in 10 healthy volunteers was investigated, both under baseline conditions and during physical and mental stress (standing up and mental arithmetic). It was concluded that the combined administration of caffeine and nicotine showed additive effects on cardiovascular parameters during baseline conditions but less than additive effects during sympathoadrenal stimulation (49).

The effects of caffeine (1.0-30.0 mg/kg) and nicotine (0.1-3.0 mg/kg) administered alone and in combination on ventilation in unanesthetized rhesus monkeys was investigated. Caffeine produced marked, dose-dependent increases in ventilation. In contrast, acute administration of nicotine had less pronounced respiratory-stimulant effects. The joint effects of caffeine and nicotine on ventilation generally did not differ from those obtained with caffeine alone. Chronic administration of nicotine (1.0 mg/kg/day) for 4 consecutive weeks via osmotic pumps significantly decreased the half-life of caffeine but had little effect on ventilation or on sensitivity to the respiratory-stimulant effects of caffeine. Two primary metabolites of caffeine, theophylline and paraxanthine, were active as respiratory stimulants and were equipotent to caffeine, and the joint effects of caffeine and its metabolites were additive. The results indicate that caffeine and nicotine stimulate respiration through different pharmacological mechanisms, in contrast to caffeine and its metabolites, which exhibit a similar pharmacological profile. Moreover, significant pharmacokinetic interactions may be obtained when caffeine and nicotine are coadministered (50).

Critical assessment
Chemical
By heating/combustion nitrous gases are formed. Caffeine is able to react with strong oxidants, resulting in radicals. It also forms complexes with compounds.
In vivo
Caffeine shows interaction effects with agonists/antagonists of the adenosine receptors, the liver enzyme system and phosphodiesterase. It has also mutagenic interaction effects.

Conclusion
Chemical
Caffeine is able to form complexes with several chemicals; it forms also reactive radicals after oxidation.
In vivo
Caffeine has several systemic interaction effects in the body. Based on the low caffeine dose in cigarettes, it is unlikely whether these interactions play a role in the health effects of smoking. Of importance is the potential mutagenic effect of caffeine; the question is whether the low caffeine dose is able to display local mutagenic effects in the pulmonary system.

DEPENDENCY

After sudden caffeine cessation, withdrawal symptoms develop in a small portion of the population but are moderate and transient. Tolerance to caffeine-induced stimulation of locomotor activity has been shown in animals. In humans, tolerance to some subjective effects of caffeine seems to occur, but most of the time complete tolerance to many effects of caffeine on the central nervous system does not occur. In animals, caffeine can act as a reinforcer, but only in a more limited range of conditions than with classical drugs of dependence. In humans, the reinforcing stimuli functions of caffeine are limited to low or rather moderate doses while high doses are usually avoided. The classical drugs of abuse lead to quite specific increases in cerebral functional activity and dopamine release in the shell of the nucleus accumbens, the key structure for reward, motivation and addiction. However, caffeine doses that reflect the daily human consumption, do not induce a release of dopamine in the shell of the nucleus accumbens but lead to a release of dopamine in the prefrontal cortex, which is consistent with caffeine reinforcing properties. Moreover, caffeine increases glucose utilization in the shell of the nucleus accumbens only at rather high doses that stimulate most brain structures, non-specifically, and likely reflect the side effects linked to high caffeine ingestion. That dose is also 5-10-fold higher than the one necessary to stimulate the caudate nucleus, which mediates motor activity and the structures regulating the sleep-wake cycle, the two functions the most sensitive to caffeine. In conclusion, it appears that although caffeine fulfils some of the criteria for drug dependence and shares with amphetamines and cocaine a certain specificity of action on the cerebral dopaminergic system, the methylxanthine does not act on the dopaminergic structures related to reward, motivation and addiction (51, 52).

The pharmacology of caffeine in cocoa products has been thoroughly reviewed and the conclusion seems to be that this agent is not responsible for the craving qualities of chocolate (15, 53).

Effects of smoking cessation

There is a strong, significant relationship between coffee consumption and smoking. In six epidemiological studies reviewed and analyzed, 86.4 % of smokers consumed coffee versus 77.2 % of non-smokers. Ex-smokers use more coffee than non-smokers do, but somewhat less than smokers do. Seventeen experimental studies suggest that the pharmacological effect of caffeine in coffee may be partially but not totally responsible for the relationship. Conditioning, a reciprocal interaction (caffeine intake increases anxiety/arousal--nicotine decreases it), or joint effect of a third variable (e.g., stress, alcohol) may account for the relationship. In abstinent smokers, blood caffeine levels increase and remain elevated for as long as 6 months. These higher caffeine plasma levels may be sufficient to produce caffeine toxicity syndrome (54).

Critical assessment

Caffeine has low addictive properties and some causal relationship exists between caffeine intake from coffee and smoking. However, the low doses in the cigarettes is marginal compared with the high intake from other caffeine sources, such as coffee. At the other hand, caffeine could increase the nicotine availability through bronchodilatation, which subsequently could increase the addictive property of

tobacco. As the bronchodilatation effects of caffeine are expected to be negligible at the caffeine dose in tobacco, it seems unlikely that caffeine plays a role in tobacco addiction through the bronchodilatory effect. However, long-term effects of caffeine on the pulmonary system are not known and furthermore, the additive effects of other methyxanthines, such as theobromine, in cigarette smoke on the pulmonary system are not known.

Conclusion
Although caffeine does not seem to play a role in smoking addiction, some caution has to be made for long term use of caffeine via inhalation and additive effects of other methylxanthines on the pulmonary system and subsequently on the addictive properties of cigarettes.

COMMERCIAL USE
Approximately 80-90 % of caffeine extracted from green coffee is used in the beverage industry and most of the remainder and synthetic caffeine is used in the pharmaceutical applications. Caffeine is permitted in the USA at a content up to 0.02 % by weight in beverages. It may be used as a flavour enhancer in several foods. Caffeine is an ingredient in many (non-) prescription drugs, including stimulant tablets, headache and cold remedies, tablets for the relief of menstrual pain, weight control aids and diuretics (1).

BENEFICIAL EFFECTS
Caffeine (64 mg), when added to aspirin (800 mg), improved vigilance performance and increased self-reported efficiency when compared with either placebo or aspirin alone. Apparently, the addition of caffeine to aspirin, in a dose commonly employed in over-the-counter drugs, has significant beneficial consequences with respect to mood and performance (55).
Caffeine may improve utilization of fatty acids as a fuel source thereby sparing muscle glycogen (56).
Caffeine has a beneficial effect on bronchospasm (57).
Some studies showed that caffeine was able to produce significant alerting and long-lasting beneficial mood effects in individuals deprived of sleep (58, 59).
Attention has long been drawn to the potentially harmful effects of coffee on health, however recent epidemiological studies have suggested unexpected, possibly beneficial effects of coffee against the occurrence of alcoholic liver cirrhosis and upon serum liver enzyme levels (60, 61).

Critical assessment
The use of caffeine is widely spread; it is used as a drug or for elevating the mood.

Conclusion
In view of cigarette smoking, the caffeine doses are likely too low to have the above expected beneficial effects.

SUMMARY AND FINAL CONCLUSION

A source of caffeine in tobacco is cocoa powder, which is used as a flavouring agent. Little is known about the profile of the pyrolysis/combustion products of caffeine. The daily intake via cigarettes smoke (estimated to be 0.5 mg/day) is low compared to the oral intake via coffee, tea, chocolate and cocoa drinks (estimated 12 – 405 mg/day).

Caffeine affects the adenosine receptor sites (A_1 and A_2) and antagonize the effect of adenosine. Caffeine has various effects in the body. It has a relaxation effect on the smooth muscles, notably on the bronchial muscle, stimulates the CNS, stimulates the cardiac muscle and increases the diuresis. Caffeine has contradicting effect on the vascular system, which is explained by the central action of caffeine. Relatively large oral doses are needed (> 200 mg) to exert effects on the respiration system. There are no data on pharmacology in animals and humans from respiratory studies of caffeine. Based on the respiratory effects of the caffeine derivative theophylline it is concluded that the pharmacological effects of caffeine doses occurring in cigarettes should have negligible effects on the respiratory system. As other methylxanthines (theobromine) also occur in cigarettes, the combined effects with these methylxanthines on the pulmonary system is not known.

The oral data indicate a high bioavailabilty (99 %) and extensive distribution (crosses the blood brain-barrier, the placenta and is present in milk) and metabolism (mediated by microsomal CYP450 reductase system) of caffeine. The average half-life of caffeine range from 4 – 6 hours, which is shorter in smokers. There are no data on pharmacokinetics in animals and humans from respiratory studies.

Acute toxicity of caffeine is very uncommon; adverse effects that are observed are gastric symptoms, insomnia and diuresis, tremor, tinnitus and headache. The lowest human toxic dose was 2 – 3 g. Animal lethal dose (LD_{50}) (I.V.) range from 105 mg/kg body weight for rats to 175 mg/kg body weight for dogs. Semichronic (100 days) administration of caffeine (110 mg/kg body weight) daily to rats evoked several clinical manifestation. Chronic consumption of coffee in moderate amounts does not seem to cause persistent increase in blood pressure in normotensive human subjects. There is inadequate evidence for the carcinogenicity of caffeine in humans and animals. Caffeine may have mutagenic properties. No data on the toxicological effects of caffeine exposure through inhalation are available.

Caffeine is able to react with strong oxidants, resulting in radicals. It also forms complexes with compounds. Caffeine shows interaction effects with agonists/antagonists of the adenosine receptors, the liver enzyme system and phosphodiesterase. It has also mutagenic interaction effects. Based on the low caffeine dose in cigarettes, it is unlikely whether these interactions play a role in the health effects of smoking.
Caffeine has some addictive properties and some causal relationship exists between caffeine intake from coffee and smoking. However, the low doses in the cigarettes is marginal compared with the high intake from other caffeine sources, such as coffee. At the other hand, caffeine could increase the nicotine availability through bronchodilatation, which subsequently might increase the addictive property of tobacco. As the bronchodilatation effects of caffeine are expected to be negligible at the caffeine dose present in tobacco, it seems unlikely that caffeine plays a role in tobacco addiction through the bronchodilatory effect.

It can be concluded that caffeine exerts various pharmacological and toxicological effects in the body. There are no data available on the pharmacodynamics, pharmacokinetics and toxicology after inhalation exposure.

Assuming similar systemic effects after oral and inhalation exposure, the additional risk for systemic effects of caffeine by cigarette smoking (estimated to be 0.5 mg/day) will be low compared with the oral intake via coffee, tea, chocolate and cocoa drinks (estimated 12 – 405 mg/day).

Since no data on the toxicological effects of caffeine exposure through inhalation are available, the influence of exposure to caffeine through smoking on the respiratory system cannot be established.

For smoking the bronchodilatory effect seems to be relevant, but the doses occurring in cigarettes seem not sufficient to evoke such an effect, and therefore it is unlikely that caffeine plays a role in taboaccoa addition via this mechanism.

Of importance is the potential mutagenic effect of caffeine; the question is whether the low caffeine dose is able to display local mutagenic effects in the pulmonary system. Since no data on the local toxicological effects of caffeine exposure through inhalation are available, the shortterm and longterm effects of exposure to caffeine through smoking on the respiratory system cannot be established. Furthermore, its additive effects on other methylxanthines present in cigarette smoke are also not known and have to be studied.

More studies are needed on:
the determination of pyrolysis and combustion products of caffeine in cigarette smoke;
the local (respiratory system) and the systemic effects of long-term use of caffeine alone and in combination with other xanthines via inhalation.

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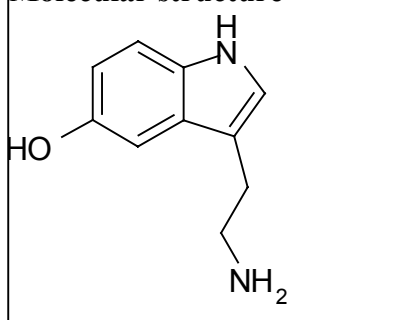
3.3 Serotonin

GENERAL
IUPAC systemic name: 3-(2-Aminoethyl)-1H-indol-5-ol (1).
Synonyms: 3-(2-Aminoethyl)-1H-indol-5-ol; 5-hydroxytryptamine; 3-(.beta.-aminoethyl)-5-hydroxyindole; 5-hydroxy-3-(.beta.-aminoethyl)indole; enteramine; thrombocytin; thrombotonin (2).
Molecular formula: $C_{10}H_{12}N_2O$ (1, 2).

Molecular structure

Molecular weight: 176.22 g/mol (1).
Alifatic: 2 C-atoms (1).
Aromatic: yes, indol structure (1).
N containing: Yes (1).
Halogen containing:No; the commercial serotonin compound is the hydrochloric salt (2) .
CAS registry no.: 50-67-9 (2).
Storage: Hydrochloride serotonin, $C_{10}H_{12}N_2O.HCl$ is a hygroscopic crystal and is sensitive to light (2). Therefore this compound should be stored in an airtight container and protected from light.
R/S classification: for the HCl salt: R: 20/21/22,36/37/38,40, S: 26,36,22 (1).
dangercode (transport): No data available
Properties:
➢ melting point: 167.5 ºC (1).
➢ boiling point: no data available
➢ density: no data available
➢ refractive index: no data available
➢ solubility: in water : 20 g/l (1).
➢ substance description:
  •   color: no data available
  •   liquid/gas/powder: powder (1).
  •   odor/taste: no data available
➢ volatility: no data available
➢ pK_a: pK1' = 4.9; pK2' = 9.8 (2).
➢ PA: kcal/mol: no data available
➢ flammability: no data available
  •   FP = no data available
  •   FL Limits = no data available
  •   IT = no data available
➢ decomposition temperature: no data available

➢ Stability: Hydrochloride serotonin, $C_{10}H_{12}N_2O.HCl$ is a hygroscopic crystal and is sensitive to light (2).
➢ vapour pressure/ vapour tension (20 °C): no data available
➢ vapour pressure (50 °C): no data available
➢ relative density: no data available
➢ octanol water partition coefficient, logP, log K_{OW}: log P = 0.21 (1).
➢ conversion factor: not relevant

Critical assessment

Serotonin contains the characteristic heterocyclic indole structure, accounting for the aromatic properties (electrophilic substitution). In addition the chemical; the presence of the ring bound hydroxyl group accounts for its polar character. An additional characterising chemical feature is the presence of the aliphatic amino-group.

Conclusion

Serotonin is a polar, Nitrogen-containing heterocyclic compound, containing an aliphatic amino-group.

FUNCTION IN TOBACCO

No data available.

AMOUNT IN TOBACCO PRODUCTS

A typical casing concentration of cocoa powder for cigarette tobacco is 1% (3).
The average amount of serotonin in cocoa powder varies from 1.25 µg/g to 60 µg/g (4, 5).
Assuming one cigarette weights approximately 1 g, the maximum serotonin amount from cocoa powder in one cigarette is estimated to be ± 0.6 µg.

AMOUNT IN SMOKE

• **main stream** no data available
• **side stream** no data available

SOURCE

(tobacco, combustion product or other)
A source of serotonin is cocoa powder, which is added to tobacco products as a flavour enhancer (3, 4).

ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

Serotonin is widely distributed in animals and plants. It occurs in vertebrates; in tunicates, mollusks, arthropods and coelenterates; and in fruits and nuts. Numerous synthetic or naturally congeners of serotonin have varying degrees of peripheral and central pharmacological activity. N,N-dimethyltryptamine (DMT) and its 5-hydroxy derivative (bufotenine) are active principles of the cahobe bean found along the offshores of the Carribean. Both of these compounds can be formed in the mammal by N-methylation of tryptamine and serotonin, respectively. LSD and several active ingredients of hallucinogenic mushrooms are 4-substituted tryptamine (6).

Serotonin amount in banana is 50-150 µg/g, tomatoes is 12 µg/g, prunes (red) is 10 µg/g, avocado is 10 µg/g, walnuts is 170 – 340 µg/g (3).

COMBUSTION PRODUCTS
No data available

CONSENSUS REPORTS
No data available

STANDARDS AND RECOMMENDATIONS
ADI: No data available
TWA$_{NL}$ = MAC: No data available
TWA$_D$ =MAK: No data available
TWA$_{USA}$: No data available
STEL$_{NL}$: No data available
STEL$_{USA}$: No data available
LTEL: No data available
TLV-C: No data available
TLV-CARCINOGENICITY: No data available
MAK-REPRODUCTION: No data available

Others:

Reference value:
The normal level of serotonin in the whole blood of a fasting subject depends on the analytical technique used. Serotonin level in whole blood measured in highly acid media gives values of 100 to 300 µg/l. Measurements at pH 4 give levels of 200 to 500 µg/l (7). The basal mean values of plasma serotonin and serum serotonin were 0.79 +/- 0.44 µg/l and 92.2 +/- 46.3 µg/l, respectively (8).
Results demonstrated unimodal distribution of individual frequencies of platelet/circulatory serotonin in the human population with mean values of 0.579 +/- 0.169 µg serotonin/10^9 platelets; 332 +/- 90 µg serotonin/g protein and 130 +/- 42.3 µg serotonin/l blood (mean +/- standard deviation). The serotonin level shows a progressive decrease with age (18-65 years), reaching statistical significance between the extreme age groups. There are no significant differences in the serotonin level between the sexes. The platelet/circulatory serotonin is not affected by seasonal oscillation (9).
Platelet serotonin level of smokers (128 ± 27.5 µg per 10^9 platelets (mean $\pm$ standard error on the mean (SEM), n = 11)) were significantly higher than those of nonsmokers (62.2 ± 27.5 µg per 10^9 platelets (mean $\pm$ SEM, n = 11)) (10).

CLASS
EG Carc. Cat.: No data available
IARC-category: No data available
CEC: No data available

Critical assessment
Comparison of smoking related daily intake of serotonin (µg) with daily intake from other sources:

| | SMOKING | COCOA INTAKE BY EATING | | BANANA |
	25 cig. (1 % cocoa)	3 chocolate bars of 60 g	cocoa powder (25 g)	(100 g)
Serotonin (μg)	15	35 (milk)[4] 174 (dark)[4]	18.75[4]	5000 – 15000[3, 11]

Little is known about the profile of the pyrolysis/combustion products of serotonin.

Conclusion
The daily intake of serotonin from cocoa added to cigarettes is marginal compared with that of serotonin intake from other sources, like chocolate or fruit (banana). Assuming similar bioavaibility, the plasma concentration reached after ingestion of serotonin from chocolate sources or plants is expected to be significantly higher, than after exposure from cigarettes. Since, serotonin is an endogenous compound, it is not expected that the inhaled amount will significantly affect the plasma concentration. However, the different route of application via smoking as compared to other sources should be taken into account. Therefore, local effect of serotonin on the respiratory system might be a point of concern.

PHARMACODYNAMICS
Mechanism of action
Over the past ten years, evidence obtained from molecular, biochemical and physiological studies has revealed the existence of fifteen serotonin receptor subtypes, which can be subdivided into seven major families (5-HT_{1-7} subtypes) (12). Serotonin both stimulates and inhibits nerves and smooth muscles in the cardiovascular, respiratory and gastrointestinal systems. Platelet membrane contains serotonin receptors (5-HT_2) that enhance aggregation when stimulated (6). Serotoninergic neurons are found in the brain stem where they are concentrated in the raphe nuclei. Projections from serotonin neurons reach the cortical forebrain, olfactory bulb, septum, hippocampus, thalamus, hypothalamus, basal ganglia (caudate, putamen and globus pallidus), substantia nigra, cerebellum, and spinal cord. Serotonin produces numerous pharmacological effects mainly because of the diversity of its receptors that are either ionotropic (ligand-gated ion channel receptors) or metabotropic (G-protein-coupled receptors). Serotonin is an autacoid that acts both at microdistances as neurotransmitters and at long distances as a hormone. The majority of serotonin receptors are metabotropic G protein-linked. The exception is the 5HT_3 receptor, which is ionotropic. This ligand-gated monovalent cation channel is present in high density in the brain region that contains the emetic centre and its antagonists (e.g., ondansetron) are potent anti-emetics. The metabotropic serotonin receptors are important targets in the brain for action of numerous therapeutics including antidepressants, anxiolytic, and antimigraine drugs. By analogy with neural antiacetylcholine receptors these drugs are likely to act as channel blockers. The metabotropic serotonin receptors are linked to either Gp-protein and their activation decreases cAMP synthesis (5-HT_{1A-F}) or to G_s protein, that activates phospholipase C and increases synthesis of IP_3 and diacylglycerol (5-HT_{2A-C}). Although there are many high-affinity agonists and antagonists for all subtypes, there are none that are totally selective for one subtype (13).

Pulmonary system

- **breathing frequency**: Afferent nerves to the bronchi may be stimulated by serotonin, causing an increase in respiratory rate (6).
- **tidal volume**: no data available
- **lung compliance**: no data available
- **airway resistance**:

Serotonin exhibits a broad diversity of effects on airway smooth muscle contraction, which seems to implicate the presence of a wide variety of serotonin receptor subtypes in both airway smooth muscle and efferent nerves and which also appears to be species-dependent. In several animal airways, serotonin acts directly on airway smooth muscle, causing contraction at low doses and relaxation at high doses. Both contraction and relaxation are mediated by stimulation of the $5-HT_{2A}$ receptor on airway smooth muscle. The effects of serotonin on airway smooth muscle contraction may also be attributed, in part, to the ability of serotonin to modulate the contractile and relaxing response to other neurotransmitters, such as neuropeptides in the sensory nerve endings and acetylcholine in the presynaptic neurons (12). Some serotonin (inhalation) studies performed on animals are described in the literature. The effect and mechanism of action of serotonin was studied in the pulmonary circulation of rabbits. Serotonin (1.76 µg, 8.8 µg and 17.6µg/l) produced a concentration-dependent increase in rabbit pulmonary arterial tension (14). Serotonin aerosols (1.5 ml/min) were generated by a nebulizer, which introduced serotonin aerosol (0.07 – 1.2 mg/ml tidal air) in cats. The pulmonary resistance increased significantly when the serotonin aerosol concentration was higher than ± 0.3 mg/ml (15).

Although the effects of serotonin on the pulmonary system have been extensively studied in several animal species, both *in vivo* and *in vitro*, the situation is less well established in humans. A possible relationship between serotonin and airway obstruction has been suggested on the basis of the association of wheezing with carcinoid syndrome (tumor of neuroendocrine cells), although it is now obvious that other mediators such as histamine, bradykinin and tachykinins are also released in this pathology (12). Inhaled serotonin does not produce bronchoconstriction in normal human subjects. It has been demonstrated in some studies, however, that inhalation of serotonin causes bronchoconstriction in 10 - 65% of asthmatic patients, whereas another study did not find the bronchoconstrictory effect of serotonin in asthmatics (16). In that study, serotonin up to a maximum concentration of 13.6 g/l had no consistent effect on FEV-1, the maximum expiratory flow at 30 % of vital capacity (V-max-30) or the specific airways conductance (sGaw) in any of the subject groups (asthmatics and non-asthmatics). That study concluded that in contrast to a variety of animals, serotonin is unlikely to serve as a significant bronchoconstrictor mediator in man. Furthermore, an elevated plasma level of 5-HT has been documented in symptomatic asthmatic patients when compared to nonasthmatics. In the former group, the 5-HT level significantly correlated with clinical severity rating and forced expiratory volume in one second (FEV1) (17).

Cardiovascular system

- **blood pressure**:

Serotonin plays a role in primary pulmonary hypertension; probably through the $5-HT_{1B/1D}$- and $5-HT_{2A}$ -receptors (18, 19). Coronary vessels in human subjects showed a biphasic response to intracoronary serotonin infusion: dilation at

concentrations up to 1.76 mg/l, but constriction at 17.6 mg/l (20).
see also heart rate

- **heart rate**:
  Serotonin does not appear to regulate blood pressure in the normal animal.
  However, when platelets become activated in certain disease states, serotonin may
  increase blood pressure. Serotonin exerts complex effects in the cardiovascular
  system, including hypotension or hypertension, vasodilatation or vasoconstriction,
  and/or bradycardia or tachycardia; the eventual response depends primarily on the
  nature of the serotonin receptors involved. Serotonin produces positive inotropic
  and chronotropic effects on the heart that are mediated by $5-HT_1$ receptors. These
  effects may be blunted by stimulation of $5-HT_3$ receptors on afferent nerves of
  baroreceptors and chemoreceptors. $5-HT_3$ receptors are also present on vagal
  nerve endings in the coronary chemoreflex, characterized by inhibition of
  sympathetic outflow and increased activity of the cardiac (efferent) vagus, leading
  to profound bradycardia and hypotension (6, 21).

Renal system
- **diuresis**: An intrarenal infusion of serotonin at a dose of 5 µg/min in anesthetized
  dogs resulted in a biphasic response of renal blood flow which decreased
  transiently then increased above the control level during prolonged infusion. The
  prolonged infusion of serotonin also increased urine flow and urinary excretion of
  Na^+. Serotonin may exert its antidiuretic action via a $5-HT_1$-like receptor in the
  tubules but the renal hemodynamic changes induced by serotonin may overcome
  its antidiuretic action and evokes subsequently diuresis (22).

- **saluresis**: After direct application of serotonin to the central nervous system
  (CNS), increases in urinary excretion of Na+ and in the Na^+/K^+ ratio were
  observed, concomitant with depressor effects. Therefore, central serotoninergic
  mechanisms are involved in the control of Na+ excretion in the hydrated rat (23).

Nervous system
- **central nervous system**:
  Serotonin exerts numerous effects on the CNS through the large family of
  serotonin receptors. Serotonin plays a role in depression, agression, long term
  memory, mental fatigue during endurance exercise (24-27). Serotonin is
  furthermore involved in regulation of sleep, circadian rhythms, food intake (fat
  and energy intake) and regulation of the BBB (brain blood barrier) function (13,
  28, 29). The serotoninergic system is also involved in the nicotine dependency
  (30, 31).

autonomic system: Serotonin can stimulate or inhibit nerves, depending on the site
  and the type of receptor involved. Activation of $5-HT_1$ receptors on adrenergic
  nerve terminals inhibits the release of the norepinephrine elicited by stimulation of
  the sympathetic nervous system. $5-HT_3$ receptors located on various sensory
  neurons mediate a depolarizing response, which may account for the ability of
  serotonin to cause pain and itching, as well as respiratory stimulation and
  cardiovascular reflex (6). Serotonin released from intestinal enterochromaffin cells
  may act either directly on vagal afferents and/or pass to the circulation and

stimulate central emetic centre (32).

Other

Serotonin has a differential effect on gastric emptying. Low and high doses (0.1, 0.3 and 30 mg/kg, i.p.) significantly inhibited the gastric emptying in rats while doses ranging from 1 to 10 mg/kg, i.p., had no significant effect on the gastric emptying (33).

Critical assessment

Serotonin has various effects in the body, through the large family of serotonin receptors. Some contradictory results were obtained about the bronchoconstrictory effect of serotonin in humans, but it is concluded that it is unlikely that serotonin serves a significant bronchoconstrictor mediator in man. Serotonin has also a pulmonary hypertension effect on the pulmonary system. Depending on the serotonin level, it exerts complex effects on the cardiovascular system, including hypotension or hypertension, vasodilatation or vasoconstriction, and/or bradycardia or tachycardia. It also has complex effects on the CNS and is involved in the nicotine dependency.

Conclusion

Serotonin, an endogenous compound, exerts various effects in the body through the large family of serotonin receptors. The inhalation studies of serotonin did not show any significant bronchoconstrictory effect in normal human subjects. Due to its negligible effect on the bronchi in normal human, it is unlikely that the cigarette serotonin will exert any bronchoconstrictory effect.

PHARMACOKINETICS

There are no oral data available on the pharmacokinetics of exogenous serotonin. Pharmacokinetics data are only available on endogenous serotonin.

Absorption

No data are available on serotonin uptake from inhalation studies.

Bioavailability

No data available on bioavailability from exogenous serotonin intake via inhalation.

Distribution

About 90% of endogenous serotonin (±10 mg) is located in the enterochromaffin cells of the gastrointestinal tract; most of the remainder is present in platelets and the CNS (6). Most of the serotonin in the body is synthesized and stored in enterochromaffin-tissue associated with the gastrointestinal tract, and is released in the blood as a potent vasoconstricting agent, with >90% of it sequestered in platelets. It is also synthesized and released by neurons, serving as a neurotransmitter in both the central and peripheral nervous system (13). Less than 1 % of serotonin in the blood is extracellular (34).

Smoking of a single cigarette caused a transient increase in platelet serotonin levels by about 350% in non-smokers, but had no additional effect in smokers. Similarly,

chewing of nicotine gum (4-8 mg nicotine) resulted in a transient increase in platelet 5-HT by about 100% in non-smokers, but not in smokers. In conclusion, smoking of cigarettes can cause an increase in platelet serotonin, most likely via enhanced supply of serotonin from enterochromaffin cells, which can be stimulated via nicotine receptors (10).

Metabolism
Serotonin found in enterochromaffin cells and neurons is synthesized in situ from tryptophan. Tryptophan is first hydroxylated to 5-hyroxytryptophan by enzym tryptophan-5-hydroxylase and is then decarboxylated to serotonin by non-specific aromatic L-amino acid decarboxylase. Serotonin is then taken up into secretory granules and stored.
Most of the serotonin, endogenous or ingested, undergoes oxidative deamination by monoamine oxidase to form 5-hydroxyindoleacetaldehyde. This is promptly degraded, mainly by further oxidation, to 5-hydroxyindoleacetic acid (5-HIAA) by aldehyde dehydrogenase; 5-hydroxyindoleacetalhyde is also reduced (by alcohol dehydrogenase) to 5-hydroxytryptophol (5-HTOL). The three enzymes are present in liver and various tissues that contain serotonin, including the brain and the lung (6). Serum serotonin is inactivated by pulmonary and vascular endothelial monoamine oxidase, hepatic inactivation and cellular reuptake. Rapid inactivation of unbound serotonin appears to be an important part of normal serotonergic activity (13, 34).

Excretion
The principal metabolite, 5-HIAA, is excreted in the urine, along with much smaller amounts of 5-HTOL, mainly as the glucuronide or sulfate. About 2 to 10 mg of 5-HIAA is excreted daily by normal adults as a result of metabolism of endogenous serotonin. Patients with malignant carcinoid (tumor of neuroendocrine cells) excrete larger amounts. Ingestion of ethyl alcohol diverts 5-hydroxyindoleacetaldehyde from the oxidative route to the reductive pathway, because of the elevated concentration of NADH. This greatly increases excretion of 5-HTOL and correspondingly reduces that of 5-HIAA (6). Ingestion of serotonin rich food (banana or walnuts) elevated the excretion of 5-HIAA in the urine. Smoking of 20- 30 cigarettes per day had no influence on the 5-HIAA urinary excretion (3).
The pulmonary microvascular endothelium has been shown to be a very important component in the clearance of many circulating bioactive compounds through the pulomonary tissue. It was discovered that serotonin is extensively removed (by about 70%) during a single passage through the lungs of dogs as well as in humans (13).

Kinetic parameters
An amount of serotonin roughly equal to that present in the body is synthesized each day. Turnover times of serotonin in brain and gastrointestinal tract have been estimated at about 1 and 17 hours, respectively (6).

Critical assessment
Serotonin is an endogenous compound, which is distributed throughout the body. It is mainly stored in the enterchromaffin cells (90 %), in the platelets and the CNS. It is synthesized in situ from tryptophan and is metabolised in various tissues. The pulmonary microvascular endothelium has been shown to be very important in the clearance of endogenous serotonin from the plasma; about 70 % serotonin is cleared in a single passage through the lung. Considering the large endogenous serotonin pool (10 mg), it seems unlikely that the low serotonin dose from cigarette smoke (estimated 15 µg/day) will affect the serotonin level in the body. However, the pharamacokinetics on exogenous serotonin, like serotonin inhalation, is not known.

Conclusion
Only pharmacokinetics data based on endogenous serotonin are known. Conclusions on kinetics from respiratory administration can not be drawn based on the endogenous serotonin kinetics.

TOXICOLOGY
The toxicity data on serotonin that are available in the literature are mostly related to increased endogenous serotonin or its metabolites level by metabolic or medication effect.

Acute toxicity
Human
Acute toxicity of serotonin is displayed when the endogenous serotonin level or its metabolites is raised by exogenous factors (drugs (serotonin reuptake inhibitor, monoamine oxidase inhibitor), food (banana or walnuts) in combination with alcohol, tryptophan) or by serotonin hyperproduction in the body (carcinoid tumor). The most common clinical symptoms observed are: nausea, vomiting, headache, diarrhea and uremic anorexia (11, 35, 36).
The serotonin syndrome (SS) is a toxic reaction to a (relative) hyperserotonergic condition in the brainstem and the spinal cord. Motoric restlessness and anxiety, fever, diaphoresis, and myoclonus characterize the syndrome. The syndrome is probably an extreme form of well-known adverse effects. A particular high risk is seen at combination treatments with monoamine oxidase inhibitors and serotonergic agents (37).
Animal
  sc-rat LD_{50}: 285 mg/kg (38)
  iv-rat LD50: 30 mg/kg (38)
  oral-mouse LD_{50}: 60 mg/kg (38)
  ipr-mouse LD_{50}: 160 mg/kg (38)
  sc-mouse LD_{50}: 601 mg/kg (38)
  iv-mouse LD_{50}: 81 mg/kg (38)
  ims-mouse LD_{50}: 750 mg/kg (38)
  iv-guinea-pig LD_{50}: 12,8 mg/kg (38)

Local tolerance
Human
No data available
Animal
No data available

Repeated dose toxicity

Subacute
No data available
Semichronic
No data available
Chronic
Serotonin is a vasoactive amine, which has been suggested to be a mediator in a wide number of vascular pathologies in human. Alterations in peripheral serotonin have been related to a major risk in suffering vascular diseases in the diabetic population. Also, the valvular thickening seen in carcinoid heart syndrome could be associated with serotonin. The mechanism of the plaque formation is poorly understood, and may involve either kinins or serotonin and its metabolite, 5-hydroxyindoleacetic acid. Its role in hemostasis and thrombosis is not clear. It does amplify aggregation induced by other aggregating agents and in certain individuals can induce aggregation alone.

It has also been shown to constrict coronary arteries in patients with coronary artery disease (13, 39).

Furthermore, serotonin is involved in the hypothalamic control of pituitary secretion, in sleep/arousal states, in regulation of circadian rhythms and inhibition of food intake. Disturbances of these serotoninergic systems have been linked to clinical depression and obsessive-compulsive disorder (13).

No data are available on repeated dose toxicity of animals.

Carcinogenicity

Human

No data available

Animal

Serotonin have mitogenic effect on vascular smooth muscle cells and on megakaryocytopoiesis (at serotonin concentration of 100 nmol/L) (40, 41), which is mediated by the 5-HT_2 receptors. However, no data are available on the carcinogenicity effect of serotonin.

Reproduction toxicology

Human

No data available

Animal

Serotoninergic pathways are involved in the neuroendocrine regulation of the sex hormones (42, 43).

Serotonin was intraperitoneally injected to adult male rats. Serotonin injected with a single dose for 2 h (10 mg kg^{-1} bodyweight) showed an inhibition of serum concentrations of luteinizing hormone (LH) and of inhibin and testicular interstitial fluid (IF) volume and intratesticular testosterone concentrations. After four daily injections of serotonin (10 mg kg^{-1}), the testis weight was decreased, and IF volume was increased nearly three-fold. Testis concentrations of inhibin and serum testosterone were reduced, whereas serum concentrations of both LH and follicle-stimulating hormone (FSH) were elevated. Although serotonin also inhibited pituitary LH release and Leydig cell steroidogenesis, these effects appeared to play only a minor role in the induction of spermatogenic damage (44).

Several serotonin reuptake inhibitors caused craniofacial malformations by inhibition of serotonin uptake into craniofacial epithelia of whole mouse embryo in culture (45).

Mutagenicity

Human

No data available

Animal

No data available

Other

Critical assessment

The serotonin toxicity is observed by elevated local or systemic serotonin level, that is induced by several exogenous agents or by carcinoid tumor. Several pathologies are related to the increased serotonin level. No toxicity data on the effects of serotonin administered through inhalation are available. It is unlikely that exposure to serotonin through smoking leads to systemic serotonin levels that exert toxicologically relevant effects.

Conclusion

Since no data on the toxicological effects of serotonin exposure through inhalation are available, the influence of exposure to serotonin through smoking on the respiratory system cannot be established. Given the high endogenous serotonin levels as compared to the exposure via smoking, it is unlikely that systemic effects will be induced.

INTERACTIONS

Chemical

One-electron oxidation of serotonin with N-3(**.**) and Br-2(**.**) radicals resulted in the formation of an indoloxyl radical with a pK(a) value much less than 3. The reactions of OH radicals ((OH)-O(**.**)) with serotonin lead to the formation of (OH)-O(**.**)-adducts, which decay by acid catalyzed water elimination to give almost quantitatively the corresponding indoloxyl and indolyl radicals, respectively. The first-order rate constants determined for water elimination are pH dependent, suggesting that the dehydration reaction is acid and base catalyzed. The (OH)-O(**.**)-adduct of serotonin reacts with oxygen in competition with the dehydration reaction to yield a peroxyl radical adduct, which is tentatively suggested to eliminate HO2(**.**). On the basis of the above findings, the mechanisms for the (OH)-O(**.**)-induced formation of indoloxyl from serotonin is proposed (46).

In vivo

Numerous agents affect the serotonin level in the body, by inhibition of tryptophan or serotonin metabolism and by inhibition of serotonin re-uptake in the presynaps. Fructose malabsorption is associated with lower tryptophan levels that may play a role in the development of depressive disorders. High intestinal fructose concentration seems to interfere with L-tryptophan metabolism, and it may reduce availability of tryptophan for the biosynthesis of serotonin (47).The effect of changes in chronic protein intake on plasma and cerebrospinal fluid (CSF) concentrations of tryptophan and 5-hydroxyindoleacetic acid (5HIAA), the principal serotonin metabolite, was studied in monkeys. The variation in CSF 5HIAA suggested that chronic protein intake may influence serotonin synthesis and turnover, perhaps via changes in tryptophan concentrations (26). Ethanol and food (banana) affect the metabolic pathway of serotonin. The urinary excretion products of serotonin are 5-hydroxyindole-3-acetic acid (5HIAA) and 5-hydroxytryptophol (5HTOL), and the ratio of 5HTOL to 5HIAA is normally very low (< 0.01) in man. During metabolism of ethanol there is a shift in the catabolic pattern of serotonin, and the formation of 5HTOL increases appreciably at the expense of 5HIAA. This increased is more pronounced with concomitant intake of serotonin rich food (3 –4 bananas) and unpleasant symptoms symptoms (diarrhea, headache, and fatigue) are observed, which are associated with the serotonin system (11).

Inhibition of serotonin metabolism or inhibition of serotonin re-uptake in the synaps

results in "serotonin syndrome". The serotonin syndrome has increasingly been recognised in patients who have received combined serotonergic drugs. This syndrome is characterised by a constellation of symptoms (confusion, fever, shivering, diaphoresis, ataxia, hyperelflexia, myoclonus or diarrhoea) in the setting of the recent addition of a serotonergic agent. The most common drug combinations causing the serotonin syndrome are monoamine oxidase inhibitors (MAOIs) and serotonin selective reuptake inhibitors (SSRIs), MAOIs and tricyclic antidepressants, MAOIs and tryptophan. This syndrome is caused by excess serotonin availability in the CNS at the $5-HT_{1A}$-receptor (48). Propranolol increased the level of serotonin in the incubation medium of cultured Leydig cells. This serotonergic action of the drug could contribute to the impairment of sexual function reported during propranolol treatment in man (49). Epidemiological studies proved that newer anorexigen, fenfluramine (or its stereoisomer, dexfenfluramine) considerably increases the risk of pulmonary hypertension through inhibition of the serotonin receptor. The development of pulmonary hypertension is probably due to the increased plasma serotonin concentration (50).

Furthermore, serotonin can affect the toxicity of drugs. Rats are more sensitive to the nephrotoxicity of the antituberculosis drug capreomycin, than mice, rabbits, hamsters, cats, or guinea pigs. This difference in sensitivity may be related to species differences in serotonin concentrations in mast cells. Rats have a relatively high concentration of serotonin in their mast cells. Capreomycin degranulates mast cells leading to the release of serotonin which is nephrotoxic (13).

Critical assessment
Chemical
Serotonin can be oxidized and thereby radicals are formed.

In vivo
Several compounds affect the metabolic pathway of serotonin. Several agents interact with the large serotonin receptor family and affect thereby the local or systemic serotonin level and cause typical serotonin clinical effects. No data were available on respiratory interaction effects via inhalation.

Conclusion
Chemical
Serotonin can form radicals by oxidation.

In vivo
Serotonin showed several systemic interaction effects in the body. The contribution of serotonin in cigarette to the systemic interaction effects can not be established and need to be studied.

DEPENDENCY
The involvement of serotonin in the nicotine dependence was shown in the following study. Chronic nicotine administration (nicotine in water during 50 days) to male NMRI mice altered the serotonin metaboles in the brain. This alteration found in the brain indicated that serotonin might be involved in nicotine dependence and withdrawal (30).
Various studies have shown a link between tobacco dependency and serotonin in

human. Nicotine binds to nicotinic receptors in the brain, augmenting the release of numerous neurotransmitters, including serotonin. Cigarette smoke has other psychoactive properties apart from nicotinic receptor stimulation. For example, it inhibits monoamine oxidase (the enzyme responsible for breaking serotonin) in the brain. Serotonin plays a role in de reward mechanism of smoking and the antidepressive effect of smoking (51, 52) Epidemiological studies on humans have shown that tobacco smoking is being prevalent in patients with depressive disorder (31). The craving qualities of chocolate have been thoroughly reviewed and the conclusion seems to be that the pharmacological active compounds (including serotonin) in cocoa do not contribute to chocolate craving (52).

Effects of smoking cessation
Serotonin reuptake inhibitors (SSRIs) and 5-HT antagonist was shown to be effective in diminishing the smoking withdrawal negative effects. It was shown in rats that sertraline (SSRI) can counteract the hyperphagia and rapid weight gain associated with nicotine withdrawal, and might therefore be a useful adjunct to smoking cessation (53). In another study it was shown that ondansetron, a selective 5-HT$_3$-receptor antagonist, may attenuate the aversion effect associated with nicotine withdrawal, and may be useful for the treatment of nicotine dependence (54).

Critical assessment
The serotoninergic system in the brain is affected by tobacco smoking and this system plays a role in the tobacco dependency and smoking cessation. From literature on chocolate craving, it seems that exogenous serotonin does not contribute to chocolate craving quality. Considering the large endogenous serotonin pool (estimated 10 mg), it seems unlikely that the low serotonin dose from cigarette smoke (estimated 15 µg/day) will affect the serotonin level in the body.

Conclusion
Serotonin released in the brain through nicotine stimulation plays a role in the nicotine dependency. It seems unlikely that serotonin from cigarette smoke could play a significant role in the addiction process due to the large endogenous serotonin pool. However, the longterm effects of serotonin and its interaction effects with other agents in the cigarette smoke on the pulmonary system and in the tobacco addiction process is not known and need to be studied.

COMMERCIAL USE
Serotonin itself is used in the treatment of myoclonus.
Tryptophan is a precursor of serotonin. Because CNS depletion of serotonin is considered to be involved in depression, tryptophan has been used in its treatment. Although it has been given alone, evidence of effectiveness is scant and tryptophan has generally been used as adjunctive therapy in depression. Pyridoxine and ascorbic acid are involved in the metabolism of tryptophan to serotonin and have sometimes been given concomitantly. In the treatment of depression the usual dose of tryptophan is 1 g given three times daily, but some patients may require up to 6 g daily in divided doses. Lower doses may be required in the elderly especially those with renal or hepatic impairment. (55).

BENEFICIAL EFFECTS
Serotonin itself can be used as a drug in the treatment of myoclonus (48).

Critical assessment
Not relevant.

Conclusion
Not relevant.

SUMMARY AND FINAL CONCLUSION
Serotonin contains the characteristic heterocyclic indole structure, accounting for the aromatic properties (electrophilic substitution). In addition the chemical; the presence of the ring bound hydroxyl group accounts for its polar character. An additional characterising chemical feature is the presence of the aliphatic amino-group.

A source of serotonin in tobacco is cocoa powder, which is used as a flavouring agent. Little is known about the profile of pyrolysis/combustion products of serotonin.
The daily intake via cigarettes smoke (estimated to be 15 µg/day) is low compared to the oral intake via chocolate, cocoa drinks and banana (estimated 19 – 15000 µg/day), and to the endogeneous pool of serotonin (10 mg).

Serotonin binds to a large family of serotonin receptors ($5\text{-}HT_{1\text{-}7}$ subtypes). Serotonin stimulates and inhibits nerves and smooth muscles in the cardiovascular, respiratory and gastrointestinal systems. Some contradictory results were obtained about the bronchoconstrictory effect of serotonin in humans in respiratory studies. The main conclusion seems to be that serotonin has a negligible effect on the bronchi. It has a pulmonary hypertension effect on the pulmonary system. Depending on the serotonin level, it exerts complex effects on the cardiovascular system, including hypotension or hypertension, vasodilatation or vasoconstriction, and/or bradycardia or tachycardia. It also has complex effects on the CNS and is involved in the nicotine dependency.

Serotonin is an endogenous compound. It is widely distributed in the body and about 90 % is stored in the enterochromaffin cells of the gastrointestinal tract; the remainder is present in platelets and in CNS. Serotonin is metabolized by monoamine oxidase; it is extensively removed from the plasma (70%) by the pulmonary microvascular endothelium during a single passage through the lungs. The turnover of serotonin is 1h in the brain to 17 h in the gastrointestinal tract. Pharmacokinetics on exogenous serotonin through the respiratory and the intestinal tract are not available.

The toxicity data on serotonin that are available in the literature are mostly related to increased endogenous serotonin or its metabolites level in the body by metabolic or medication effect. Acute toxicity of serotonin is displayed when the endogenous serotonin level is raised by exogenous factors (drugs, food (banana or walnuts), tryptophan) or by serotonin hyperproduction (carcinoid tumor). The most common clinical symptoms observed are: nausea, vomiting, headache, diarrhea and uremic anorexia. Animal I.V. LD50 varied between 12.8 mg/kg bodyweight for the guinea pig to 81 mg/kg body weight for the mouse. Chronic serotonin toxicity is seen in

elevated plasma serotonin level; it evokes several vascular pathologies e.g. in the diabetic population. It is also involved in several CNS effects. There are no data available on the carcinogenicity effect of serotonin. Serotonin displays some reproduction toxicity effects through its neuroendocrine effect.

Serotonin can form radicals by oxidation. Several compounds affect the metabolic pathway of serotonin. Several agents interact with the large serotonin receptor family and affect thereby the local or systemic serotonin level and cause typical serotonin clinical effects. No data were available on respiratory interaction effects via inhalation.

The serotoninergic system in the brain is affected by tobacco smoking and this system plays a role in the tobacco dependency and smoking cessation. From literature on chocolate craving, it seems that exogenous serotonin does not contribute to chocolate craving quality.

It can be concluded that serotonin exerts various pharmacological and toxicological effects in the body through the large serotonin receptor family. There are no data available on the pharmacodynamics, pharmacokinetics and toxicology of exogenous serotonin after oral and inhalation exposure. Assuming similar systemic effects after oral and inhalation exposure, the additional risk for systemic effects of serotonin by cigarette smoking (estimated to be 15 μg/day) will be low compared with the oral intake via chocolate, cocoa drinks and banana (estimated 19 – 15000 μg/day). Due to its negligible effect on the bronchi in normal human subjects, it is unlikely that the cigarette serotonin dose will exert any bronchoconstrictory effect. Considering the large endogenous serotonin pool (estimated 10 mg), it seems unlikely that the low serotonin dose from cigarette smoke (estimated 15 μg/day) will affect the serotonin level in the body. Since no data on the local toxicological effects of serotonin exposure through inhalation are available, the shortterm and longterm effects of exposure to serotonin through smoking on the respiratory system cannot be established. Furthermore, its additive effects on other biogenic amines present in cigarette smoke are also not known and have to be studied.

More studies are needed on:
- the determination of pyrolysis and combustion products of serotonin in cigarette smoke;
- the local (respiratory system) effects of long-term use of serotonin alone and in combination with other biogenic amines via inhalation.

Date this sheet was generated
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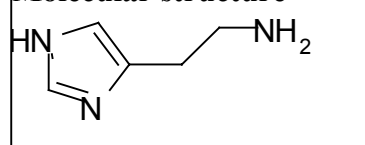   2.00.000. 2001. Micromedex Inc. Electronic Version.

3.4   Histamine

GENERAL
IUPAC systematic name: 1H-Imidazole-4-ethanamine (1)
Synonyms: 2-(4-imidazolyl)ethylamine; 4-imidazoleethylamine; 5-imidazoleethylamine; ß-aminoethylimidazole; ß-aminoethylglyoxaline (1)
Molecular formula: $C_5H_9N_3$ (1)

Molecular structure

Molecular weight: 111.15 g/mol (2)
Alifatic: yes (1)
Aromatic: yes, imidazole ring (1)
N containing: yes, imidazole and amine group (1)
Halogen containing: no (1)
CAS registry no.: 51-45-6 (2)
Storage:
R/S classification: histamine dihydrochloride: R20/21/22-36/37/38-42/43; S26-36 (3)
dangercode (transport): no data available.
Properties:
➢ melting point: 83 – 84 ºC (2)
➢ boiling point: 209 –210 ºC at 2.4 kPa(2)
➢ density: no data available.
➢ refractive index: no data available.
➢ solubility: freely soluble in water (1 g in 4 ml), alcohol and hot chloroform; sparingly soluble in ether (2)
➢ substance description:
   •   color: colorless (2)
   •   liquid/gas/powder: needles from chloroform and prisms from ethanol (2)
   •   odor/taste: odorless (2)
➢ volatility: no data available.
➢ pK_a: pK_a1= 9.68; pK_a2= 5.88 (2)
➢ PA: no data available.
➢ flammability:
   •   FP = no data available.
   •   FL Limits = no data available.
   •   IT = no data available.
➢ decomposition temperature: no data available
➢ stability: stable in air but is affected by light /phosphate (2). A study concluded that solutions of histamine phosphate could be sterilised by heating in an autoclave with little degradation. Autoclaved solutions could be stored for a minimum of 4 months (4).
➢ vapour pressure/ vapour tension (20 °C): no data available.
➢ vapour pressure (50 °C): no data available.
➢ relative density: no data available.

➢ octanol water partition coefficient, log K_{OW}: no data available.
➢ conversion factor: no data available.

Critical assessment
Histamine is composed of a heterocylic, two nitrogen atoms containing imidazole
ring (five membered ring with aromatic properties) and a short aliphatic chain ending
with a free amino group.The free amino group is a potential group to react with
aldehydes and ketones and with monoamino-oxydase (MOA).
For the nitrogen atoms in the ring: the extra pairs of electrons are involved in the pi-
cloud of the ring and are not available for sharing with acids.

Conclusion
Histamine is a nitrogen containing heterocyclic compound, linked to a short aliphatic
chain with a free amino group. The compound contains three nitrogen atoms, each
with a different character; especially the ring nitrogen atoms differ in character from
the nitrogen atom present in the amino group.
Hiastamine potentially acts as a competitor for nicotine with respect to the oxidation
reaction with monoamino-oxydase.

FUNCTION IN TOBACCO
No data available.

AMOUNT IN TOBACCO PRODUCTS
Histamine is a natural component of cocoa, which is added to tobacco as a flavouring
agent. A typical casing concentration of cocoa for cigarette tobacco is 1% (5). The
average amount of histamine in cocoa varies from $0.41 - 1.3$ µg/g (6). Assuming one
cigarette weights approximately 1 g, the maximum histamine amount from cocoa in
one cigarette is estimated to be 13 ng.

AMOUNT IN SMOKE
- **main stream**
  No data available.
- **side stream**
  No data available.

SOURCE
Histamine is natural component of cocoa, which is added to tobacco as a flavouring
agent (5).

ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE
Histamine is found in fermented foods (yeast, lactic acid fermentation). The
histamine level of some foods is: wine (1.5 mg/kg), sherry (3 mg/kg), sauerkraut (38
mg/kg), Dutch cheese (52 mg/kg) and fermented sausage (11 mg/kg) (7).

COMBUSTION PRODUCTS
By combustion of the dihydrochloride salt of histamine, toxic nitrous gasses are
generated (3). No data are available on histamine combustion products in cigarette

smoke.

CONSENSUS REPORTS
No data available.

STANDARDS AND RECOMMENDATIONS
ADI: An intake of > 40 mg biogenic amines (histamine, tryptamine, tyramine, phenylethylamine, etc.) per meal has been considered potentially toxic. Switzerland considered 10 mg histamine per liter wine a permissible limit. The European Economic Community has put a maximum limit for histamine in for fresh fish (200 mg/kg) and for enzymatically ripened fish (400 mg/kg) belonging to Scombridae and Clupedae families (8).
TWA$_{NL}$ = MAC: no data available.
TWA$_D$ =MAK: no data available.
TWA$_{USA}$: no data available.
STEL$_{NL}$: no data available.
STEL$_{USA}$: no data available.
LTEL: no data available.
TLV-C: no data available.
TLV-CARCINOGENICITY: no data available.
MAK-REPRODUCTION: no data available.

Others:

Reference value:
Skin histamine concentrations (2.09 ± 0.31 µg/l) were found to be significantly higher than plasma histamine concentrations (0.48 ± 0.08 µg/l) (9). Median plasma histamine concentration was reported to be 0.53 (range 0.21-1.59) µg/l (n = 18). Median total cell-bound histamine content was 46.3 (range 19.6 – 101.1) µg/l in whole blood and 52.8 (range 40.0 – 173.4) µg/l in plasma-reduced whole blood (10). The mean histamine content ranged from 2.5 ± 0.5 pg/mast cell for the smallest diameter mast cells (8-10 µm) to 10 ± 2.5 pg/mast cell for the largest (16-20 µm) (11). It was shown that plasma histamine levels followed biorhythmic changes with 3 maxima and 3 minima. The acrophases of the maxima are 12.77 ± 0.61, 19.33 ± 0.78 and 5.42 ± 1.83 h. The most important rise in plasma histamine levels was found in the early hours of the morning (12).

CLASS
EG Carc. Cat.: No data available.
IARC-category: No data available.
CEC: No data available.

Critical assessment
Comparison of smoking related potential daily intake of histamine with histamine daily intake from other sources

	SMOKING	**HISTAMINE INTAKE BY EATING OR DRINKING**		
	25 cigarettes (1 % cocoa)	3 chocolate bars of 60 g	wine (125 g)	Dutch cheese (50 g)
Histamine (µg)	0.33[6]	200[6]	185[7]	2600[7]

Little is known about the profile of the pyrolysis/combustion products of histamine.

Conclusion
The daily intake of histamine from cigarettes (from added cocoa) is about 500 times less than histamine intake from other sources such as chocolate, wine or Dutch cheese. Assuming similar bioavailability, the plasma concentration reached after ingestion of histamine from chocolate sources or other food sources is expected to be significantly higher, than after exposure from cigarettes. However, the different route of application via smoking as compared to other sources should be taken into account. Therefore, the systemic and the local effect of smoking related exposure to histamine might be a point of concern. Since nothing is known about the pyrolysis/combustion products of histamine in cigarette smoke, this may be an additional point of concern.

PHARMACODYNAMICS
Mechanism of action
Histamine is an autacoid that is closely associated with mast cells and functions as a mediator of inflammation. Like serotonin, it is also a neurotransmitter in the central and peripheral nervous systems. Its effects are mediated by three receptor subtypes with differential selectivities for both agonists and antagonists (H_1, H_2 and H_3) (13, 14) They share structural and membrane topography features with other metabotropic receptors. Histamine receptors H_1 and H_2 are postsynaptic whereas H_3 is presynaptic. There are no known natural toxins or toxicants of histamine receptors. The H_1 and H_2 antagonists are potent therapeutics. The potent therapeutic H_1 agonists are effective for the treatment of allergies, but their side effects and toxicities include sedation and anticholinergic actions. Antagonists for H_2 receptor are excellent therapeutics for peptic and gastric ulcers, because of their ability to block histamine-induced gastric acid production (15).
Recently a new histamine receptor, H_4, was discovered in bone marrow and it may be a therapeutic target for the regulation of immune function, particularly with respect to allergy and asthma (16).
H_1-receptors have been detected in a wide variety of tissues including: mammalian brain, smooth muscle from airways, gastrointestinal tract, genito-urinary system, cardiovascular system, adrenal medulla, endothelial cells and lymphocytes. The primary mechanism by which histamine H_1-receptors produce functional responses in cells is the activation of phospholipase C. An H_1-receptor mediated increase in either inositol phosphate accumulation or intracellular calcium mobilization has been described.
Histamine H_2-receptors have a potent effect on gastric acid secretion. This receptor occurs in cardiac tissues, smooth muscle of the airway, uterine and vascular system in high densities and is widely distributed in the brain. H_2-receptors in basophils and mast cells have been shown to negatively regulate the release of histamine. Histamine H_2-receptors is coupled to the adenylyl cyclase via the GTP-binding protein G_s. H_2-receptor mediated effects on cAMP accumulation have been observed in brain cells, gastric mucosa, cardiac myocytes, vascular smooth muscle and neutrophils.
Histamine H_3-receptors have inhibitory effects on the neurotransmitter release in the CNS and in the periphery. The signal transduction pathway of the H_3-receptor is

unclear, but it is suggested that this receptor belongs to the superfamily of G-protein-coupled receptors (14).

Pulmonary system

- **breathing frequency**: In spontaneously breathing dogs, the inhalation of histamine caused an increased respiratory frequency, decreased tidal volume, and decreased dynamic lung compliance (17). However, some conflicting results were obtained about the breathing frequency in humans after histamine inhalation. In one study the breathing frequency remained unchanged after histamine inhalation in both nonsmokers and smokers (18). In another study the effects of inhalation of histamine on respiratory frequency (fR) were evaluated in 63 humans. Forty four subjects were hyperresponsive (BHR+). In each of these subjects, the doses of histamine applied for the present study (mean 3.5 mg/ml) caused a decrease in forced expiratory volume in one second (FEV1) that was greater than 20% of the control value. The dose of histamine applied in the 19 nonhyperresponsive subjects (BHR-) was substantially larger (8.0 mg/ml) whilst for this dose the decrease in FEV1 was less than 20% of control value. After histamine, fR was significantly increased in both subgroups of subjects, BHR+ and BHR-. In general, the changes in fR were not uniform; 40 subjects responded with an increase and 23 with a decrease (19).

- **tidal volume**: The respiratory response to bronchospasms induced by histamine inhalation was measured in nonsmokers and asymptomatic smokers. In each subject, tidal volume (VT) and inspiratory time (TI) were measured. The respiratory responses to histamine were the same in both groups: the tidal volume (VT) increased and the inspiratory time (TI) remained unchanged. Thus, VT/TI, an index of respiratory drive also increased (18).
  In another study, the effects of inhalation of histamine on respiratory frequency (fR), tidal volume (VT), minute ventilation (V'E), and functional residual capacity (FRC) were evaluated in 63 humans. Forty four subjects were hyperresponsive (BHR+). In each of these subjects, the doses of histamine applied for the present study (mean 3.5 mg/ml) caused a decrease in forced expiratory volume in one second (FEV1) that was greater than 20% of the control value. The dose of histamine applied in the 19 nonhyperresponsive subjects (BHR-) was substantially larger (8.0 mg/ml) whilst for this dose the decrease in FEV1 was less than 20% of control value. After histamine, fR was significantly increased in both subgroups of subjects, BHR+ and BHR-. The increase in V'E was significant in BHR- but not significant in BHR+. In general, the changes in V'E, fR and VT were not uniform; comparable numbers of subjects responded with increases (n=33) and decreases (n=30) in V'E. For fR 40 subjects responded with an increase and 23 with a decrease, and for VT these numbers were 26 and 37, respectively. The increase in FRC after histamine application was significantly larger in BHR+ subjects than in BHR-. These findings may be interpreted to indicate that different mechanisms with opposite effects may be operating simultaneously, e.g. excitation of central inspiratory activity by stimulation of rapidly-adapting pulmonary stretch receptors, which will promote increases in respiratory frequency, tidal volume and minute ventilation, and bronchoconstriction with increased airway resistance, which will promote decreases in these parameters. As a consequence, depending on the net result of these opposite contributions to, e.g. minute ventilation, administration of

histamine will cause an increase in minute ventilation in one subject and a decrease in another (19).

Both hypercapnic (n = 7) and normocapnic (n = 6) patients with chronic obstructive pulmonary disease were exposed to doubling concentrations of aerosolized histamine, and FEV1 was measured 30 and 90 s after each 2-min exposure. A provocative dose (PD_{20}) of histamine was defined as that which produced a 20% decrease in FEV1. At PD_{20}, minute ventilation and tidal volume (VT) decreased in both groups. The decrease in VT was significantly greater in the normocapnic patients. Inspiratory flow (VT/TI) did not change in either group (20) (the dosage is not mentioned in the abstract).

- **lung compliance**: In spontaneously breathing dogs, the inhalation of histamine caused a decreased dynamic lung compliance (17) (the dosage is not mentioned in the abstract).

- **airway resistance**: The airway resistance is increased by histamine. Histamine inhalation dose causing a 20% fall in forced expiratory volume in one second (PD_{20}) has been described by several studies. It was shown in young normal adults that the optimal cut-off point for PD_{20} was 0.73 mg (21). Another study found a mean histamine PD_{20} dose of 1.20 mg in young normal adults. In asthmatics the histamine PD_{20} dose was 0.23 mg (22). In 6 subjects in whom dose-response curves were obtained for mass of histamine deposited in the lungs and the FEV1, the mean deposited histamine mass required to decrease the FEV1 by 10% was 0.11 mg (23).

Cardiovascular system

Histamine is stored in large amounts in human cardiac tissue, where it is contained in cytoplasmatic granules of mast cells (24). Histamine content in human heart tissue was found to be 1.7 ± 0.1 µg/g wet weight (mean ± standard error on the mean). Spontaneous release of histamine from heart tissue is negligible. The local concentration of histamine appears to be high enough to play some role in the modulation of several cardiac functions in vivo (25).

- **blood pressure**: Histamine characteristically causes dilatation of the finer blood vessels, resulting in flushing, lowered total peripheral resistance and a fall in systemic blood pressure. In addition histamine tends to increase capillary permeability. Its effects on the heart are generally less important. The vasodilatation involves both H_1 and H_2-receptors, distributed throughout the resistance vessels in most vascular beds. Activation of H_1-receptors mediates a dilatation that is relatively rapid in onset and short-lived. Activation of H_2-receptors mediates a dilatation that develops more slowly and is more sustained (13).

  Intracerebroventricularly (i.c.v.) injection of histamine in rat produced a prompt dose-dependent (0.01 – 11µg/dose) and long-lasting (1-11 µg/dose) increase in mean arterial pressure (MAP), pulse pressure (PP) and heart rate (HR). It was concluded that histamine H_2 receptors were involved in the histamine induced central cardiovascular effects (26). Experiments have been made in anaesthetised cats and dogs and in healthy, human volunteers to compare the changes in blood

pressure and heart rate during systemic administration of histamine. Histamine, 0.1 – 11.1 µg/kg/min, lowered blood pressure in a similar dose-dependent fashion in all three species. In man and in cat this was accompanied by clear dose-dependent tachycardia whereas in dog the heart rate changes were minimal. Pharmacological analysis of the depressor responses to histamine in all three species and the reduction in total peripheral resistance in cat and dog showed that the immediate responses to histamine in all three species involved H_1-receptors and that sustained responses involved H_2-receptors (27).

In pithed guinea pigs, the general characteristics and origin of the pressor response to intravenous injection of histamine were examined. Histamine (5-80 µg/kg) produced a rapid, short-lasting, constant, prominent and dose-dependent pressor response, followed by a secondary slight and prolonged depressor response. The vascular response to histamine was accompanied by a marked tachycardia. The pressor effect of histamine (30 µg/kg) was strongly reduced or abolished in animals pretreated with nicotine, reserpine, bretylium or 6-hydroxydopamine. Furthermore, pyrilamine, a histamine H_1-receptor antagonist, antagonized in a dose-dependent manner the pressor response to histamine. On the contrary, metiamide, a histamine H_2-receptor antagonist, as well as hexamethonium and atropine, cholinergic antagonists, did not suppress the pressor effect of histamine. Those experiments provide evidence that in guinea-pigs, the pressor component of the vascular response to histamine results predominantly from the activation of histamine H_1-receptors in the sympathetic ganglia with consequent release of noradrenaline at postganglionic sympathetic nerve terminals (28).

- **heart rate**: Histamine is released into the systemic circulation during anaphylaxis by drugs and by surgical procedures. Studies in animal models have conclusively demonstrated that released cardiac histamine is a major mediator of arrhythmias that occur during anaphylaxis and following the administration of histamine-releasing drugs. Several lines of evidence suggest a similar arrhythmogenic role for cardiac histamine in humans: (1) The human heart is rich in histamine; (2) cardiac histamine can be readily released from human heart in vitro by therapeutic concentrations of drugs; (3) histamine has potent arrhythmogenic effects on the human heart in vitro. Arrhythmogenic effects of histamine include enhancement of normal automaticity, induction of abnormal automaticity, induction of triggered tachyarrhythmias, depression of atrioventricular conduction, and increase in the vulnerability of the ventricles to fibrillation (24, 29).

Renal system
It is suggested that 1) H_1 and H_2 receptors are present in the renal vasculature, 2) changes in intrarenal blood flow distribution are not responsible for histamine-induced diuresis, and 3) H_1 receptors are primarily postglomerular while H_2 receptors exhibit both pre- and postglomerular distribution (30, 31).

- **diuresis**: Histamine, when given intracerebroventricularly (i.c.v.), has been reported to produce antidiuresis in the rabbit. Histamine (H), 100 µg/kg i.c.v., produced antidiuresis with decreases in renal plasma flow and glomerular filtration rate in urethane-anesthetized rabbits. With larger doses, a tendency towards increased electrolyte excretion was noted in spite of decreased filtration.

In the denervated kidney, marked diuresis and natriuresis were observed following i.c.v. histamine, whereas the contralateral innervated kidney responded with typical antidiuresis. It was suggested that histamine, given i.c.v., influences renal function in dual ways, i.e., antidiuresis by increasing the sympathetic tone to the kidney and diuresis due to some humoral natriuretic factor, the latter becoming apparent only when the former influence has been removed. Further it is suggested that H_1-receptors might be involved in the nerve-mediated antidiuresis, whereas H_2-receptors might mediate the humorally induced natriuresis and diuresis (30). The actions of intracerebroventricularly-infused (i.c.v.) (11 – 89 µg/dose) histamine and selective histamine H_1, H_2 and H_3 receptor agonists on urine flow were studied in rats. It was found that both metoprine and thioperamide, which increase histaminergic activity through different mechanisms, also reduced food intake. This finding indicates that the brain histaminergic system is associated with feeding behavior. The same is true for body water homeostasis. Histamine (i.c.v.) caused a long-lasting diuresis. Also H_2 agonists dimaprit and metoprine increased urine flow and the blockade of H_2 receptors abolished the diuretic responses to histamine and dimaprit. On the other hand, the H_3 agonist (R)-alpha-methylhistamine elicited drinking and this effect could be prevented by thioperamide pretreatment. The results imply that activation of H_3 receptors predominantly provokes drinking, whereas central H_2 receptors mediate the diuretic effect of histamine (32).

- **saluresis**: see section diuresis

Nervous system
- **central nervous system**: Histamine receptors are widely distributed in the CNS. (13, 14). The central histamine receptors may regulate the cardiovascular system (24, 26) diuresis (30, 32) and food intake (32, 33).

- **autonomic system**: No data available.

Other

Maximal gastric secretion was induced in 122 control subjects (without peptic ulcer) and 201 preoperative duodenal ulcer patients by intravenous histamine acid phosphate (14.4 µg/kg/h), and measured as gastric secretory volume (ml/h) and maximal acid output (mmol/h). In both groups, men secreted more than women, and smokers secreted more than non-smokers. Significant correlations were found between maximal gastric secretion on the one hand, and height, age, and chronic smoking on the other (34).

Critical assessment

Histamine is an autacoid that is closely associated with mast cells and functions as a mediator of inflammation. Histamine is a neurotransmitter in the central and peripheral nervous systems. It mediates its effects through three receptor subtypes with differential selectivities for both agonists and antagonists (e.g., mepyramine for H_1, ranitidine for H_2, and thiperamide for H_3). Through these receptors, histamine evokes several physiological effects. Histamine characteristically causes dilatation of the finer blood vessels, resulting in flushing, lowered total peripheral resistance and a fall in systemic blood pressure. The released cardiac histamine is a major mediator of arrhythmias that occur during anaphylaxis and following the administration of

histamine-releasing drugs. Histamine receptors are widely distributed in the CNS. The central histamine receptors may regulate the cardiovascular system, diuresis and food intake. Histamine also induces gastric secretion.

Histamine provokes bronchoconstriction, but some conflicting results were found about the breathing frequency and the tidal volume. It seems that different mechanisms with opposite effects are acting simultaneously.

The histamine inhalation dose causing a 20% fall in forced expiratory volume in one second (PD_{20}) was shown to be between 0.73 mg -1.20 mg in young normal adults. In astmatics the histamine dose for PD_{20} was significantly lower (about 0.23 mg). The estimated daily histamine intake through cigarette smoking is about 2000 times less than the PD_{20} in normal adults. Therefore it is expected that the histamine dose in cigarette will not evoke any bronchoconstrictory effects.

Conclusion
It seems that the histamine dose of cigarette smoking is not high enough to evoke any bronchoconstrictory effects. However, the (longterm) effects of histamine and/or its pyrolysis/combustion products on the pulmonary system are unknown and need further study.

PHARMACOKINETICS
Absorption
In four Ascaris-sensitive rhesus monkeys, the fractional absorption of ^{3}H-histamine (^{3}HH) and airway response, as pulmonary resistance (R1), was measured of standard histamine aerosols containing trace amounts of ^{3}HH for control runs (Run 1) and of runs after Ascaris antigen challenge (Run 2). The mean rate of accumulation of radioactivity in the plasma volume as a function of delivered dose during histamine exposure (2 min) was fivefold greater for Run 2 (0.047% delivered dose/min) as compared with Run 1 (0.009% delivered dose/min). These data are consistent with the hypothesis that airway mucosal hyperpermeability induced by an allergic reaction is one of the factors contributing to airway hyperreactivity by increasing flows of inhaled bronchoactive agents to effector sites in the airway wall (35).

In a double blind oral test histamine-rich (22.8 mg/l) and histamine free wine to eight healthy subjects were given. Blood samples were taken at 0, 10, 30 and 45 minutes after ingestion of the wine for measurement of plasma histamine and methylhistamine. Urine was collected 5 hours before and 5 hours after ingestion for measurement of urinary methylhistamine. No change in plasma histamine and plasma and urinary methylhistamine was seen. This study shows that the amount of histamine in wine has no clinical or biological effect in healthy subjects, and this emphasised the efficiency in man of the systems for degradation of histamine that is absorbed by the alimentary tract (36).

Bioavailability
The bioavailability through the pulmonary system seems to be high. In a study, ^{14}C-histamine was administered intrabronchially to asthmatic patients and controls. The urinary excretion of total radioactivity, ^{14}C-histamine and its radioactive metabolites was measured. It was found that the excretion of total radioactivity was complete within 24 h. The excretion rate was equal to that observed after intravenous injection

of ^{14}C-histamine, indicating a rapid penetration of the bronchial mucosa and high bioavailability (37). Histamine that is ingested or formed by bacteria in the gastrointestinal tract is rapidly metabolised and excreted in the urine (13).

Distribution

Almost all mammalian tissues contain histamine in amounts ranging from less than 1μg up to more than 100 μg/g tissue. Concentrations in plasma and other body fluids are generally very low, but human cerebrospinal fluid contains significant amounts. The mast cell is the predominant storage site for histamine in most tissues, especially in the skin, the mucosa of the bronchial tree and the intestinal mucosa (13).

Metabolism

Every mammalian tissue that contains histamine is able to synthesise it from histidine by virtue of its contents of L-histidine decarboxylase. Since this enzym is inducible, the histamine-forming capacity at non-mastcell sites is subject to regulation by various physiological and other factors. There are two major paths of histamine metabolism in man. The more important of these involves methylation and is catalysed by the enzym histamine-N-methyltransferase, which is widely distributed. Most of the product, N-methylhistamine is converted by monoamine oxidase (MAO) to N-methyl imidazole acetic acid. Alternatively, histamine undergoes oxidative deamination catalyzed mainly by the nonspecific enzyme diamine oxidase (DAO). The products are imidazole acetic acid and eventually its riboside (13).

Excretion

In mammals, the metabolites resulting from catalysation are excreted in the urine (13). ^{14}C-histamine was administered intrabronchially to asthmatic patients and controls. The urinary excretion of total radioactivity, ^{14}C-histamine and its radioactive metabolites was measured. It was found that the excretion of total radioactivity was complete within 24 h. The excretion rate was equal to that observed after intravenous injection of ^{14}C-histamine, indicating a rapid penetration of the bronchial mucosa. However, the diuresis seemed to be of importance for the excretion rate (37). The urinary excretion of histamine and its metabolites, methylhistamine, methylimidazoleacetic acid and imidazoleacetic acid, was measured under standardized dietary conditions in 24 women with normal pregnancies and in eleven patients with toxaemia of pregnancy. A slight increase in the urinary excretion of methylimidazoleacetic acid was observed in normal pregnancy as well as in toxaemia of pregnancy compared to non-pregnant women. In two toxaemic patients and in one of the healthy subjects the urinary excretion of unmetabolized histamine was moderately increased. Despite the very high diamino oxidase activity in the plasma and in the uterus during pregnancy, there were no signs of altered catabolism of endogenous histamine in the pregnant women. Smoking increased the urinary excretion of the quantitatively dominant histamine metabolite, methylimidazolacetic acid (38).

Kinetic parameters

Histamine was co-administered with interleukin-2 (IL-2) in a phase III study in patients with metastatic melanoma, offering a survival advantage over IL-2 treatment alone. In order to characterize any drug-drug interactions between IL-2 and histamine, a phase I pharmacokinetic (PK) study was initiated. Histamine and IL-2

were administered to twelve patients (8 with metastatic melanoma and 4 with metastatic renal cell carcinoma). Histamine was administered slowly by subcutaneous injection over 20 minutes. Serial blood samples were collected during the first 240 min for analysis of serum histamine. The patient population was predominantly Caucasian (92%) and male (83%) with an average age of 52.3 years. Histamine had $t_{1/2}$ 12.7 min and Vd 66.0 l (39) (the histamine dose was not mentioned in the abstract).

Critical assessment
^{14}C and ^{3}H-Histamine studies showed that histamine is absorbed through the pulmonary system. Almost all mammalian tissues contain histamine in amounts ranging from less than 1μg up to more than 100 μg/g tissue.

All mammalian tissues that contain histamine are able to synthesise it from histidine by means of their contents of L-histidine decarboxylase. Histamine kinetic parameters determined in patients with melanoma's had $t_{1/2}$ 12.7 min and Vd 66.0 l. The small $t_{1/2}$, seems to implicate a rapid histamine metabolisation.

Conclusion
Histamine is absorbed through the respiratory system. However, due to the rapid histamine metabolisation it is not expected that the histamine dose in cigarette will be high enough to affect the plasma histamine level.

TOXICOLOGY
Acute toxicity
Human
Injection of histamine can produce a wide range of adverse effects that includes headache, flushing of the skin, general vasodilatation with a fall in blood pressure, tachycardia, bronchial constriction and dyspnoea, visual disturbances, vomiting, diarrhoea, and other gastrointestinal effects. These reactions may be serious and excessive dosage can produce collapse and shock, and may be fatal. Reactions may occur at the injection site (14).

In a study a case of occupational histamine poisoning by spoiled fish flour via inhalation, skin and eye contact was described. Twenty harbour workers handled shipments of fish flour transported in black or blue bags. Ten workers handling blue bags developed allergy-like skin, eye, gastrointestinal, respiratory and cardiac symptoms within 30 min. Workers handling black bags were symptom-free, except for minimal eye irritation. The histamine content was 10-fold higher in samples from the blue than from the black bags (510 mg/100 g flour compared with 50 mg/100 g flour, respectively) (40). It is often stated that ingestion of foods rich in histamine can result in absorption of sufficient histamine to provoke signs and symptoms reminiscent of an allergic reaction. Histamine ingestion in excess of 36 to 250 mg may result in a clinical response, which includes abdominal complaints, feelings of warmth, flushing and headache. (41).

Several foods contain histamine at levels potentially toxic for man; amongst the most frequently incriminated products is fish, especially the scombroid species (tuna, mackerel), which plays a pre-eminent role in the etiology of the so called scombrotoxic fish poisoning. This syndrome begins from a few minutes to two hours

from incriminated meals and presents itself with the characteristic signs and symptoms of histamine activity on various organs and is very rarely, if ever, life threatening. Histamine formation in food is due to the decarboxylase activity of some microorganisms, mainly enterobacteria; they can be part of its normal flora or represent a secondary contamination and find a favourable environment for outgrowth if food is not stored or processed in proper conditions (42).

Animal
No data available

Local tolerance
Human
Eye effect of an 8% (w/w) solution of histamine hydrochloride have been described in case of girl who spilled some on her handkerchief and contaminated her right eye. In 10 min conjunctivae became hyperaemic and lids oedematous, without discomfort. Reaction had nearly disappeared in 5 hr and completely gone next day. In some human glaucomatous eyes, application of 3% (w/w) histamine hydrochloride eyedrops has been known to cause rise in ocular pressure, particular in cases of acute glaucoma. Injected intradermally a triple response follows: red spot, flare developing and more slowly a localised oedema. These effects are due to local dilatation of minute blood vessels, the dilatation of neighbouring arterioles and the direct action on walls of vessels to increase their permeability (2).

Animal
scu-rat LDLo: 250 mg/kg (43)
ivn-rat LD_{50}: 630 mg/kg (43)
orl-mouse LD_{50}: 220 mg/kg (43)
ipr-mouse LD_{50}: 725 mg/kg (43)
scu-mouse LD_{50}: 2500 mg/kg (43)
ivn-mouse LD_{50}: 385 mg/kg (43)
scu-dog LDLo: 28.5 mg/kg (43)
ivn-dog LD_{50}: 7 mg/kg (43)
ivn-monkey LDLo: 50 mg/kg (43)
scu-cat LDLo: 34 mg/kg (43)
scu-rabbit LDLo: 12 mg/kg (43)
ivn-rabbit LDLo: 2 mg/kg (43)
orl-guinea pig LDLo: 200 mg/kg (43)
ipr- guinea pig LD_{50}: 5 mg/kg (43)
scu- guinea pig LDLo: 8 mg/kg (43)
ivn- guinea pig LD_{50}: 0.18 mg/kg (43)
scu- guinea pig LDLo: 1700 mg/kg (43)

Repeated dose toxicity
Subacute
No data available.

Semichronic
No data available.

Chronic

Hyperresponsiveness to histamine is a key feature of a variety of pathological conditions, including bronchial asthma, food allergy, colitis ulcerosa, and topical allergic disorders. Several mediators enhance the cellular reaction to histamine in cell types involved in pathological and immunological histamine hyperresponsiveness (44).

Pathological histamine levels are correlated with several disorders. Normal and pathological plasma histamine levels vary considerably in the literature. The normal range for human plasma histamine as 0-1.0 ng/ml. Values greater than 1 ng/ml have to be considered as pathological (45).

Patients with B cell chronic lymphocytic leukemia (B-CLL) have decreased capacity to mount relevant antibody responses upon immunization, and development of hypogammaglobulinemia is part of the natural history of the disease. Plasma histamine levels determined in B-CLL patients were 2-fold to 20-fold higher in 23 out of 31 B-CLL patients, compared to normal controls and these levels showed a significant positive correlation to disease duration. The increased plasma histamine levels, strongly suggests the involvement of histamine in the pathogenesis of B-CLL immunodeficiency (46).

In one study the basal plasma histamine level and eosinophil count in the peripheral blood in patients with a history of allergy (allergic patients) were examined and compared with those in patients without any history of allergy (non-allergic patients). The mean basal plasma histamine level in non-allergic patients (n = 70) and allergic patients (n = 70) were 0.31 ± 0.27 ng/ml and 0.47 ± 0.30 ng/ml, respectively ($p < 0.01$). The mean eosinophil counts in non-allergic patients and allergic patients were $3.3 \pm 3.0\%$ and $5.3 \pm 3.4\%$ of total white blood cells, respectively ($p < 0.01$). The patients who had asthma, atopic dermatitis or a food-induced allergy showed a high level of basal plasma histamine compared to that in non-allergic patients. The patients with asthma, allergic rhinitis or atopic dermatitis all demonstrated a higher eosinophil count than non-allergic patients. In addition, the correlation between the plasma histamine level and the eosinophil count was statistically significant ($p < 0.05$). It was concluded that the allergic patients had both higher basal plasma histamine levels and eosinophil counts than non-allergic patients ($p < 0.01$) (47).

There was also a positive correlation between basal plasma and total blood-histamine levels (r = 0.67, p less than 0.01) in normal and asthmatic subjects suggesting that basophils contribute significantly to plasma histamine. The spontaneous basophil release of histamine was greater in asthmatic ($13.4 \pm 2\%$) than in normal subjects ($6.46 \pm 7\%$, p less than 0.005), which is consistent with the higher resting plasma-histamine levels in the asthmatic subjects (48).

Carcinogenicity
Human
Endogenous histamine has been shown to affect growth mechanisms in experimental mammary carcinomas via cellmembrane containing H_2 receptors. Both H_1 and H_2 binding sites are present in human mammary glands. About 75% of malignant carcinomas express H_2 receptors. The presence of mast cells around tumour tissue raises questions concerning the source of histamine in breast tumour tissue (49).

Animal

No data available.

Reproduction toxicology
Human
No data available.
Animal
No data available.

Mutagenicity
Human
No data available.
Animal
Ames tests have been performed with imidazole and its principal metabolites, hydantoin and hydantoic acid, N-acetyl-imidazole and histamine. Imidazole and histamine were also tested in the unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes. Imidazole gave consistently negative results in the Ames test, the UDS assay and the transformation assay. The three metabolites of imidazole, namely hydantoin, hydantoic acid and N-acetyl-imidazole, all gave negative results in the Ames test. Histamine gave no evidence of mutagenic activity in the Ames test or of genotoxicity in the UDS assay. These results indicate that imidazole and its metabolites are unlikely to present a mutagenic or carcinogenic hazard (50).

Other

Critical assessment
Hyperresponsiveness to histamine is a key feature of a variety of pathological conditions, including bronchial asthma, food allergy, colitis ulcerosa, and topical allergic disorders. Several mediators enhance the cellular reaction to histamine in cell types involved in patho-immunological histamine hyperresponsiveness. Epidemiologic reports on food-borne diseases from different countries show frequent outbreaks due to histamine toxicity. Workers exposed to high histamine dose, developed allergy-like skin, eye, gastrointestinal, respiratory and cardiac symptoms within 30 min. The histamine dose in cigarette smoking does not seem to be high enough to exert toxicological effects.

Conclusion
The histamine dose of cigarette smoking does not seem to be high enough to exert toxicological effects. However, the long-term effects of this compound via the respiratory system are not known and need to be studied.

INTERACTIONS
Chemical
No data available.

In vivo
Maternal ethanol consumption during pregnancy results in an increase in the cerebral histamine levels of the fetus. An increase in the brain histamine levels is also observed in the newborn rats suckling on the ethanol-fed mothers compared to the corresponding controls. Acute administration of ethanol or acetaldehyde resulted in

significant increase in brain histamine levels after 20 minutes. This increase in the brain histamine levels seems to be a direct result of brain histamine release due to ethanol or acetaldehyde metabolism in the body. The effect of ethanol on brain histamine levels may have important implications in view of the fact that both histamine and ethanol influence diuresis, EEG activity, and thermoregulation in the body (51).

The plasma histamine concentrations after oral food challenges in 13 patients who were positive to food antigen-specific IgE, increased significantly. No significant change in plasma histamine concentrations was observed after the challenges in the controls. The results confirmed the strong connection between food allergy and the elevation of plasma histamine concentration. Therefore, plasma histamine concentration following food challenges might be a useful marker in the detection of food allergy (52).

Since many factors may alter lung epithelial permeability (LEP) to water soluble molecules, the effect of histamine on the absorption and clearance of inhaled sodium cromoglycate (SCG) was examined in seven mildly asthmatic patients with hyperresponsive airways and eight normal subjects. When compared with inhaled saline, histamine increased the initial pulmonary absorption of SCG without influencing the total amount of drug absorbed in both asthmatics and normals. These observations suggest that the pharmacokinetics of inhaled sodium cromoglycate may be altered significantly by inflammatory mediators present at the site of drug absorption from the airways (53). However, another study showed that histamine did not increase the absorption of tracer chromium-51 labelled EDTA, which was instilled into one nasal cavity for 15 minutes, with a nasal pool-device (total volume 14 ml). The present data agree with previous observations in guinea pig tracheobronchial airways, where histamine and other exudative agents did not increase the mucosal absorption of solutes from the airway lumen. The data in the mentioned study suggest that the potent protein systems of blood plasma can transverse the endothelial-epithelial linings and operate on the surface of the airway mucosa without compromising its integrity as a barrier to luminal material (54).

Normal CFW mice, when exposed to tobacco smoke, showed a significantly increased susceptibility to the lethal effects of histamine. The LD_{50} for mice subjected to smoke was 45 mg/kg of histamine, whereas in normal CFW mice the LD_{50} was 1,100 mg/kg. Injecting the mice with isoproterenol markedly diminished the histamine susceptibility of tobacco smoke. Normal CFW mice, as well as sham control mice, exhibited an epinephrine-induced hyperglycemia, whereas the blood glucose values for smoked mice given epinephrine were essentially the same as those for sham mice given only saline. This observation indicates that tobacco smoke may contain a component, which causes an autonomic imbalance, hence rendering the mice more susceptible to histamine. This tobacco smoke-induced allergy is probably related to a blockade of adrenergic receptors and not to an immunologic phenomenon (55).

Both S-(-)- and R-(+)-nicotine enantiomers are inhibitors of histamine N tau-methylation activity in guinea-pig pulmonary alveolar macrophage cultures, exhibiting IC_{50} values of 7 and 8 μM, respectively. S-(-)-Nicotine is not biotransformed under the conditions of the experiment, however, R-(+)-nicotine

undergoes significant N-methylation to produce N-methylnicotinium ion. S-(-)-Nicotine appears to inhibit the N-methylation of its optical antipode by the alveolar nicotine N-methyltransferase. The results indicate that a contributing factor in the toxicology of cigarette smoke inhalation may be due to the inhibition of pulmonary metabolism of histamine by nicotine (56).

In vitro studies with rat intestines showed that the potentiation of histamine toxicity by putrefactive amines, such as cadaverine, results from the inhibition of histamine metabolism which leads to increased uptake of unmetabolized histamine (57).

The airway response to histamine has been shown to be related to the 24 hour urinary excretion of sodium. To assess whether this relation is likely to represent a direct causal association a randomised double blind crossover trial of slow sodium (80 mmol/day) was compared with placebo in 36 subjects having a low sodium diet. The dose of histamine causing a 20% fall in FEV1 (PD_{20}) was 1.51 doubling doses lower when the men were taking sodium than when they were taking placebo (p less than 0.05). On the basis of PD_{10} values, the difference in men was 1.66 doubling doses of histamine (p less than 0.05). There was no corresponding effect in women. Regressing PD_{10} against urinary excretion of electrolytes with data from the two occasions during the trial and the measurements made before the trial showed a significant association with sodium excretion after allowance had been made for any effect associated with potassium or creatinine excretion, the latter being a marker of the completeness of the urine collection. Again there was no corresponding effect among women. These findings are compatible with the differences in regional mortality data for England and Wales, which show a relation between asthma mortality and regional per person purchases of table salt for men but not for women (58).

Critical assessment
Chemical (see critical assessment of the general section)
The free amino group is
- a potential group to react with aldehydes and ketones and with monoamino-oxydase (MOA);
- a base group, i.e. a potential group to react with acids.

The ring nitrogen atoms:
The extra pairs of electrons are involved in the pi-cloud of the ring and are not available for sharing with acids.
Substitution reactions may occur in which the stabilized ring is retained.

In vivo
Histamine level in the body is increased either by mediators (at food allergy) or by inhibition of the histamine metabolism (by nicotine or putrefactive amines). Increased sodium intake seems to increase the hyperresponsiveness to histamine reactions in asthmatic men. It is unclear whether histamine increases the permeability of the respiratory mucosa to other compounds.

Conclusion
Chemical
Especially the free amino group has the potential of a reactive site.

In vivo
The increased histamine level in the body induced by mediators may have important physiological and toxicological implications.

DEPENDENCY
No data available.

Effects of smoking cessation
No data available.

Critical assessment
Not possible.

Conclusion
Not possible.

COMMERCIAL USE
Histamine is used as a diagnostic and for therapeutic purposes. Intradermal injection of histamine produces the characteristic `triple response' of erythema, flare, and wheal. This is utilised as a control response in skin testing for hypersensitivity. Also, since it is mediated in part by axon reflexes, it has been used to test the integrity of sensory nerves, for example in leprosy.
Inhalation of histamine causes bronchoconstriction and is used as a test of bronchial reactivity. Histamine has also been given subcutaneously to identify the causes of achlorhydria and intravenously in the diagnosis of phaeochromocytoma, but safer tests are generally preferred. Histamine is included in some combination topical preparations for musculoskeletal disorders. (4).

BENEFICIAL EFFECTS
Histamine dihydrochloride is under investigation as an adjunct in the management of acute myeloid leukaemia and malignant melanoma. It has also been tried as an adjunct to interferons and other drugs in the management of hepatitis C (4).

Critical assessment
Histamine is used as a diagnostic and for therapeutic purposes. It does not seem to have any beneficial effects on the respiratory system.

Conclusion
Histamine does not seem to have any beneficial effects on the respiratory system.

SUMMARY AND FINAL CONCLUSION
The potential daily intake of histamine from cigarettes (from added cocoa; 0.33 μg/day) is about 500 times less than histamine intake from other sources such as chocolate or wine or Dutch cheese (185 – 2600 μg). Assuming similar bioavaibility, the plasma concentration reached after ingestion of histamine from chocolate sources

or other food sources is expected to be significantly more, than after exposure to cigarette smoking. However, the different route of application via smoking as compared to other sources should be taken into account. Therefore, the systemic and the local effect of smoking related exposure to histamine might be a point of concern. Since nothing is known about the pyrolysis/combustion products of histamine in cigarette smoke, this may be an additional point of concern.

Histamine is an autacoid that is closely associated with mast cells and functions as a mediator of inflammation. Histamine is a neurotransmitter in the central and peripheral nervous systems. It mediates its effects through three receptor subtypes with differential selectivities for both agonists and antagonists (e.g., mepyramine for H_1, ranitidine for H_2, and thiperamide for H_3). Through these receptors, histamine evokes several physiological effects. Histamine characteristically causes dilatation of the finer blood vessels, resulting in flushing, lowered total peripheral resistance and a fall in systemic blood pressure. The released cardiac histamine is a major mediator of arrhythmias that occur during anaphylaxis and following the administration of histamine-releasing drugs. Histamine receptors are widely distributed in the CNS. The central histamine receptors may regulate the cardiovascular system, diuresis and food intake. Histamine also induces gastric secretion. Histamine provokes bronchoconstriction, but some conflicting results were found concerning the breathing frequency and the tidal volume. It seems that different mechanisms with opposite effects are acting simultaneously.

The histamine dose causing a 20% fall in forced expiratory volume in one second (PD_{20}) was shown to be between 0.73 mg -1.20 mg in young normal adults. In astmatics the histamine dose for PD_{20} was 0.23 mg. The estimated daily histamine intake through cigarette smoking is about 2000 times less than the PD_{20} in normal adults.

[14]C and ^{3}H-histamine studies showed that histamine is absorbed through the pulmonary system. Almost all mammalian tissues contain histamine in amounts ranging from less than 1µg up to more than 100 µg/g tissue. Every mammalian tissue that contains histamine is able to synthesise it from histidine by virtue of its contents of L-histidine decarboxylase. There are two major pathways of histamine metabolism in man. The more important of these involves methylation and is catalysed by the enzym histamine-N-methyltransferase, which is widely distributed. Most of the product, N-methylhistamine, is converted by monoamine oxidase (MAO) to N-methyl imidazole acetic acid. The metabolites resulting from catalysation are excreted in the urine. Histamine kinetic parameters determined in patients with melanoma's had $t_{1/2}$ 12.7 min and Vd of 66.0 l. The small $t_{1/2}$ seems to implicate a rapid histamine metabolisation.

Hyperresponsiveness to histamine is a key feature of a variety of pathological conditions, including bronchial asthma, food allergy, colitis ulcerosa, and topical allergic disorders. Several mediators enhance the cellular reaction to histamine in cell types involved in patho-immunological histamine hyperresponsiveness. Epidemiological reports on food-borne diseases from different countries show frequent outbreaks due to histamine toxicity. Workers exposed to high histamine dose developed allergy-like skin, eye, gastrointestinal, respiratory and cardiac

symptoms within 30 min.

Histamine level is increased either by mediators (at food allergy) or by inhibition of the histamine metabolism (by nicotine or putrefactive amines). Increased sodium intake seems to increase the hyperresponsiveness to histamine reactions in asthmatic men. It is unclear whether histamine increases the permeability of the respiratory mucosa to other compounds.

Histamine is used as a diagnostic and for therapeutic purposes. It does not seem to have any beneficial effects on the respiratory system.

It seems that histamine dose in cigarette smoking is not high enough to evoke any bronchoconstrictory effects. However, the (longterm) effects of histamine or its pyrolysis/combustion products on the pulmonary system are unknown and need further study. Histamine is absorbed through the respiratory system. However, due to the rapid histamine metabolisation it is not expected that the histamine dose in cigarettes will be enough to affect the plasma histamine level. The histamine dose of cigarette smoking does not seem to be high enough to exert toxicological effects. Therefore, more studies are needed on:

- the determination of pyrolysis/combustion products of histamine in cigarette smoke;
- the local (respiratory system) effects of long-term use of histamine and their pyrolysis/combustion products or other biogenic amines via inhalation.

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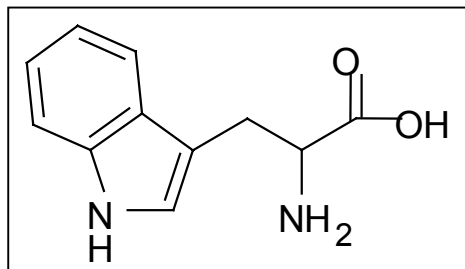
Thorax, 1989; 44(1):36-41.

3.5   Tryptophan

GENERAL
IUPAC systematic name: L-2-Amino-3-(indol-3-yl)propionic acid (1).
Synonyms: L-Tryptophan; Tryptophanum (1) (S)-alpha-amino-1H-indole-3-propanoic acid; l-alpha-aminoindole-3-propionic acid; l-alpha-amino-3-indolepropionic acid; 2-amino-3-indolylpropanoic acid; l-beta-3-indolylalanine (2).
Molecular formula: $C_{11}H_{12}N_2O_2$ (1-4)

Molecular weight: 204.2 g/mol (1-4)
Alifatic: propyl-chain (4)
Aromatic: indole ring (4)
N containing: yes (4)
Halogen containing: No (4)
CAS registry no.: 73-22-3 (3).
Storage: Not stable in light (3).
R/S classification: No data available.
dangercode (transport): No data available.
Properties:
➢ melting point: E 280 ºC (3).
➢ boiling point: No data available
➢ density: E 1340 kg/m^3 (3).
➢ refractive index: No data available
➢ solubility: Sparingly soluble in water; slightly soluble in alcohol; practically insoluble in ether; dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides (1). Moderate soluble in water (E 1.1 g/100 ml) (3).
➢ substance:
  • color: white/light yellow (3).
  • liquid/gas/powder: a white or almost white crystalline or amorphous powder (1).
  • odor/taste: odourless (3).
➢ volatility: No data available
➢ pK_a: pK_a = 2.38, pKb = 9.34 and pI = 5.89 (4).
➢ PA: kcal/mol: No data available
➢ flammability: No data available
  • FP =
  • FL Limits =
  • IT =
➢ decomposition temperature: E 280ºC (3), 290 – 295ºC (5).
➢ vapour pressure/ vapour tension (20 °C): No data available
➢ vapour pressure (50 °C): No data available

> ➢ relative density: E 1.3 (3)
> ➢ log P octanol/water: No data available
> ➢ octanol water partition coefficient, log K_{OW}: No data available
> ➢ conversion factor: No data available

Critical assessment

Tryptophan is in the first place an amino acid. Its three characteristic structural features are: the heterocyclic indol part, linked to the short aliphatic chain with the amino group and the carboxy group. The carboxy group supplies polarity to the compound. The amino acid feature means that it is a potential component of proteins. The available free amino group is a potential group to react with aldehydes and ketones.

Conclusion

Tryptophan is a nitrogen-containing heterocyclic compound, linked to a short aliphatic chain with a free amino-group and a free carboxy-group. The compound belongs to the group of amino acids, the basic components for proteins.

FUNCTION IN TOBACCO

No data available.

AMOUNT IN TOBACCO PRODUCTS

No data are available on the amount of natural occurring tryptophan in tobacco.
A source of tryptophan in cigarettes is cocoa powder. A typical casing concentration of cocoa powder for cigarette tobacco is 1% (6).
The average amount of tryptophan in cocoa powder is 3 mg/g (7).
Assuming one cigarette weights approximately 1 g, the tryptophan amount from cocoa powder in one cigarette is estimated to be ± 30 µg.

AMOUNT IN SMOKE

- **main stream:** No data available
- **side stream:** No data avaialable

SOURCE

(tobacco, combustion product or other)
Tryptophan is an endogenous compound of tobacco and is also added exogenously as cocoa powder (8, 9).

ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

Tryptophan amount per 100 g is in milk 42 mg, in eggs 165 mg, wheat flour 110 mg, sausage 93 mg, potato 28 mg, cheese (Edam) 325 mg and beef 230 mg. It is estimated that the daily Finnish intake is 900mg/day per person, which exceeds the required amount of 250 mg/day (10).

COMBUSTION PRODUCTS

By combustion generation of toxic/corrosive damps/gases: nitrous gasses and carbon monoxide and dioxide (3). Pyrolysis of tryptophan results in carcinogenic products such as 3-amino-1,4-dimethyl-5H-pyrido(4,3-b)indole (Trp-P-1) and 3-amino-1-

methyl-5H-pyrido-(4,3-b)indole (Trp-P-2) (11-13).

CONSENSUS REPORTS
No data available

STANDARDS AND RECOMMENDATIONS
ADI: An adult requires 3.5 mg tryptophan per kg body weight per day or about 250
      mg per day to maintain nitrogen balance (10).
TWA_{NL} = **MAC**: no data available
TWA_D =**MAK**: no data available
TWA_{USA}: no data available
$STEL_{NL}$: no data available
$STEL_{USA}$: no data available
LTEL: no data available
TLV-C: no data available
TLV-CARCINOGENICITY: no data available
MAK-REPRODUCTION: no data available

Others:

Reference value:
Mean free tryptophan in plasma is 9.8 mg/l (range 5.1 – 14.9 mg/l) (14). The amount
of tryptophan in whole blood is 2.0 g/ 100 g protein (15).

CLASS
Tryptophan is not classified as carcinogenic (EG, IARC, TLV and MAK) (3).

EG Carc. Cat.: no data available
IARC-category: no data available
CEC: no data available

Critical assessment
Comparison of smoking related potential daily intake of tryptophan (mg) with daily
intake from other sources:

	SMOKING	DAILY TRYPTOPHAN ORAL INTAKE FROM		
	25 cig./day (1% cocoa)	3 chocolate bars of 60 g	cocoa powder (25g)	Milk (250 ml)
TRYPTOPHAN (mg)	0.75 [7]	98 (milk)[7] 41 (dark)[7]	75[7]	105[10]

Conclusion
The daily intake of tryptophan from cocoa added to cigarettes is marginal compared
with that of tryptophan intake from other sources, like chocolate or milk. The plasma
concentration reached after ingestion of tryptophan from chocolate sources or food is
expected to be significantly higher, than intake from cigarettes. Since tryptophan is an
endogenous compound, it is not expected that the inhaled amount from cigarette

smoking will significantly affect the tryptophan plasma level.

PHARMACODYNAMICS

Mechanism of action

Tryptophan is an essential constituent of the diet. It plays an important role in protein synthesis and is also precursor of a variety of biologically active compounds including serotonin, melatonin, tryptamine, quinolinic acid and kynurenic acid. In addition, tryptophan is precursor to the coenzymes NAD and NADP and can replace niacin as an essential nutrient (10).

A compound in cigarette tar, possibly an oxidised tryptophan, exhibits an affinity for the aryl-hydrocarbon receptor, which induces the biotransformation enzymes (16).

Pulmonary system

- **breathing frequency**: no data available
- **tidal volume**: no data available
- **lung compliance**: no data available
- **airway resistance**: no data available

Cardiovascular system

- **blood pressure**: In humans, single oral doses of L-tryptophan (50 mg/kg body weight) lowered blood pressure significantly 90-120 min after administration in patients with essential hypertension, but not in normotensive controls. The tryptophan-induced lowering of blood pressure could be attributable to the enhancement of central serotonin synthesis (17).

  Chronic oral administration of L-tryptophan (1.26 g/kg/day) attenuated the elevation of systolic blood pressure in deoxycorticosterone salt-treated rats (18).

- **heart rate**: No data available.

Renal system

- **diuresis**: Intra peritoneal injected tryptophan in rats showed the same antidiuretic effect as serotonin in rats; it has an initial antidiuretic effect and evokes subsequently diuresis (19).
- **saluresis**: Tryptophan injected in the median raphe nucleus of rats (200 mg/kg) increased the Na^+ and K^+ excretion (20).

Nervous system

Tryptophan affects the CNS due to alternations of brain tryptophan levels, which influence serotonin synthesis (10). Both excessive intake and deficiency of tryptophan affects the CNS.

- **central nervous system**: Tryptophan deficiency (due to chronic dietary insufficiency) may lead to pellagra. Pellagra is associated with diarrhoea, dermatitis and mental symptoms. Mild cases of pellagra can be associated with headache, sleep disturbances and depression and severe cases are associated with hallucinations, catatonia, dementia and seizures. Tryptophan is used in mild to moderate depression, mild insomnia, pain and aggression. High oral doses (> 1 g) are needed to obtain these pharmacological effects (10).
- **autonomic system**: No data available.

Other

Critical assessment

Tryptophan is essentiel in the diet and is a precursor for several biological active compounds. High tryptophan doses are needed to observe any effects on the cardiovascular, the renal system and the CNS. Tryptophan deficiency affects also the CNS. No data are available on the effect of tryptophan on the pulmonary system.

Conclusion

High doses of tryptophan are needed to observe any pharmacological effect. Therefore it is unlikely that the tryptophan dose occurring in cigarettes is enough to exert any systemic pharmacological effect. The (longterm) effect of tryptophan on the pulmonary system is unknown and needs further study.

PHARMACOKINETICS

Absorption

L-Tryptophan is well absorbed from the mammalian small intestine and to some extent from the stomach (1, 21).

Bioavailability

The tryptophan bioavailability depends on the tryptophan source. In rats the tryptophan bioavailability ranged between 85 % and 100 % for several products. Lower bioavailability was obtained for wheat cereal (73 %) and pinto beans (59 %) (22). Tryptophan bioavailability from soybean in ducks was 92 % (23).

Distribution

Tryptophan is distributed throughout the body and is extensively bound to plasma albumin (85 %)(21, 24). Tryptophan uptake in the brain is affected by plasma levels of other large neutral amino acids (LNAA). All these LNAA, including tryptophan, share a common transport system that moves them from blood to brain. Hence the ratio of other LNAA and tryptophan must be low before relatively large amount of tryptophan is able to cross the blood brain barrier and enter the brain (10).

Metabolism

Quantitatively, the most important pathway for tryptophan metabolism, after protein synthesis, is the kynurenine pathway which is responsible for over 90% of tryptophan catabolism. Two enzymes initiate this pathway, tryptophan-2,3-dioxygenase in the liver and indoleamine-2,3-dioxygenase which is present in a variety of tissues including intestine, stomach, lungs and brain. The former is induced by glucocorticoids and tryptophan. The latter is induced by interferon gamma. There are several important metabolites along the kynurenine pathway including kynurenic acid, which is a glutamate receptor antagonist and quinolinic acid, which is a

glutamate agonist. The majority of tryptophan is eventually converted to carbon dioxide but a small amount can act as a precursor of the coenzymes NAD and NADP (10).
Of the fraction of tryptophan that enters the brain, part is metabolised to tryptamine and possibly to kynurenine in addition to serotonin. It has been estimated that only 1 % of ingested tryptophan is metabolised to serotonin. Biosynthesis of serotonin requires two enzymatic steps: L-tryptophan is first hydroxylated by tryptophan hydroxylase to L-5-hydroxytryptophan (L-5HTP) and L-5HTP is then decarboxylated into serotonin by decarboxylase. Tryptophan hydroxylase is restricted to serotonin neurons and therefore oral administration of tryptophan gives rise to selective increases in serotonin synthesis and release. A range of 6-8 g/day, given in divided doses, seems sufficient to keep tryptophan hydroxylase reasonably close to saturation throughout most of the day. Higher doses would only increase synthesis of tryptamine and induce tryptophan dioxygenase enzyme (25).

Excretion
No data available

Kinetic parameters
The half-life of plasma tryptophan in healthy individuals is 2.0 ± 0.1 h (26). After oral administration of L-tryptophan, 100 mg per kg body weight, the peak concentration of tryptophan in plasma occurred after 1 to 2 h. Tryptophan disappeared linearly from 2 to 5 h and exponentially from 5 to 8 h after administration (27).

Critical assessment
The oral data indicate a high bioavailabilty, extensive distribution and metabolism of tryptophan. There are no data on pharmacokinetics in animals and humans from respiratory studies on tryptophan. Tryptophan is bound extensively to albumin in plasma. Tryptophan is extensively metabolised resulting in several biologically active compounds.

Conclusion
There are no data available on kinetics after respiratory exposure. Conclusions on potential differences in pharmacokinetics between respiratory and oral administration can neither be drawn based on the pharmacological and toxicological data.

TOXICOLOGY
Acute toxicity
Human
Tryptophan alone seems to produce no more side effects than placebo when given at a moderate dose (3 g per day orally). (10).
Nausea, headache, lightheadedness and drowsiness have been reported as side effects of tryptophan (21).
Single intake of high doses of tryptophan (> 2 g) with monoamine oxidase inhibitors may lead to development of neurological complications. These symptoms are related to serotonin syndrome (25).

Animal
LD$_{50}$ oral rat: 1.6 g/kg (3, 5).
LD$_{50}$ intraperitoneal rat: 1.63 g/kg body weight (21).
LD$_{50}$ intraperitoneal mouse: 4.8 g/kg body weight (21).

Local tolerance
Human
No data available

Animal
No data available

Repeated dose toxicity
Subacute
No data available

Semichronic
Human
Tryptophan-containing products have been associated with the eosinophilia-myalgia
syndrome in humans, but contamination of tryptophan during the manufacturing
process may have been responsible. Evidence pointed to contamination coming from
a single manufacturer and the syndrome was probably caused by a bacitracin-like
peptide. Tryptophan probably promoted this disorder. Tryptophan dosage ranged
from 150 mg/day to 8.4 g/day, with duration of tryptophan use ranging from 2 weeks
to 8 years induced the eosinophilia-myalgia syndrome in humans (25).

Chronic
Animal
Chronic administration of tryptophan doses below the LD$_{50}$ reduced the food intake
and the growth of rats, due to amino acid imbalance.
Large groups of rats and mice were given greatly elevated amounts of tryptophan (2.5
% or 5% (w/w) in food, equivalent to 6.25 or 12.5 g/kg BW and 0.94 or 1.88 g/kg
BW respectively for mice and rats) in their diets for most of their lives (104 – 105
weeks). In this study neither cancer incidence was increased nor gross microscopic
changes in the tissue were observed at autopsy (10).
A potential side effect of chronic tryptophan use includes the risk of diabetes
mellitus. Since xanthurenic acid, which is increased on tryptophan loading, has
diabetogenic action in animnals, tryptophan may promote glucose intolerance. In
addition there is some evidence that photooxidation of tryptophan and some of its
metabolites, such as kynurenine, may be involved in cataract formation (10, 25).

Carcinogenicity
Human
While tryptophan itself seems to be relatively safe, during heating several pyrolysis
products are formed which are mutagens, carcinogens and comutagens. No details are
available on tryptophan data from this study (10).
It has been suggested that long-term tryptophan use may promote bladder cancer.
Elevated urinary levels of tryptophan metabolites has been reported in both bladder
cancer patients relative to controls, and in patients who had recurrence of cancer

relative to those who did not (10, 25).

Animal
The National Toxicology Programme tested rats and mice via feed (2.5 % or 5 % w/w tryptophan). No evidence of carcinogenicity was seen in either species of either sex. However in another study when tryptophan was administered subcutaneous to rats (2 years, 20 mg per week), malignant tumours in the uterus, mammary gland fibroadenomas, salivary gland adenomas, mesenteric reticulosarcomas and reticuloleukosis were observed (21).
Tryptophan (6 g/day) is a promoter or cocarcinogen of urinary bladder tumors in dogs treated with an initiating dose of 4-aminobiphenyl or 2-naphthylamine for 0.3 – 7 years (15, 28). Based on longterm studies on rats (80 weeks) with 2% tryptophan diet and vitamin B_6 intake, it was concluded that tryptophan promoted tumor formation when vitamin B_6 intake was marginal but not when vitamin B_6 was adequate. Using pellets with crude tryptophan pyrolysates, 3-amino-1,4-dimethyl-5H-pyrido(4,3-b)indole (Trp-1) or 3-amino-1-methyl-5H-pyrido-(4,3-b)indole (Trp-2), high incidence of transitional cell carcinomas in the bladders of female mice were found after 40 weeks. In another study when a pellet diet containing Trp-1 and Trp-2 (0.2 %) were fed to mice for up to 621 days, a high incidence of hepatocellular carcinomas was observed in the female mice (28).

Reproduction toxicology
Human
No data available

Animal
Tryptophan, given as 1.8 % of the diet to pregnant hamsters, caused significant reduction in embryo and neonate survival and in neonatal weight of the pups (10).

Mutagenicity
Human
No data available

Animal
Indole derivates (tryptophan derivates included) which are present in cigarette smoke were shown to have a strong mutagenicity effect to Salmonella typhimurium TA100 and TA98 after nitrite treatment (29).
Tryptophan reduces sister chromatid exchange incidence in rats treated with cyclophosphamide (21).

Other

Critical assessment
In human tryptophan alone seems to produce no more side effects than placebo when given at a moderate dose (3 g per day). Nausea, headache, lightheadedness and drowsiness have been reported as side effects of acute tryptophan exposure in human. The LD_{50} in rats is high. Animal studies have indicated that tryptophan may act as a co-carcinogen or tumor promoter. During heating several pyrolysis products are formed which are mutagens, carcinogens and comutagens. Tryptophan is probably

involved into glucose intolerance and into cataract formation. No toxicological data are available from tryptophan inhalation studies. While tryptophan itself seems to be relatively safe, during heating several pyrolysis products are formed which are mutagens, carcinogens and comutagens. As the pyrolyse products of tryptophan in cigarette smoke is reported to be hazardous, the long-term effect of these compounds on the respiratory system needs to be studied.

Conclusion
Tryptophan itself seems to be safe, but the pyrolyse products of tryptophan in cigarette smoke are reported to be hazardous. As no data are available on inhalation effects of tryptophan and its pyrolyse products, the long-term effect of these compounds via the respiratory system needs to be studied.

INTERACTIONS
Chemical
Both the amino group and the carboxy group of tryptophan form potential sites for a wide variety of reactions. Numerous compounds react with tryptophan in cigarettes during smoking, generating several hazardous compounds. One of these compounds is peroxyacetyl nitrate (PAN), which is a common gaseous photochemical compound in polluted air and cigarette smoke. 5-Hydroxytryptophan is produced from the reaction of PAN with tryptophan in cigarette smoke (30). L-Kynurenine is also formed from the reaction of nitrite with free tryptophan. This compound is linked to cataract formation (31). Beta.-carbolines, the condensation products of tryptophan and indole alkylamines with aldehydes or amines, are found in cigarette smoke but not in tobacco itself (32-34).

In vivo
The combination of tryptophan and monoamineoxidase inhibitors (MAOIs) oral intake may potentiate the adverse effects of MAOIs. Use of tryptophan with drugs that inhibit the reuptake of serotonin may exacerbate the adverse effects of the latter and precipitate the serotonin syndrome. There have been occasional reports of sexual disinhibition in patients taking tryptophan in conjunction with phenothiazines or benzodiazepines (1). Some compounds like valproate, benzoate and acetylsalicylic acid reduce serum-protein binding of tryptophan in man, causing rise in free serum tryptophan (10, 35-37). The blood-brain transport is shared by several large neutral amino acids (LNAA), including tryptophan. A protein meal will increase the plasma level of large neutral amino acids (LNAA) and relatively less tryptophan will be available for the brain uptake. However, carbohydrate meals will decrease some of the LNAA plasma level, but not tryptophan and therefore relatively more tryptophan is available for brain uptake. Tryptophan pyrrolase (tryptophan-2,3-dioxygenae) is induced by tryptophan and glucocorticoids. Several agents that induce glucocorticoids can induce this enzyme and thus affect the tryptophan level in the plasma and the brain. When the immune system is stimulated there can be an induction of indoleamine-2,3-dioxygenase by interferon gamma (10). Furthermore paroxetine and vitamin B6 inhibit the basal tryptophan pyrrolase activity, which subsequently increases the tryptophan availability to the brain (38, 39). The daytime administration of the heme precursor 5-aminolevulinate (5-ALA) has been shown to reduce brain tryptophan and serotonin levels owing to saturation of liver tryptophan pyrrolase. Saturation of this enzyme with heme results in enhanced

activity, leading to increased catabolism of tryptophan and thus making less tryptophan available to the brain. Allopurinol, an inhibitor of hepatic tryptophan pyrrolase activity, prevented the reduction in the indole levels induced by 5-ALA (40).

The tryptophan degradation in the brain is reduced by methamphetamine due to inhibition of tryptophan hydroxylase (41).

Critical assessment
Chemical
Both the amino group and the carboxy group form potential sites for a wide variety of reactions. It has been shown that numerous compounds react with tryptophan in cigarettes during smoking and resulting in complex compounds with potential hazardous effect in the body.
In vivo
Several compounds affect the tryptophan level in plasma or brain either by inducing or inhibiting the tryptophan degradation or by interaction with the binding site of tryptophan in albumin or in the transport system through the blood-brain-barrier.

Conclusion
Chemical
Tryptophan can react with numerous compounds during smoking resulting in potentially hazardous compounds for the body.
In vivo
Several compounds affect the tryptophan level in plasma and brain.

DEPENDENCY
Effects of smoking cessation
It is known that nicotine enhances the serotonin release in the brain and that nicotine withdrawal has the opposite effect. Serotonin-releasing brain neurons are unique in that the amount of neurotransmitter they release is normally controlled by food intake: carbohydrate consumption--acting via insulin secretion and the 'plasma tryptophan ratio'--increases serotonin release; protein intake lacks this effect. This ability of neurons to couple neuronal signalling properties to food consumption is a link in the feedback mechanism that normally keeps carbohydrate and protein intakes more or less constant. Hence many patients learn to overeat carbohydrates (particularly snack foods, like potato chips or pastries, which are rich in carbohydrates and fats) to make themselves feel better. This tendency to use certain foods as though they were drugs is a frequent cause of weight gain, and can also be seen in people who are attempting to give up smoking (42). Serotonin-enhancing substances, such as tryptophan and high-carbohydrate diets, have been used in clinical disorders to relieve negative affect, a classic symptom of cigarette withdrawal. In a study it was investigated whether the use of tryptophan (50 mg/kg/day) and high-carbohydrate diets, together with more traditional smoking cessation treatment techniques, was able to ameliorate the smoking withdrawal syndrome and to improve abstinence rates. Subjects were randomly assigned to receive either tryptophan (n = 16) or placebo (n = 15). Standard smoking cessation treatment was identical for the experimental and control groups and consisted of four 2-hr weekly sessions of multicomponent group therapy. Smoking behaviour, symptoms of nicotine withdrawal, and negative effect were assessed during a 2-week

withdrawal period. Tryptophan-treated subjects who could not fully abstain were able to smoke fewer daily cigarettes. Reported anxiety and other withdrawal symptoms were lower in the tryptophan group compared with control subjects. These data suggest that serotonin-enhancing substances show promise for use as an adjunct to existing smoking cessation programs (43).

Critical assessment
The tryptophan level in the plasma or the brain affects also the brain serotonin level and showed promising results in a smoking cessation study. That means that tryptophan in cigarettes may potentially decrease the addiction potential of cigarette. However, due to the small cigarette tryptophan dose and the large tryptophan pool in the body, it is unlikely that cigarette tryptophan will affect smokking cessation.

Conclusion
Tryptophan doses in cigarette are too small to play a role in smoking cessation.

COMMERCIAL USE
Tryptophan is used medically in parental nutrition, as antidepressant, against pain and myoclonus, as sleep inducer or as dietary. In the treatment of depression the usual dose of tryptophan is 1 g given three times daily, but some patients may require up to 6 g daily in divided doses. Lower doses may be required in the elderly especially those with renal or hepatic impairment (15). When tryptophan is concomitantly administered with monoamine oxidase inhibitor, the initial dose of tryptophan should be 500 mg daily and increased gradually after one week (35).

BENEFICIAL EFFECTS
Mostly therapeutic beneficial effects of tryptophan are observed in man. Tryptophan can be used against several disorders, such as myoclonus, depression, pellagra and insomnia (35).

Critical assessment
Commercially, tryptophan is used as medicine and in diets. Large amount of tryptophan is used as therapeutics, which could be indicated as beneficial effect of tryptophan. Therefore, it is unlikely that tryptophan dose in cigarette will be sufficient to be beneficial.

Conclusion
The tryptophan doses in cigarette are considered to be insufficient to have any beneficial effects in the body.

SUMMARY AND FINAL CONCLUSION
Tryptophan is an endogenous compound of tobacco and is also added exogenously as cocoa powder, which is used as a flavouring agent. The daily intake of tryptophan from cocoa added to cigarettes is marginal compared (estimated 0.75 mg/day) with that of oral tryptophan intake from other sources, like chocolate or milk (estimated 900 mg/day) or to that from tryptophan pool in the body. The plasma concentration reached after ingestion of tryptophan from chocolate sources or food is expected to be

significantly higher, than intake from cigarettes. Since tryptophan is an endogenous compound, it is not expected that the inhaled amount will significantly affect the tryptophan plasma level.

Tryptophan is an essential constituent of the diet. It plays an important role in protein synthesis and is also precursor of a variety of biologically active compounds including serotonin, melatonin, tryptamine, quinolinic acid and kynurenic acid. In addition, tryptophan is precursor to the coenzymes NAD and NADP and can replace niacin as an essential nutrient. Large tryptophan doses are needed to observe any effects on the cardiovascular, the renal system and the CNS. Tryptophan deficiency affects also the CNS. No data were available on the effect of tryptophan on the pulmonary system.

Tryptophan is well absorbed from the mammalian small intestine and to some extent from the stomach. The bioavailability is between 85 % and 100 % for most of tryptophan products. Tryptophan is extensively bound to plasma albumin. Quantitatively, the most important pathway for tryptophan metabolism, after protein synthesis, is the kynurenine pathway, which is responsible for over 90% of tryptophan catabolism. About one percent of ingested tryptophan is metabolised in the brain to serotonin, which is a neurotransmitter of a large family of receptors. After two hours of ingestion a plasma peak for tryptophan is observed. Pharmacokinetic data from respiratory studies were not available.

Tryptophan alone seems to produce no more side effects than placebo when given at a moderate dose (3 g per day). The LD_{50} of rat (1.6 g/kg body weight) would result in a LD_{50} of 100 g in human. Nausea, headache, light-headedness and drowsiness have been reported as side effects of tryptophan. Animal studies have indicated that tryptophan may act as a co-carcinogen or tumor promoter. Tryptophan is probably involved in glucose intolerance and into cataract formation. No toxicological data were available from tryptophan inhalation studies. During heating several pyrolysis products are formed which are mutagens, carcinogens and comutagens..

During smoking tryptophan reacts with other reactive compounds in cigarettes, generating complex and potentially hazardous compounds. Several compounds interact with the metabolisation of tryptophan in the body and bind competitively to the binding site of tryptophan with plasma albumin or with the binding site of the transport system from blood to brain, thereby affecting the free plasma/brain tryptophan level.

Large tryptophan doses are used in diets or as medicine.

The tryptophan level in the body affects the serotonin level in the brain. By increasing the tryptophan availability to the brain through carbohydrate diets or tryptophan intake, the brain serotonin level can be increased. A decreased serotonin level is related with substance abuse. Therefore tryptophan intake seems to reduce the negative withdrawal effect of cigarette smoking. However the tryptophan level in cigarette is likely insufficient to affect the brain serotonin level and subsequently will not play any role in smoking cessation.

It can be concluded that the tryptophan amount in cigarette is negligible compared with the large amount of tryptophan daily intake. Tryptophan is an essentiel compound in the diet and it plays an important role in the body. Tryptophan itself seems to be safe but of concern are the pyrolysis products of tryptophan that are produced during smoking. These products seem to be hazardous. There are no data available on the pharmacodynamics, pharmacokinetics and toxicology after inhalation exposure.

Since no data on the toxicological effects of tryptophan exposure through inhalation are available, the influence of (long-term) exposure to tryptophan through smoking on the respiratory system cannot be established. For smoking the complex and potential hazardous derivatives of tryptophan in smoke seems to be relevant.
More studies are needed on:
- the determination of pyrolysis and combustion products of tryptophan in cigarette smoke and their health risk.
- the local (respiratory system) and the systemic effects of long-term use of tryptophan.

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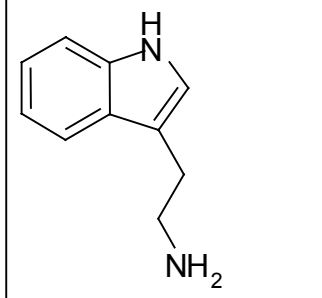
3.6   Tryptamine

GENERAL
IUPAC systematic name: 1H-Indole-3-ethanamine (1)
Synonyms: 3-(2-aminoethyl)indole; 2-(3-indolyl)ethylamine (1)
Molecular formula: $C_{10}H_{12}N_2$ (1)

Molecular structure

Molecular weight: 160.22 g/mol (1)
Alifatic: yes, ethylamine group (1)
Aromatic: yes, indole group (1)
N containing: yes, indole and a primary amine group (1)
Halogen containing: no
CAS registry no.: 61-54-1 (1)
Storage:
R/S classification: R 11-23/24/25 and S16-27-45 (2)
dangercode (transport): no data available
Properties:
➢ melting point: 118 ºC (1, 3).
➢ boiling point: 136 ºC – 138 ºC (2)
➢ density: no data available
➢ refractive index: no data available
➢ solubility: soluble in ethanol, acetone. Practically insoluble in water, ether, benzene (1).
➢ substance description:
   • color: orange (2)
   • liquid/gas/powder: crystal needles (1, 2)
   • odor/taste: no data available
➢ volatility: no data available
➢ pK_a: 10.2 (3).
➢ PA: kcal/mol: no data available
➢ flammability:
   • FP = 185 ºC (2)
   • FL Limits = no data available
   • IT = 491 ºC (2)
➢ decomposition temperature: no data available
➢ stability: no data available
➢ vapour pressure/ vapour tension (20 °C): 0.17 Pa at 25 ºC (4).
➢ vapour pressure (50 °C): no data available
➢ relative density: no data available

➢ octanol water partition coefficient, log P, log K_{OW}: log P = 1.55 (4).
➢ conversion factor: no data available

Critical assessment
Tryptamine contains the characteristic heterocyclic indole structure, accounting for aromatic properties (electrophilic substitution). The hydrogen atom linked to the cyclic N-atom is sensitive for reaction. An additional characterising chemical feature is the presence of the aliphatic amino-group.
Remarkable is the low solubilty of tryptamine both in water (polar solvent) as well as in benzene (aromatic solvent).
Conclusion
Tryptamine is a nitrogen-containing heterocyclic compound, linked to a short aliphatic chain with a free amino-group, resulting in an overall low polar compound that is practically insoluble in water and benzene.

FUNCTION IN TOBACCO
No data available

AMOUNT IN TOBACCO PRODUCTS
Tryptamine is a natural component of tobacco leaves. In a transgenic tobacco species, more than 1 mg of tryptamine/g fresh weight was reported, a 260-fold increase over controls (5). Therefore, we conclude that the estimated tryptamine amount in fresh tobacco leaves is ± 4 µg/g fresh weight. Tryptamine is also added to tobacco as a component of cocoa, which is used as a flavouring agent. A typical casing concentration of cocoa for cigarette tobacco is 1% (6). The average amount of tryptamine in cocoa varies from 0.69 - 0.83 µg/g (7). Assuming one cigarette weights approximately 1 g, the maximum tryptamine amount from cocoa in one cigarette is estimated to be ± 8 ng. The natural tryptamine amount in tobacco leaves is significantly higher compared with the tryptamine amount from added cocoa.

AMOUNT IN SMOKE
• **main stream:** no data available
• **side stream:** no data available

SOURCE
(tobacco, combustion product or other)
Tryptamine is an natural tobacco component and is also added to tobacco as a component of cocoa powder, which is used as flavouring agent (6).

ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE
Tryptamine is found in plants such as: orange (0.1 µg/g) and in tomato (4 µg/g). In Egyptian dry sausage, tryptamine was found in 68% of the tested sausages. The average concentration was 12.7 mg/kg (8). After 75 days of ageing, typical Italian dry sausages made with nitrite contained tryptamine 23.9 mg/kg. Corresponding values for sausage manufactured without nitrite was 16.4 mg/kg (9).

COMBUSTION PRODUCTS

No data available

CONSENSUS REPORTS
No data available

STANDARDS AND RECOMMENDATIONS
ADI: An intake of > 40 mg biogenic amines (histamine, tryptamine, tyramine, phenylethylamine, etc.) per meal has been considered potentially toxic (10)

TWA_{NL} = **MAC**: No data available
TWA_D =**MAK**: No data available
TWA_{USA}: No data available
$STEL_{NL}$: No data available
$STEL_{USA}$: No data available
LTEL: No data available
TLV-C: No data available
TLV-CARCINOGENICITY: No data available
MAK-REPRODUCTION: No data available

Others:

Reference value:
No data are available on the human tryptamine reference value in blood. The tryptamine excretion in urine in 24 h was estimated to be 33.5 µg ± 25.8 µg (11). Tryptamine level in the whole brain of human was 0.1 – 1.5 ng/g wet weight tissue. The tryptamine level in whole rat brain ranged between 0.2 – 155 ng/g wet weight tissue (12). Tryptamine levels of 0.04 µg/ml in blood and 0.06 µg/ml in rumen fluid were found in buffalo calves (13).

CLASS
EG Carc. Cat.: No data available
IARC-category: No data available
CEC: No data available

Critical assessment
Comparison of smoking potential related daily intake of tryptamine with daily intake from other sources:

	SMOKING	TRYPTAMINE INTAKE BY EATING		
	25 cigarettes per day	3 chocolate bars of 60 g	Tomato (50g)	Italian dry sausage (50 g)
TRYPTAMINE (µg)	1000[5]*	149[14]	200[15]	820[9]

* = assuming the dry tobacco leaves weight 10 % of fresh leaves and there is no loss on tryptamine during processing

Little is known about the profile of the pyrolysis/combustion products of tryptamine. Reference value in humans is not available.

Conclusion
The estimated natural tryptamine amount in tobacco leaves is at least 5000 times higher than the tryptamine amount from added cocoa. Therefore, it is debatable whether tryptamine should be considered as an additive to tobacco. The daily potential intake of tryptamine from cigarettes (from tobacco leaves and from cocoa) is higher than tryptamine intake from other sources such as chocolate or tomato, and is comparable with Italian dry sausages. Assuming similar bioavailability and no loss by combustion, the plasma concentration reached after ingestion of tryptamine from chocolate sources or other food sources is expected to be lower than after exposure from cigarettes. Also the different route of application via smoking as compared to other sources should be taken into account. Therefore, the systemic and the local effect of smoking related exposure to tryptamine might be a point of concern. Since nothing is known about tryptamine's pyrolysis/combustion products, this may also be a point of concern.

PHARMACODYNAMICS
Mechanism of action
Tryptamine is a neurotransmitter (12) or a modulator of neurotransmission (12, 16, 17). Studies with [^{3}H]-tryptamine have shown [^{3}H]-tryptamine-binding sites in various brain regions and in several visceral organs. Three active classes of compounds, tryptamine analogues, ß-carbolines and substituted phenylethylamines, were shown to displace [^{3}H]-tryptamine binding (12, 18). Tryptamine evokes physiological effects through interaction with the large family of serotonin receptor by means of modulation. It is suggested that synthesis of tryptamine occurs in terminals of dopaminergic neurons and these neurons are seen as allosteric regulator of serotonin receptors. The modulatory effects of tryptamine are mediated either directly at presynaptic and/or postsynaptic tryptamine binding sites of serotonergic neurons or by inducing allosteric changes at serotonin receptor (12).
Furthermore, tryptamine derivatives, such as ß-carbolines, inhibit monoamine oxidase and the monoamine uptake and bind to benzodiazepine receptor (7).

Pulmonary system
- **breathing frequency**: Tryptamine produces pharmacological effects in man which are similar to those produced by LSD and other tryptamine derivatives. One of these effects is tachypnea. No details were available on tryptamine data (19).
- **tidal volume**: no data available
- **lung compliance**: no data available
- **airway resistance**: no data available

Cardiovascular system
- **blood pressure**: see below
- **heart rate**:
Tryptamine has a biphasic effect on the serotonin receptors, regulating the arterial tone (12). Tryptamine (2-20 µg/dose), administered into the lateral cerebral ventricle of the rat, evoked a pressor response, which was sometimes followed by a prolonged depressor response. The intracisternal administration of tryptamine (7-20 µg/dose) caused a slow progressive and long-lasting depressor effect without or with an initial pressor effect. The pressor response was accompanied by variable changes in heart

rate, whilst the pure depressor response was accompanied by a decrease in heart rate. Tryptamine, injected centrally, causes both increases and decreases in arterial blood pressure and heart rate. The pressor response to tryptamine results from the activation of central noncholinergic, methysergide-sensitive, receptor sites and the depressor response to tryptamine may be due to a centrally induced reduction in sympathetic nervous activity. It is tentatively suggested that tryptamine participates in the physiological regulation of the cardiovascular system of the rat, as both a central excitatory and inhibitory regulator (20). Tryptamine produces pharmacological effects in man that are similar to those produced by LSD and other tryptamine derivatives. The cardiovascular effect is tachycardia (19).

Renal system
- **diuresis**: no data available
- **saluresis**: no data available

Nervous system
central nervous system:
It is proposed that tryptamine induces behavioural effect as a result of antagonism of central serotonin systems. It has been shown that serotonin antagonists blocked the certain tryptamine mediated effects, suggesting the possibility of serotonin receptor-mediated tryptamine responses (12). Tryptamine produces pharmacological effects in man which are similar to those produced by LSD and other tryptamine derivatives. The CNS effects are behavioral changes and hallucinations (19).
The effects of intraperitoneal administration of tryptamine to rats pretreated with iproniazid, on the acquisition of an unsignalled one-way active avoidance task, were examined. Tryptamine at 2.5 and 5 mg/kg significantly increased the number of trials required to perform this task. The iproniazid pretreatment had no affect on acquisition, or any other performance variable, of the task. The acquisition deficit induced by tryptamine may involve a direct stimulation of central serotonin receptors since it was not induced by systemically administered serotonin. This effect was reversed by the serotonin antagonists methysergide and metergoline, but was not affected by depletion of brain serotonin, with p-chlorophenylalanine, or by the dopamine antagonist haloperidol (21). Tryptamine given via intracerebroventricular (i.c.v.) injection to mice produced a significant hypothermia at a dosage above 1 μg. The hypothermic effect of tryptamine was inhibited by methysergide whereas ketanserin and p-chlorophenylalanine did not affect it. That study demonstrated that the hypothermia induced by tryptamine i.c.v. was produced by direct activation of the 5-HT1 receptor (22). Tryptamine induces serotonin syndrome (head weaving and hindlimb abduction) and head twitch in mice through induction of the serotonin (5-HT_1 and 5-HT_2) receptors in the brain (23). When tryptamine was injected (2 – 16 μg/dose) into the paraventricular nucleus of the hypothalamus after pre-treatment with a monoamineoxidase inhibitor or with serotonin, it induced an anorectic effect. This effect may be due to a prolongation of the activity of serotonin resulting from tryptamine competing with serotonin for the same reuptake system (24).

- **autonomic system**:
No data available

Other
Tryptamine has been shown to increase a dose-related plasma glucagon level of mice,

which is mediated by the peripheral serotonin (5-HT$_2$) receptor (25). Another study showed a tryptamine induced apparent increase of serum insulin level in mice, mediated also by the same serotonin receptor (16).

Critical assessment

Tryptamine is a neurotransmitter or a modulator of neurotransmission. Tryptamine produces pharmacological effects in man that are similar to LSD and other tryptamine derivatives. Such effects are tachypnea, tachycardia, behavioral changes and hallucinations. Experiments with rats showed that tryptamine evoke effects, which are related with the serotonin receptors. It has a biphasic effect on the arterial tone, induces acquisition deficits, hypothermia and anorectic effect and affected the glucose plasma level. The tryptamine dose used to show these effects were in the range of 1 µg (i.c.v.) (22) and 5 mg/kg body weight (ipr.) (21). However, no data are available on tryptamine pharmacological effects by respiratory studies. It is not clear whether the estimated potential tryptamine dose in cigarette (1000 µg/day) exerts any respiratory effects, as only data are available via other routes.

Conclusion

No conclusion can be made whether the tryptamine dose in cigarettes is high enough to exert any systemic pharmacological effects. The (longterm) effects of tryptamine or its pyrolysis/combustion products on the pulmonary system are unknown and need further study.

PHARMACOKINETICS

Absorption

No data are available on absorption through the respiratory and gastrointestinal system.

Bioavailability

No data are available on the oral and respiratory bioavailability.
Oral tryptamine administration seems to be inactive, due to deamination by monoamine oxidase (26).

Distribution

Tryptamine is found in the brain, liver, kidney and other tissues (12).
Human platelets show an active and saturable uptake of serotonin and tryptamine. The uptake of both substrates appears to be mediated by the same carrier (27).

metabolism

The major route of catabolism for tryptamine is one of enzymatic inactivation. Sequential action by monoamine oxidase and aldehyde dehydrogenase results into formation to indole-3-acetic acid (IAA) via indole acetaldehyde. It has been shown that this pathway produces 70 % of IAA. A minor portion of the aldehyde is reduced to indole-3-ethanolamine by aldehyde reductase. N-methyltransferase, has been shown to exist in human brain, lung and blood and is linked to the formation of hallucinogenic N-methyl and N,N-dimethyl derivatives of tryptamine. In addition to methylation of tryptamine, this enzyme is also linked to the formation of harmalan derivatives (a condensed product of tryptamine with aldehydes) (12). Tryptamine metabolism is sensitive to changes in brain tryptophan. This is especially apparent

after a tryptophan load (28).

Excretion
No data are available for tryptamine excretion after tryptamine loading. Tryptamine is excreted in the urine after oral loading with L-tryptophan (30 mg/kg body weight). The urinary excretion of tryptamine increases immediately after loading and reaches a maximum in approximately 45 min (29).Tryptamine in the unconjugated form in urine collected from human volunteers was 82 ± 11 µg/g creatinine (mean $\pm$ standard error of the mean) (30).

Kinetic parameters
Intraventricular injection into the rat brain tryptamine resulted in rapid exponential decrease of it in the first 30 min after injection. Tryptamine showed a biphasic decrease with half-lives of 4.7 min (over the 5-10 min period) and 14.1 min (10-30 min) (31). The turnover rate is high in the rat brain (38 ng/ g brain tissue/h) (12).

Critical assessment
Little is known about tryptamine pharmacokinetics in man from oral and respiratory studies on tryptamine. The major route of catabolism for tryptamine is one of enzymatic inactivation.

Conclusion
There are no pharmacokinetic data available after tryptamine respiratory and oral loading.

TOXICOLOGY
Acute toxicity
Human
Tryptamine produces pharmacological effects in man which are similar to those produced by LSD, mescaline, psilocin and other tryptamine derivatives. These effects include tachycardia, tachypnea, mydriasis, hyperreflexia, behavioral changes and in man, hallucinations. No details were available on the tryptamine data in that study (19).

Animal
Tryptamine induced serotonin (5-HT) syndrome (head weaving and hindlimb abduction) in rats through the 5-HT1A receptor. The 5-HT syndrome may also be associated with the 5-HT1A receptor in mice, as it is in rats (32). However, another study stated that the serotonin syndrome was attributed to the binding of tryptamine to 5-HT2 receptors and subsequent agonistic actions. Intravenous doses of 25 mg/kg to mice induced the 5-HT syndrome of head weaving and hind limb abduction (33).

The behavioural effects of intravenously administered tryptamine were examined in mice. Tryptamine in a dose greater than 15 mg/kg induced distinct head-weaving and hindlimb abduction. These behavioural syndromes appeared immediately after the injection and disappeared within 3 min. The changes in time course of the behaviour induced by tryptamine were consistent with those of the levels of tryptamine in the brain (34).

The effects of tryptamine on behavior were investigated in mice. Tryptamine at a

dose of 50 mg/kg i.p. induced an inhibition of locomotor activity and, at doses ranging from 150 to 300 mg/kg, induced peculiar behaviors such as head twitch, head weaving, forepaw treading, hindlimb abduction and Straub tail. These behavioral effects were continuous, although tryptamine rapidly disappeared from the brain. It was concluded that tryptamine induced both the depression and excitation in the behavior of mice depending on the dosage and tryptamine -induced excitatory behaviors may be attributed to both its direct stimulation of serotonin receptors and facilitation of serotonin release (35).

LD_{50} ipr rat: 223 mg/kg (33, 36)
LD_{50} ipr. mouse: 100 mg/kg (33, 36)
LD_{50} sc. mouse: 500 mg/kg (33, 36)

Local tolerance
Human
No data available
Animal
No data available

Repeated dose toxicity
Subacute
No data available

Semichronic
No data available

Chronic
No data available

Carcinogenicity
Human
No data available
Animal
No data available

Reproduction toxicology
Human
No data available

Animal
No data about reproduction toxicology on mammals were available.
A study on drosophila reproduction showed 15% reduction of controls when adult insects mated and the young were allowed to develop on medium containing 75 mM tryptamine. Tryptamine-induced depression in reproductive success was due to decreased oviposition rate and preadult survival. Preference tests indicated that tryptamine may act as an antiattractant or antifeedant in this species (37).

Mutagenicity
Human
No data available

Animal
Tryptamine inhibited or enhanced the S9-mediated mutagenesis of 2-amino-3-methylimidazo-[4,5-f]quinoline (IQ) and methyl-2-amino-3-methylimidazo-[4,5-f]quinoline (MeIQ) in Salmonella strain TA98 as a function of amine concentration and also the strain of rat used as the S9 source, and the IQ-type mutagen tested (38). Tryptamine became highly mutagenic upon nitrosation in Salmonella typhimurium strain TA100 (39).

Other

Critical assessment
No data on human tryptamine toxicological doses are available. No toxicological data are available from tryptamine inhalation studies. Intravenous data on tryptamine in rats, indicate that 15 mg/kg dose induced toxicological effects. The LD_{50} mice ranged from 100 mg/kg body (ipr.) weight to 500 mg/kg bodyweight (sc.).

Conclusion
As no data are available on inhalation effects of tryptamine the long-term effect of this compound via the respiratory system needs to be studied.

INTERACTIONS
Chemical
One-electron oxidation of tryptamine with N-3(**.**) and Br-2(**.**) radicals resulted in the formation of an indolyl radical with a pK(a) value of 4.2. The reactions of OH radicals ((OH)-O(**.**)) with tryptamine lead to the formation of (OH)-O(**.**)-adducts, which decay by acid catalyzed water elimination to give indolyl radicals (40). A reaction of tryptamine and other biogenic amines 5-hydroxytryptamine, dopamine, histamine, p-tyramine, ß-phenylethylamine with components of cigarette smoke was observed. Both formaldehyde and cyanide, which are known to be present in cigarette smoke, were involved in the reaction with the primary amines. The reaction was time dependent and was enhanced by an increase in temperature or by incubation under alkaline condtions. Cyanomethyl adduct formation was increased when smoke from cigarettes with higher tar and nicotine content was used. When the amines were incubated with human saliva obtained after cigarette smoking, cyanomethylamine products were readily detected (41). Tetrahydro-ß-carbolines are naturally occurring indole alkaloids produced from indoleamines such as tryptamines and aldehydes and/or alpha-ketoacids through Pictet-Spengler condensation (7).

In vivo
Acetylenic analogues of tryptamine, in which the side chain is attached at the 2 position of the heterocyclic ring, were shown to be inhibitors of MAO-A and MAO-B (42). Tryptamine was degraded by incubation with rat brain homogenate to an unknown product. The same results were obtained with pig brain and bovine brain. The monoamine oxidase inhibitor pargyline inhibited the reaction strongly, indicating the participation of the enzyme to the reaction. Chromatographic and electrophoretic properties as well as the chemical reaction of the product with specific reagents suggested that the compound consisted of an indole part and an amino acid part. It is formed by enzymatic oxidation of tryptamine producing indole-3-acetaldehyde which spontaneously cyclizes with free L-cysteine from the tissue. The results suggest that the reaction of biogenic aldehydes with brain macromolecules may proceed via an

analogous reaction (43).

Tryptamines and ß-carbolines are two classes of psychoactive indoles found in plants and animals (44). ß -carboline alkaloids are derived as a result of condensation between indoleamine (e.g. tryptamine) and short-chain carboxylic acid (e.g. pyruvic acid) or aldehyde (e.g. acetaldehyde), a reaction that occurs readily at room temperature. These compounds have been found endogenously in human and animal tissues and may be formed as a byproduct of a secondary metabolisation (45). Also exogenous aldehydes in may react with tryptamine to form ß-carbolines. When human saliva obtained after cigarette smoking was incubated in the presence of tryptamine, the formation of 1,2,3,4-tetrahydro-ß-carboline (TBC) and 1-methyl-1,2,3,4-tetrahydro-ß-carboline (MTBC) was observed in a short time. After incubation with tryptamine (2.5 µg/ml) for 10 min, the concentrations of TBC and MTBC formed were 3.27 and 0.35 ng/ml, respectively. The analysis of cigarette smoke solution and immersion solutions of denture-base acrylic resins showed that ng- µg/ml levels of formaldehyde and acetaldehyde were contained in cigarette smoke and leached from dental resins. These results indicate that both precursors, tryptamine and aldehydes, coexist in oral environments and that their interaction to form TBC and MTBC potentially occurs in human saliva without participation of salivary enzyme (46).

When tryptamine was injected into the paraventricular nucleus of the hypothalamus after pretreatment with a monoamineoxidase inhibitor or with serotonin, it induced an anorectic effect. This effect may be due to a prolongation of the activity of serotonin resulting from tryptamine competing with serotonin for the same reuptake system (24).

The sequential injection of the dopamine and serotonin receptor agonists, apomorphine and tryptamine, in rats at time intervals with minimal direct behavioral interference, was used to observe response changes with respect to a single challenge. When tryptamine was preceded by an apomorphine challenge the effective doses of the serotonin (5-HT$_2$) antagonists ritanserin and risperidone for 50% inhibition of the seizures increased by a factor of 2.5. When apomorphine was preceded by a tryptamine challenge, the total agitation score of the control animals increased by 59% on the average. Mutual enhancement of tryptamine and apomorphine appears to occur even at a time when the behavioral effects of the first agonist are no longer manifest (47).

CYP2A6 is the principle enzyme metabolizing nicotine to its metabolite cotinine. Tryptamine is specific and relatively selective inhibitor for CYP2A6 and it is suggested that is may be useful in vivo to decrease smoking by inhibiting nicotine metabolism (48).

Critical assessment
Chemical
Tryptamine can be oxidized and thereby radicals are formed. Tryptamine can react with aldehydes and ketones. It forms adducts with other cigarette components and forms also carbolines via Pictet-Spengler condensation.

In vivo
Tryptamine derivatives, such as carbolines, which are readily formed in cigarette smoke, affect the monoamine oxidase system. Tryptamine inhibits the CYP2A6 enzyme and could be therefore inhibit the nicotine degradation. No data were

available on respiratory interaction effects via inhalation.

Conclusion
Chemical
Tryptamine can react with several compounds, such as aldehydes and ketones.

In vivo
Tryptamine derivatives seem to affect the monoamine oxidase system and the CYP2A6 enzym. The contribution of tryptamine in cigarette smoking with respect to these mechanisms can not be established from available data and need to be studied.

DEPENDENCY
It is suggested that tryptamine is seen as allosteric regulator of serotonin receptors. The modulatory effects of tryptamine are mediated either directly at presynaptic and/or postsynaptic tryptamine binding sites of serotonin neurons or by inducing allosteric changes at serotonin receptors (12). Several studies have shown some relationship between nicotine or tobacco dependency and serotonin activity in the brain (49-52). The tryptamine affected serotonin activity may implicate that tryptamine could play a role in the tobacco dependency process.
On the other hand, the craving qualities of chocolate have been thoroughly reviewed and the conclusion seems to be that the pharmacological active compounds in cocoa do not contribute to chocolate craving (53).

Effects on smoking cessation
CYP2A6 is the principle enzyme metabolizing nicotine to its metabolite cotinine. Tryptamine is specific and relatively selective for CYP2A6 and it is suggested that is may be useful in vivo to decrease smoking by inhibiting nicotine metabolism (48).

Critical assessment
The regulation of the serotoninergic system in the brain by tryptamine and the role of this system in the tobacco dependency seems to indicate that tryptamine may has a role in the tobacco dependency process. From literature on chocolate craving, it seems that pharmacological active compounds does not contribute to chocolate craving.

Conclusion
Serotonin (which is regulated in the brain by tryptamine) plays a role in the nicotine dependency. It is not clear how the natural amount of tryptamine from tobacco (which is probably lower than the endogenous amount in the body) may contribute to the process of addiction. The longterm effects of tryptamine and its interaction effects with other agents in the cigarette smoke on the pulmonary system and in the tobacco addiction process are not known and need to be studied.

COMMERCIAL USE
Tryptamine is used as a raw material for the synthesis of the vasodilator and antihypertensive, vincamine (54).

BENEFICIAL EFFECTS
Tryptamine is an endogenous neuroactive metabolite of tryptophan. Tryptamine is a

potent inhibitor of protein biosynthesis, via the competitive inhibition of tryptophanyl-tRNA synthetase (TrpRS). The results indicate that long-term tryptamine treatment of HeLa cells led to a significant increase in the half-life of TrpRS. It was shown that tryptamine is an effective inhibitor of HeLa cell growth. In contrast to the well-characterized antineoplastic compounds, resistance to tryptamine at very low levels was difficult to achieve, i.e. the 2-fold resistant subline was selected after 19 months of treatment of HeLa cells with gradually increasing concentrations of tryptamine. It was suggested that tryptamine could be a potential anti-cancer drug (55).

Critical assessment
Not relevant.

Conclusion
Not relevant.

SUMMARY AND FINAL CONCLUSION
The natural amount of tryptamine in tobacco leaves is at least 5000 times higher than the tryptamine amount from added cocoa to tobacco. Therefore, it is debatable whether tryptamine should be considered as an additive to tobacco. The daily intake of tryptamine from cigarettes (from tobacco leaves and from added cocoa) is is higher than the intake from other sources, like chocolate or tomato. Assuming similar bioavailability, the plasma concentration reached after ingestion of tryptamine from chocolate sources or other food sources is expected to be lower, than after exposure from cigarettes. No conclusion can be drawn about the amount of tryptamine in cigarettes compared with the amount present in mammalian body.

Tryptamine is a neurotransmitter or a modulator of neurotransmission. Tryptamine produces pharmacological effects in man that are similar to LSD and other tryptamine derivatives. Such effects are tachypnea, tachycardia, behavioral changes and hallucinations. Experiments with rats showed that tryptamine evoke effects, which are related to serotonin receptors. It has a biphasic effect on the arterial tone, induction of acquisition deficit, hypothermia and anorectic effect and affected the glucose plasma level. The tryptamine dose used to show these effects were in the range of 1 µg/dose (i.c.v.) and 5 mg/kg body weight (ipr.). However, no data are available on tryptamine pharmacological effects by respiratory studies. It is not clear whether the estimated potential tryptamine dose in cigarette (1000 µg/day) exerts any respiratory effects, as only data are available via other routes.

Little is known about tryptamine pharmacokinetics in man from respiratory studies on tryptamine. It seems that oral tryptamine is inactive and tryptamine level in the brain is affected by tryptophan load. Tryptamine is synthesised by amino acid decarboxylase (AAD) from tryptophan in several tissues. The major route of catabolism for tryptamine is one of enzymatic inactivation. Tryptamine is excreted in the urine after oral loading with L-tryptophan.

Tryptamine produces pharmacological effects in man which are similar to those produced by LSD, mescaline, psilocin and other tryptamine derivatives. These effects include tachycardia, tachypnea, mydriasis, hyperreflexia, behavioral changes and in man, hallucinations. No toxicological data are available from tryptamine inhalation studies. Intravenous data on tryptamine in rats, indicate that 15 mg/kg dose

induced toxicological effects. The LD_{50} mice ranged from 100 mg/kg body weight (ipr.) to 500 mg/kg bodyweight (sc.).

Tryptamine can be oxidized and thereby radicals are formed. It forms adducts with other cigarette components and forms also carbolines via Pictet-Spengler condensation. Tryptamine derivatives, such as carbolines, which are readily formed in cigarette smoke, affect the monoamine oxidase system. Tryptamine inhibits the CYP2A6 enzyme and could therefore inhibit the nicotine degradation. No data were available on respiratory interaction effects via inhalation.

The regulation of the serotoninergic system in the brain by tryptamine and the role of this system in the tobacco dependency seems to indicate that tryptamine has a role in the tobacco dependency process. From literature on chocolate craving, it seems that exogenous tryptamine does not contribute to chocolate craving.
Tryptamine is used as a raw material for the synthesis of the vasodilator and antihypertensive, vincamine.

Since no data on pharmacodynamic, pharmacokinetic and toxicological effects of tryptamine exposure through inhalation are available, the shortterm and longterm effects of exposure to tryptamine through smoking on the respiratory system cannot be established. Furthermore, its additive effects on other biogenic amines present in cigarette smoke are also not known and have to be studied.

More studies are needed on:
- the determination of pyrolysis/combustion products of tryptamine in cigarette smoke;
- the local (respiratory system) effects of long-term use of tryptamine alone and their pyrolysis/combustion products via inhalation.
- the local (respiratory system) effects of long-term use of tryptamine in combination with other biogenic amines via inhalation

Date this sheet was generated
Based on literature available in October 2001.

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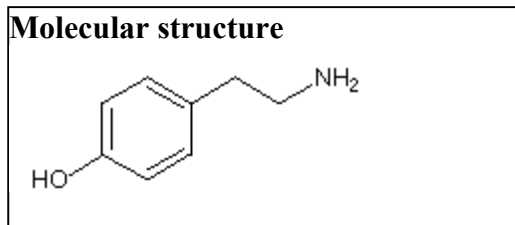
3.7   Tyramine

GENERAL
IUPAC systematic name: tyramine (is a trivial name) (1)
Synonyms: 4-(2-aminoethyl)phenol; 4-hydroxyphenethylamine; p-beta-aminoethylphenol; alpha-(4-hydroxyphenol)-beta-aminoethane (1)
Molecular formula: $C_8H_{11}NO$ (1)

Molecular structure

Molecular weight: 137.18 g/mol (1)
Alifatic: Ethylamine group (1)
Aromatic: Phenol group (1)
N containing: primary amine (1)
Halogen containing: no
CAS registry no.: 51-67-2 (1)
Storage:
R/S classification: R36/37/38; S26/36 (2)
dangercode (transport): no data available.
Properties:
➢ melting point: 161ºC (1)
➢ boilingpoint: 175 – 181 ºC at 1067 Pa (1), 205 – 207 ºC at 3333 Pa (3)
➢ solubility: water: 10 g/l at 15 ºC; soluble in organic solvent(s): benzene, ethanol (4)
➢ density: no data available
➢ refractive index: no data available
➢ substance description:
   • color: colourless (2)
   • liquid/gas/powder: crystalline solid (2)
   • odor/taste: no data available
➢ volatility: no data available
➢ pK_a: pK_{a1} is 9.74 and pK_{a2} is10.52 (3)
➢ PA: no data available
➢ Flammability: no data available
   • FP =
   • FL Limits =
   • IT =
➢ decomposition temperature: no data available
➢ stability: a 12.66 mg tyramine hydrochloride water solution (eqivalent to 10 mg tyramine base) is stable for at least 1 year stored in dark bottle at 4 ºC (5).
➢ vapour pressure/ vapour tension (20 °C): no data available
➢ vapour pressure (50 °C): no data available
➢ relative density: no data available
➢ octanol water partition coefficient, log P, log K_{OW}: log K_{OW} is 0.72 (4)

> ➢ conversion factor: no data available

Critical assessment
Tyramine can be regarded as being phenol, ring-linked to an amino group containing, aliphatic, short chain (aminoethyl-group).
Phenol contains a homocyclic six membered ring (**no** nitrogen or oxygen atoms in the ring). The ring linked hydroxyl has the potential to act as a (very weak) acid.
The free amino group in the aliphatic chain is a potential group to react with aldehydes and ketones and with monoamino-oxydase (MOA), and it adds basic properties to the compound.

Conclusion
Tyramine potentially acts as a competitor for nicotine with respect to the oxidation reaction with monoamino-oxydase.

FUNCTION IN TOBACCO
No data available.

AMOUNT IN TOBACCO PRODUCTS
Tyramine is a natural component of tobacco leaves. In *Nicotiana tabacum* plant, the amount of free tyramine was 40 µg/g fresh weight (6). Assuming the dry weight of tobacco is 10 % of the fresh weight and tyramine is not degraded during fermentation process, than we conclude that the estimated tyramine amount in dried tobacco plant is ± 400 µg/g dry weight. Tyramine is also added to tobacco as a component of cocoa, which is used as a flavouring agent. A typical casing concentration of cocoa for cigarette tobacco is 1% (7). The average amount of tyramine in cocoa varies from 0.73 – 14.7 µg/g (8). Assuming one cigarette weights approximately 1 g, the maximum tyramine amount from cocoa in one cigarette is estimated to be 147 ng. The natural tyramine amount in cigarettes from tobacco plant is ±2700 times higher compared to the tyramine amount from added cocoa.

AMOUNT IN SMOKE
- **main stream**
  No data available.
- **side stream**
  No data available.

SOURCE
Tyramine is a natural tobacco component and is also added to tobacco as a component of cocoa powder, which is used as flavouring agent (7).

ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE
Tyramine content was determined in fish and fish products, ripening and processed cheese, yeast, wine, cabbage and sauerkraut, and tomato paste. Tyramine levels found in those products were: raw fish 0.0-2.6 mg/100 g, fish products 0.0-10.0 mg/100 g, and cheeses 1.3-20.0 mg/100 g. In the remaining food products (tomato paste, yeast, wine, cabbage and sauerkraut) tyramine content fluctuated between 0.0-8.0 mg/100 g

(highest in sauerkraut) (9). Free tyramine measured in several beverages (red and white wine, chiantie and beer) showed an average tyramine concentration which ranged between 1.22 mg/l to 1.48 mg/l (10).

COMBUSTION PRODUCTS
No data available.

CONSENSUS REPORTS
No data availabe.

STANDARDS AND RECOMMENDATIONS
ADI: An intake of > 40 mg biogenic amines (histamine, tryptamine, tyramine, phenylethylamine, etc.) per meal has been considered potentially toxic. In cheese and sauerkraut it is recommended that the sum of tyramine, histamine, putrescine and cadaverine should not exceed the amount of 0.03 % (w/w) (11).
$TWA_{NL} = MAC$: no data available.
$TWA_D = MAK$: no data available.
TWA_{USA}: no data available.
$STEL_{NL}$: no data available.
$STEL_{USA}$: no data available.
LTEL: no data available.
TLV-C: no data available.
TLV-CARCINOGENICITY: no data available.
MAK-REPRODUCTION: no data available.

Others:

Reference value:
The mean basal plasma tyramine concentrations measured in 24 healthy male volunteers were 4.0 ± 1.5 ng/ml (12). Another study found a lower mean plasma tyramine concentration in eight normal subjects: 1.3 ± 0.1 ng /ml (13).

CLASS
EG Carc. Cat.: no data available.
IARC-category: no data available.
CEC: no data available.

Critical assessment
Comparison of smoking related daily intake of tyramine with daily intake from other sources:

	SMOKING	TYRAMINE INTAKE BY EATING OR DRINKING		
	25 cigarettes (1 % cocoa)	3 chocolate bars of 60 g	wine (125 g)	cheese (50 g)
TYRAMINE (µg)	10004[6, 7]	2646[8]	185[10]	10000[9]

No data are available on the profile of the pyrolysis/combustion products of tyramine.

Conclusion
The estimated natural tyramine amount from tobacco plant is at least 2700 times higher than the tyramine amount from added cocoa. Therefore, it is debatable whether tyramine should be considered as an additive to tobacco. The daily potential intake of tyramine from cigarettes (from tobacco plant and from cocoa) is higher than tyramine intake from other sources such as chocolate or wine, and is comparable with cheese. The plasma concentration reached after ingestion of tyramine from chocolate or other food sources is expected to be lower or equal to tyramine after exposure from cigarettes, assuming similar bioavailability and no loss by combustion. Also the different route of application via smoking as compared to other sources should be taken into account. Therefore, the systemic and the local effect of smoking related exposure to tyramine might be a point of concern. Since nothing is known about the pyrolysis/combustion products of tyramine, this may also be a point of concern.

PHARMACODYNAMICS
Mechanism of action
Tyramine is an indirectly acting sympathomimetic substance. It is taken up by the neural endings where it stimulates the release of noradrenaline. Tyramine does not affect plasma adrenaline. The effect of endogenous noradrenaline (released by tyramine) is characterised by increased blood pressure. This increase in blood pressure results from its myocardial positive inotrope action, mainly mediated by cardial β_1-adrenoreceptor stimulation and is not due to vasoconstrictor effects (14).

Pulmonary system
Tyramine releases noradrenaline from the neural endings. Noradrenaline is a potent agonist of α- and β_1-adrenoreceptors, but has little action on β_2-receptors. Since, the smooth musculature in the respiratory system is mainly stimulated by β_2-receptors (15), it is not expected that noradrenaline released by tyramine in the respiratory system will lead to significant bronchial dilatation.

- **breathing frequency**: No data available.
- **tidal volume**: No data available.
- **lung compliance**: No data available.
- **airway resistance**: No data available.

Cardiovascular system
- **blood pressure**: Tyramine (i.v. up to 20 µg/min/kg body weight for 15 min ≈ 21 mg) significantly lowered diastolic blood pressure (Δmax −6.8 ± 3.1 mm Hg) and induced a marked increase in systolic blood pressure (Δmax 56.9 ± 6.8 mm Hg) in healthy young male volunteers (26.1 ± 0.5 years, n = 12). The increased blood pressure by tyramine is suggested to be a result of myocardial positive inotropic action (14). Tyramine (i.v. up to 20 µg/min/kg body weight for 15 min) caused a smaller increase in systolic blood pressure in elder healthy volunteers (61 ± 2.2 years (3 females and 3 males)) than in the healthy young volunteers; in addition it slightly increased the diastolic blood pressure while it decreased diastolic blood

pressure in young healthy volunteers (16). In another study it was found that tyramine (i.v. 15.0 μg/kg/min for 30 min) elevated systolic blood pressure (SBP) from 122 ± 11 to 149 ± 4 mm Hg, without increasing diastolic blood pressure or heart rate (17). After ingestion of 400 - 600 mg tyramine added to meals by eight healthy volunteers of both sexes, it was shown that the SBP increased by more than 30 mmHg. When the subjects received moclobemide 600 mg/day (a monoamine oxidase inhibitor) for seven days, an average dose of 250 mg tyramine (range 150-400 mg) was needed to increase SBP by 36.6 mmHg (18) The pressor effect of intravenous tyramine was investigated in 19 healthy unmedicated subjects. The pressor dose (PD) that raised systolic blood pressure by 30 mm Hg (PD30) ranged from 2 to 8 mg for tyramine. Coefficients of variation ranged from 3 to 47%. A sex-related difference was found for the PD30 of i.v. tyramine: 4.4 mg for 11 males and 3.8 mg for 8 females. Additional results from supported this observation; PD30 of tyramine 4.6 mg in 34 males vs. 3.5 mg in 21 females (19).

- **heart rate**: Tyramine (i.v. up to 20 μg/min/kg body weight for 15 min) did not show any dose-related changes in heart rate during i.v. tyramine dosage; however, tyramine caused a pronounced shortening of QS_2c and pre-ejection period of the left ventricle (14). Another study confirmed that tyramine (i.v. 15.0 μg/kg/min for 30 min) does not change the heart rate (17).

Renal system
- **diuresis**: A non-pressor dose of intravenous tyramine of 4 μg/kg/min for 120 min in 8 healthy volunteers caused a significant increase in urinary flow rate ($p < 0.05$ (20).

- **saluresis**: A pressor dose of tyramine (i.v. 15 μg/ kg/min) in six normal volunteers induced increase in blood pressure and subsequent natriuresis (21).

Nervous system
- **central nervous system**: No data are available on the effect of tyramine on the human central nervous system. Most of the tyramine data on central nervous system are from animal experiments.

  Both p- and m-tyramine are found in rat brain. The p- and m-tyramine are unevenly
  distributed among the nuclei. The highest concentrations of p-tyramine were measured in the olfactory tubercle, followed by the nucleus accumbens and septal nuclei, for m-tyramine the concentrations decreased in the following order: olfactory tubercle, nucleus accumbens, amygdala, septal nuclei, and nucleus tractus diagonalis (22).
  The brain microdialysis technique was used to examine the in vivo effects of tyramine on dopamine (DA) release and metabolites in the striatum of halothane-anesthetized rats. A dose-related release of DA was also observed following addition of tyramine (1-100 μM) to the perfusing buffer. Tyramine-induced DA release appears to involve a carrier-dependent process. Tyramine induces the release of DA from vesicular stores (23).
  Tetrabenazine induced depression of performance of rats in shuttle box and is antagonized by sympathomimetic amine with catecholamine enhancers. Tyramine,

which are rapidly metabolized in vivo, was ineffective up to 40 mg/kg to antagonize the effect of tetrabenazine in the shutlle box (24).

The effect of tyramine on brain noradrenaline (NA) containing neurons in the locus coeruleus (LC) was analyzed using single unit recording techniques. In control rats, administration of high doses of tyramine caused a slight inhibition of firing. However, after pretreatment with the monoamineoxidase (MAO)-A inhibitors clorgyline (10 mg/kg, i.p., 1 h) or amiflamine (3 mg/kg, i.p., 1 h) administration of low doses of tyramine caused a consistent and dose-dependent inhibition of firing of the noradrenergic neurons. This inhibition was reversed by the α_2-receptor antagonist yohimbine and prevented by depletion of endogenous stores of noradrenaline (pretreatment with reserpine (10 mg/kg, i.p., 5 h) ad α-methyl-p-tyrosine (250 mg/kg, i.p., 30 min). Pretreatment with the MAO-B inhibitor (-)-deprenyl (10 mg/kg, i.p., 1 h) did not promote tyramine to inhibit LC units and therefore it is suggested that this is related to a re-uptake blocking effect of its metabolite, I-amphetamine. Apparently, tyramine, although known to be a rather polar agent, can inhibit central noradrenergic firing rate via an indirect, α_2-receptor mediated effect. The present results indicate that the serious "cheese effect" of MAO-inhibitors may also have a central origin. Small amounts of pressor amines, which are normally considered to be harmless, in foods (especially cheese) can lead to a hypertensive crisis in patients on MAO-inhibitor drug regimens, which is often termed the 'cheese reaction' (25).

- **autonomic system**: Administration of tyramine (i.v. 300 µg/kg) to human volunteers, increased plasma noradrenaline level by 145 ± 39 pg/ml (n = 6) from the baseline. Tyramine did not affect plasma adrenaline (14). In another study, tyramine (i.v. 15.0 micrograms/kg/min for 30 min) increased plasma noradrenaline from 547 ± 184 to 836 ± 96 pg/ml in normal human volunteers; plasma adrenaline was unchanged (17).

Other
Tyramine hydrochloride eyedrops (75 mM; 2 x 10 µl) evoked a significant mydriasis both in light and dark in healthy male subjects (aged 18 – 22 years, n = 8), which was more prominent in the light condition (change in resting pupil size; mm ± s.e.m: light 1.05 ± 0.28; dark: 0.73 ± 0.15) (26).

Critical assessment
Tyramine is an indirect acting sympathomimetic substance. It increases the release of noradrenaline from the neural endings. The main pharmacological effect of tyramine is the increase of the blood pressure. About 21 mg tyramine (i.v. 20 µg/kg/min in 15 min) increased the systolic blood pressure significantly. It is unlikely that tyramine dose in cigarettes (estimated 0.4 mg/cigarette) will exert a significant increase in systolic blood pressure.Based on the mechanism of action of tyramine by releasing noradrenaline from the neural endings, it is expected that the dose of tyramine in cigarettes will not have a significant effect on the bronchial function.

Conclusion
It seems unlikely that the tyramine dose in one cigarette (estimated 0.4 mg/cigarette) could increase the systolic blood pressure significantly. The (longterm) effects of tyramine or its pyrolysis/combustion products on the pulmonary system are unknown and need further study.

PHARMACOKINETICS

Absorption

Tyramine is rapidly absorbed from the gastrointestinal tract and is very rapidly cleared from plasma (12).

Bioavailability

Studies with everted intestines showed that at concentrations above 10 µM over 70% of tyramine was deaminated during transport (27), which means that the oral tyramine bioavailability will be reduced. As tyramine is a good substrate for MAO-A, inhibition of MAO results in enhanced bioavailability of tyramine (12).

Distribution

^{14}C- tyramine bound to plasma proteins of rabbits in dose- and time of incubation-related manner. Maximal binding capacity was 70.2 ug/g affinity for plasma proteins, much lower than that of noradrenaline (4).

Metabolism

Tyramine can be deaminated by monoamine oxidase types A and B in a variety of tissues, including the wall of the gastro-intestinal tract, liver and the central nervous system (1, 4). About 70% of the total monoamineoxidase (MAO)-enzymes in the rat intestines constitued of the A-form. A similar proportion of that form of the enzyme was found in homogenates of biopsy samples of human intestine. Studies with everted intestines showed that at concentrations above 10 µM over 70% of tyramine was deaminated during transport and the use of selective inhibitors confirmed the A-form of monoamine oxidase to play the dominant role in that process (27).Tyramine taken orally is normally detoxicated by monoamine oxidase, present in intestine and liver, to yield para-hydroxyphenylethanol, para-hydroxyphenylacetic acid and its glycine conjugate, para-hydroxyphenaceturic acid, and n-acetyltyramine (1, 4).
In a study with human hepatic microsomes, it was shown that CYP2D is capable of converting tyramine to dopamine. Those results suggest that dopamine is formed from endogenous and/or exogenous tyramine by this CYP2D isoform (28).

Excretion

Eight normal subjects ingested 125 mg of deuterium-labelled p-tyramine hydrochloride and the 3 h and following 21 h urine collections were analysed by monitoring for the deuterated metabolites: free and conjugated p-tyramine, free p-octopamine, free and conjugated p-hydroxyphenylacetic acid, and free p-hydroxymandelic acid. These metabolites accounted for 72% of the ingested label, of which conjugated p-tyramine and free p-hydroxyphenylacetic acid constituted 90%. Approximately 50% of the total deuterated tyramine and 70% of the total deuterated p-hydroxyphenylacetic acid were excreted in the first three hours, although there was considerable variation between individuals. (29)

Kinetic parameters

The elimination half-life of tyramine is 0.30 ± 0.24 h (n=46) determined in normal human male subjects (12).

Critical assessment

Tyramine taken orally, is largely metabolised by the MAO-enzymes in the intestines.

No data are available on respiratory pharmacokinetics of tyramine in man, but as MAO also occur in the lungs, probably tyramine is also metabolised by inhalation. The major route of catabolism for tyramine is one of enzymatic L-deaminohydroxylation, and oxidation of the hydroxyl moiety and glycine conjugation.

Conclusion
There are no pharmacokinetic data available on respiratory intake of tyramine, but the lung MAO will metabolise inhaled tyramine.

TOXICOLOGY
Acute toxicity
Human
Small amounts of pressor amines, which are normally considered to be harmless, in foods can lead to a hypertensive crisis in patients on monoamine oxidase inhibitor (MAOI) drug regimens, which is often termed the 'cheese reaction' Consumption of 6 mg of tyramine may produce a mild crisis whereas 10 to 25 mg may produce severe headaches with intracranial hemorrhage and its sequelae (30).

Animal
Acute oral toxicity in Wistar rats is > 2000 mg/kg (1)
LD_{50} i.v. mice, rabbits 229, 300 mg/kg, respectively (1)
LDLo i.p. mice 800 mg/kg (1)
LDLo s.c. cat, mice is 30, 225 mg/kg, respectively (1)
No-observed-adverse-effect level (6 wk) in Wistar rat 2000 ppm in diet (180 mg/kg/day) (1)
LD_{50} icv-mice: 30 mg/kg (31)
LDLo scu-cat: 30 mg/kg (31)

Local tolerance
Human
No data available.
Animal
No data available.

Repeated dose toxicity
Subacute
The acute and subacute toxicity of tyramine has been examined in Wistar rats. Tyramine caused a dose-related increase in blood pressure after intravenous administration. In 6-wk studies tyramine was administered in the diet to groups of 10 male and 10 female rats. Tyramine was given at levels of 0, 18, 180, 900 mg/kg body weight/day in the first study and at levels of 0 or 900 mg/kg body weight/day in a second study. Decreased body weights associated with diminished food intake were generally seen. The no-observed-adverse-effect level was 2000 ppm (180 mg/kg body weight/day) for tyramine (32).

Semichronic
No data available.

Chronic

No data available.

Carcinogenicity
Human
No data available.

Animal
A variety of foodstuffs including soy sauce, vegetables and smoked foods showed direct-acting mutagenicity in bacteria upon nitrite treatment. The direct-acting mutagenic products of phenolic compounds with nitrite were all diazo derivatives. The diazo compound formed from tyramine with nitrite was proved to be carcinogenic in rats (33, 34). A mutagenic nitrosation product of tyramine, 4-(2-aminoethyl)-6-diazo-2,4-cyclohexadienone (3-diazotyramine, 3-DT) preferentially induced tumors of the oral cavity. Squamous-cell carcinomas of the mucosa of the oral cavity floor developed in 19 out of 28 male F344 rats administered 0.1% (w/v) 3-DT in their drinking water. Tyramine and nitrite are found at fairly high concentrations in various foods. This demonstration of the carcinogenicity of 3-DT indicates that although the implications of 3-DT for human cancer are not clear, other nitrosable mutagen precursors need to be tested as possible risk factors in human cancer (35).

Reproduction toxicology
Human
No data available.
Animal
No data available.

Mutagenicity
Human
No data avaialble.

Animal
The mutagenic effects of tyramine have been thouroughly investigated, especially reaction products of tyramine with nitrites. In one study no mutagenicity of tyramine was found, but most studies indicated mutagenicity of tyramine.

Using the L5178Y mouse lymphoma cell thymidine kinase locus and the Salmonella his locus assays, the mutagenic potentials of tyramine and several catecholamines were examined. In the mouse lymphoma assay tyramine was inactive. Mutagenic responses in Salmonella were also negative (36).
Content of tyramine was determined in salted and dried small fish and its mutagenicity after nitrosification was assayed. Results showed content of tyramine in the fish correlated significantly with mutagenicity (r = 0.993, and P < 0.01) (37).
The acute cytogenetic effect of tyramine, precursor of the mutagen present in soy sauce, was studied on mouse bone marrow cells in vivo by the micronucleus test. The incidence of micronucleated polychromatic erythrocytes (MNPCE) in bone marrow cells gradually increased and reached a maximum level 24 h after intraperitoneal injection of tyramine and decreased within 36 h. A dose-dependent increase in MNPCE was clearly observed for tyramine. Compared to the values for the untreated control, significant positive results were obtained with 0.5 mmole tyramine/kg (68.5

mg/kg) 24 h after intraperitoneal administration. Micronuclei were significantly induced but no severe reduction in the ratio of polychromatic/normochromatic erythrocyte was observed (38).
The acute cytogenetic effects of tyramine, precursor of tyramine derived mutagen present in soy sauce, was studied with the in vivo chromosome aberration test in rat bone marrow cells. Tyramine was administered intraperitoneally. Statistically significant positive result was obtained with tyramine at a dose of 5 mmole/kg (686 mg/kg) body weight. Chromosome aberrations (CA) induced by L-proline co-administered with tyramine were significantly lower than those induced by tyramine alone. These data suggest that L-proline, after endogenous nitrosation, became nitrosoproline and suppressed CA, and that, as a result of in vivo nitrosation of tyramine, they became mutagenic nitroso compounds showing positive results. Statistically significant positive results were obtained by administration of 40 mmole NaCl/kg body weight (2338 mg/kg). The cocarcinogenic role of NaCl with tyramine was suggested because soy sauce contains about 18% NaCl (39).
Mutagenicity of nitrite treated Japanese soy sauce (4 kinds) and tyramine, which is a precursor of a mutagen (3-diazotyramine) and present in soy sauce, was studied in Chinese hamster V79. Nitrite-treated tyramine was mutagenic for the cells; it induced 8.6, 13.3, and 18.3 TG-resistant mutants per 10^{-5} clonable cells at concentrations of 20 μM, 56 μM, and 112 μM, respectively (40).

Other

Critical assessment
Small amounts of pressor amines, which are normally considered to be harmless, in foods can lead to a hypertensive crisis in patients on monoamine oxidase inhibitor (MAOI) drug regimens. Consumption of 6 mg of tyramine may produce a mild crisis whereas 10 to 25 mg may produce severe headaches with intracranial hemorrhage.
The oral NOAEL in rat was 180 mg/kg body weight/day. The tyramine dose in one cigarette (0.4 mg/cigarette) seems to be too low to have a significant systemic toxicological effect. However, no data are available on the inhalation toxicological effect of tyramine. Tyramine forms diazo-derivatives with nitrite, which are carcinogenic and mutagenic.

Conclusion
 data are available on inhalation toxicological effects of tyramine and its combustion products. The long-term effect of this compound via the respiratory system needs to be studied.

INTERACTIONS
Chemical
A reaction of p-tyramine and other biogenic amines 5-hydroxytryptamine, dopamine, histamine, beta-phenylethylamine and tryptamine with components of cigarette smoke was observed. Both formaldehyde and cyanide, which are known to be present in cigarette smoke, were involved in the reaction with the primary amines. The reaction was time dependent and was enhanced by an increase in temperature or by incubation under alkaline conditions. Cyanomethyl adduct formation was increased

when smoke from cigarettes with higher tar and nicotine content was used. When the amines were incubated with human saliva obtained after cigarette smoking, cyanomethylamine products were readily detected (41).

In vivo
The potentially fatal consequences of ingesting tyramine whilst receiving therapy with monoamine oxidase inhibitors have been well documented. In normal subjects, tyramine is rapidly inactivated by monoamine oxidase, but when the enzyme is inhibited, tyramine can cause hypertensive crises by its indirect sympathomimetic actions (1). Some monoamine oxidase inhibitors are moclobemide (18) and toloxatone (42), brofaromine, clorgyline, selegiline, phenelzine, tranylcypromine (43). In healthy volunteers, both propanolol and indenolol reduced the pressor response to tyramine, as shown by a significant increase in the dose of tyramine (effective dose) required to increase systolic blood pressure by 15% (ED_{15}). The ED_{15} (i.v., bolus injection) was 2.2 mg prior treatment and 5.5 mg and 5.2 mg respectively for indelol and propanolol (44).

Critical assessment
Chemical
The free amino group is
- a potential group to react with aldehydes and ketones and with monoamino-oxydase (MOA);
- a base group, i.e. a potential group to react with acids.

The phenolic hydroxyl group is a potential protondonor.

In vivo
The bioavailability of tyramine is affected by monoamine oxidase inhibitors. Anti-hypertension drugs reduced the pressor response to tyramine.

Conclusion
Chemical
Tyramine contains two reactive sites of different nature: the aliphatic aminogroup (base) and the phenolic hydroxylgroup (slightly acidic).

In vivo
Tyramine shows an interaction with monoamine oxidase inhibitors and anti-hypertension drugs.

DEPENDENCY
No data available.

Effects of smoking cessation
No data available.

Critical assessment
Not possible.

Conclusion

Not possible.

COMMERCIAL USE
Tyramine hydrochloride solution (Mydrial-Atropin) is used for production of mydriasis (45).

BENEFICIAL EFFECTS
No data available.

Critical assessment
Not relevant.

Conclusion
Not relevant.

SUMMARY AND FINAL CONCLUSION
Tyramine is a natural tobacco component and is also added to tobacco as a component of cocoa powder, which is used as flavouring agent. The estimated tyramine amount in dried tobacco plant is ± 400 µg/g dry weight. The average amount of tyramine in cocoa varies from 0.73 – 14.7 µg/g. Tyramine is found in fish and fish products, ripening and processed cheese, yeast, wine, cabbage and sauerkraut, and tomato paste. An intake of > 40 mg biogenic amines (histamine, tryptamine, tyramine, phenylethylamine, etc.) per meal has been considered potentially toxic. The estimated tyramine amount in cigarettes from tobacco plant is at least 2700 times higher than the tyramine amount from added cocoa. Therefore, it is debatable whether tyramine should be considered as an additive to tobacco. The daily potential intake of tyramine from cigarettes (from tobacco plant and from cocoa) (10 mg/25 cigarettes/day) is higher than tyramine intake from other sources such as chocolate (2.6 mg/3 bars) or wine (0.2 mg/glass), and is comparable with cheese (10 mg/50g). The plasma concentration reached after ingestion of tyramine from chocolate or other food sources is expected to be lower or equal to tyramine after exposure from cigarettes, assuming similar bioavailability and no loss by combustion. Also the different route of application via smoking as compared to other sources should be taken into account. Therefore, the systemic and the local effect of smoking related exposure to tyramine might be a point of concern. Since nothing is known about the pyrolysis/combustion products of tyramine, this may also be a point of concern.

Tyramine is an indirect acting sympathomimetic substance. It increases the release of noradrenaline from the neural endings. The main pharmacological effect of tyramine is the increase of the blood pressure. About 21 mg tyramine (i.v. in 15 min) increased the systolic blood pressure significantly. It is unlikely that tyramine dose in cigarettes (estimated 0.4 mg/cigarette) will exert a significant increase in systolic blood pressure. Based on the mechanism of action of tyramine by releasing noradrenaline from the neural endings, it is expected that the dose of tyramine in cigarettes will not have a significant effect on the bronchial function.

Oral tyramine is largely metabolised by the MAO-enzymes in the intestines. No data

are available on the respiratory pharmacokinetics of tyramine in man, but the lung MAO will metabolise inhaled tyramine. Tyramine taken orally is normally detoxicated by monoamine oxidase, present in intestine and liver, to yield para-hydroxyphenylethanol, para-hydroxyphenylacetic acid and its glycine conjugate, para-hydroxyphenaceturic acid, and n-acetyltyramine.

Small amounts pressor amines, which are normally considered to be harmless, in foods can lead to a hypertensive crisis in patients on monoamine oxidase inhibitor (MAOI) drug regimens. Consumption of 6 mg of tyramine may produce a mild crisis whereas 10 to 25 mg may produce severe headaches with intracranial hemorrhage. The tyramine dose in one cigarette (0.4 mg/cigarette) seems to be too low to have a significant systemic toxicological effect. However, no data are available on the inhalation toxicological effect of tyramine. The oral NOAEL from a diet study in rat was 180 mg/kg body weight/day. Tyramine forms diazo-derivatives with nitrite, which are carcinogenic and mutagenic.

Tyramine contains two reactive sites of different nature: the aliphatic aminogroup (base) and the phenolic hydroxylgroup (slightly acidic). Tyramine interacts with monoamine oxidase inhibitors and anti-hypertension drugs. There are no data available on dependency or smoking cessation.

Since no data are available on pharmacodynamic, pharmacokinetic and toxicological effects of tyramine exposure through inhalation, the shortterm and longterm effects of exposure to tyramine through smoking on the respiratory system cannot be established. Furthermore, its additive effects on other biogenic amines present in cigarette smoke are also not known and have to be studied.

More studies are needed on:
- the determination of pyrolysis/combustion products of tyramine in cigarette smoke;
- the local (respiratory system) effects of long-term use of tyramine alone and its pyrolysis/combustion products via inhalation;
- the local (respiratory system) effects of long-term use of tyramine in combination with other biogenic amines via inhalation.

Date this sheet was generated
Based on literature available in january 2002.

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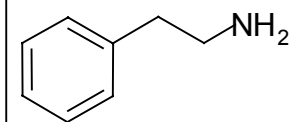
3.8   Phenylethylamine

GENERAL
IUPAC systematic name: phenethylamine (1)
Synonyms: benzenethanamine-; beta-(aminoethyl)benzene (1)
Molecular formula: $C_8H_{11}N$ (1)

Molecular structure

Molecular weight: 121.18 g/mol (1)
Alifatic: yes, ethyl group (1)
Aromatic: yes, phenyl group (1)
N containing: yes, amine group (1)
Halogen containing: no
CAS registry no.: 64-04-0 (1)
Storage:
R/S classification: R 22-34 and S (01/02)-26-28-36/37/39-45 (2)
dangercode (transport): 80 (2)
Properties:
➢ melting point: -60ºC (2)
➢ boiling point: 197 – 200 ºC (1), 198ºC (2)
➢ density: 0.958 g/ml (2)
➢ refractive index: 1.529 º at 25 ºC (3)
➢ solubility: 4.3 g/l water, soluble in ethanol, ether, tetrachloromethane (2)
➢ substance description:
    •   color: colourless to light yellow (2)
    •   liquid/gas/powder: liquid (2)
    •   odor/taste: amine-like odour, smell of fish (2)
➢ volatility: slightly volatile (2)
➢ pK_a: 9.84 (3)
➢ PA: 936.2 kJ/mol (4)
➢ flammability:
    •   FP = 90 ºC (1) and another source states 80ºC (2)
    •   FL Limits = no data available
    •   IT = 425 ºC (2)
➢ decomposition temperature:
➢ stability: unstable on exposure to air (2)
➢ vapour pressure/ vapour tension (20 °C): 0.298 mmHg (40 Pa) at 25 ºC (4)
➢ vapour pressure (50 °C): 500 Pa (2)
➢ relative density: 0.96 (2)
➢ octanol water partition coefficient, log P, log K_{OW}: log P is 1.41 (1)
➢ conversion factor: not relevant

Critical assessment
Phenylethylamine is an endogenous amine related structurally and pharmocologically
to amphetamine (5). The aliphatic bound amine group is chemically the dominant

feature, immediately followed by the presence of the phenyl ring.
The amine group supplies the compound with its base character, enabling it to react
with acids: adduct formation with hydrochloride results in the well-known salt.
At the other hand the benzene ring provides the aromatic feature to the compound.

Conclusion
Being an endogenous amine, phenylethylamine has an aliphatic base character while
at the same time the structure contains an aromatic part.
It readily forms a salt with an acid.

FUNCTION IN TOBACCO
No data available.

AMOUNT IN TOBACCO PRODUCTS
Phenylethylamine was found in several parts of *Nicotiana tabacum cv Xanthi n.c.*
plant. Depending on the development and the part of the plant it varies between 58 –
400 nmol/ g fresh weight (7.0 – 48.5 µg/g fresh weight) (6). Assuming the dry weight
of tobacco is 10 % of the fresh weight and phenylethylamine is not degraded during
fermentation process, than we conclude that the estimated phenylethylamine amount
in dried tobacco plant is between 70 - 485 µg/g dry weight. Assuming 1 g tobacco is
used in cigarette, then the phenylethylamine level in one is cigarette is estimated to
be between 70 µg and 485 µg. Phenylethylamine is also added to tobacco as a
component of cocoa, which is used as a flavouring agent. A typical casing
concentration of cocoa for cigarette tobacco is 1% (7). In cocoa phenylethylamine
ranged from 0.22 µg/g to 22.0 µg/g (8). Assuming one cigarette weights
approximately 1 g, the maximum phenylethylamine amount from cocoa in one
cigarette is estimated to be 220 ng. The maximum natural phenylethylamine amount
in cigarettes from tobacco plant is about 2200 times higher compared with the
maximum phenylethylamine amount from added cocoa.

AMOUNT IN SMOKE
* **main stream**
  No data available.
* **side stream**
  No data available.

SOURCE
Phenylethylamine is an natural tobacco component (6) and is also added to tobacco as
a component of cocoa powder, which is used as flavouring agent (7).

ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE
The mean phenylethylamine concentration which was determined in some English
cheese ranged from 6.1 – 11.3 mg/kg (8). Phenylethylamine was also found in
sauerkraut (2 mg/kg), Dutch cheese (9 mg/kg) and fermented sausage (14 mg/kg) (9).

COMBUSTION PRODUCTS
No data available.

CONSENSUS REPORTS
No data available.

STANDARDS AND RECOMMENDATIONS
ADI: A threshold value of 30 mg/kg for phenylethylamine has been reported (9).
TWA$_{NL}$ = MAC: No data available.
TWA$_D$ =MAK: No data available.
TWA$_{USA}$: No data available.
STEL$_{NL}$: No data available.
STEL$_{USA}$: No data available.
LTEL: No data available.
TLV-C: No data available.
TLV-CARCINOGENICITY: No data available.
MAK-REPRODUCTION: No data available.

Others:

Reference value:
The mean plasma phenylethylamine level in healthy volunteers was 1129.8 ± 268.1 pg/ml (n=40, age 39.3±10.3 year (mean ± standard deviation)) (10).

CLASS
EG Carc. Cat.: No data available.
IARC-category: No data available.
CEC: No data available.

Critical assessment
Comparison of smoking potential related daily intake of phenylethylamine with daily intake from other sources:

	SMOKING	PHENYLETHYLAMINE INTAKE		
		BY EATING		
	25 cigarettes /day	3 chocolate bars of 60 g	Dutch cheese (50g)	sausage (50 g)
phenylethylamine (mg)	12.1[6]*	4.0[8]	0.45[9]	0.9[9]

 * = assuming the dry tobacco leaves weight 10 % of fresh leaves and there is no loss on phenylethylamine during processing and combustion

Nothing is known about the profile of the pyrolysis/combustion products of phenylethylamine.

Conclusion
The estimated natural phenylethylamine amount from tobacco plant in cigarette is at least 2200 times higher than phenylethylamine from added cocoa. Therefore, it is debatable whether phenylethylamine should be considered as an additive to tobacco. The daily potential intake of phenylethylamine from cigarettes (from tobacco plant and from added cocoa) is higher than phenylethylamine intake from other sources such as chocolate, sausage or cheese. Assuming similar bioavailability and no loss by combustion, the plasma concentration reached after ingestion of phenylethylamine from chocolate sources or other food sources is expected to be lower than after

exposure from cigarettes. Also, the different route of application via smoking as compared with other sources should be taken into account. Therefore, the systemic and the local effect of smoking related exposure to phenylethylamine might be a point of concern. Since nothing is known about the pyrolysis/combustion products of phenylethylamine, this may also be a point of concern.

PHARMACODYNAMICS
Mechanism of action
Phenylethylamine is classified as a neuromodulator of dopaminergic and possibly serotonergic and noradrenergic synapses. At the molecular physiological level, phenylethylamine potentiates transmission by postsynaptic and possibly presynaptic action. As yet, no conclusive evidence for phenylethylamine receptor has been shown (11). However, there is growing body of evidence for the existence that trace amines (TA) such as phenylethylamine, tyramine and tryptamine, function independently of the classical amine transmitters and mediate some of their effects via specific receptors. A study with ^{3}H-phenylethylamine in rat brain suggested the possibility of a specific binding site for phenylethylamine. Recently, a family of related mammalian 15 G protein-coupled receptors was identified of which two members (TA_1- and TA_2-receptors) have been shown to specifically bind and/or be activated by trace amines, such as phenylethylamine (12).

Pulmonary system
- **breathing frequency**: no data available.
- **tidal volume**: no data available.
- **lung compliance**: no data available.
- **airway resistance**: Phenylethylamine caused an initial relaxation (at 10^{-7} - 10^{-5} M) of the guinea-pig isolated lung parenchymal strip followed by contraction at higher concentration (10^{-4} – 10^{-3} M). Phenylethylamine produced a bronchoconstriction of perfused lungs, with a mean effective concentration (EC_{50}) (n =5) of 4.53×10^{-4} M. The relaxation of phenylethylamine seems to be mediated by ß-adrenoreceptors. The contraction effect of phenylethylamine does not seem to be mediated by α-adrenergic, muscarinic, histaminergic, serotonergic or dopaminergic receptor stimulation. It is not clear which receptors are involved phenylethylamine contraction effects (13).

Cardiovascular system
- **blood pressure**: Phenylethylamine increased mean aortic blood pressure, total peripheral vascular resistance, left ventricular dP/dt, and (dP/dt)/P in chloralose-anesthetized dogs. Pretreatment with phentolamine reduced the increases in aortic blood pressure and total peripheral vascular resistance produced by phenylethylamine, whereas the effects of phenylethylamine on left ventricular dP/dt and (dP/dt)/P were abolished by propranolol, but increased after phentolamine pretreatment. Furthermore, both the cardiac and vascular effects of phenylethylamine were abolished by desipramine. These results indicate that phenylethylamine exerts both positive inotropic and vasoconstrictory effects, probably by releasing endogenous norepinephrine from the adrenergic nerve endings. (The phenylethylamine dose was not mentioned in the abstract of the article) (14).

- **heart rate**: Single i.v. dose of phenylethylamine was administered to five dogs. The dose- and time-related effects of phenylethylamine were determined on pupil diameter, heart rate and body temperature. Phenylethylamine dilated pupils, tended to produce an initial tachycardia followed by a bradycardia and elevated body temperature. Plasma levels of phenylethylamine correlated significantly only with increases in pupil diameter. (The phenylethylamine dose was not mentioned in the abstract of the article) (15).

Renal system
- **diuresis**: no data avilable.
- **saluresis**: no data available.

Nervous system
- **central nervous system**: Phenylethylamine is unique among endogenous amines in that its systemic administration produces behavioral effects. Because of it is rapidly degraded by monoamineoxidase (MAO), phenylethylamine induces pharmacological effects only at high doses or following pretreatments with MAO-inhibitors (MAO-I). Its amphetamine-like effects in rats include symphatomimetic effects, increase in nonspecific motoractivity, exploratory behavior, steoreotypic behavior, electrophysiological alerting, reinforcement of complex behavior and anorectic effects (16). All the above actions of phenylethylamine, however, occur at concentrations at least 100 times higher than its endogenous concentration, which is calculated to be ± 0.24 ng/ml by assuming an even distribution within tissues (11).
  It is suggested that endogenous phenylethylamine may contribute to the antidepressant, stimulant, or euphoriant effects of several drugs. MAO-I markedly increase the central stimulant effects of phenylethylamine administration, and it increase brain and peripheral tissue levels of endogenous phenylethylamine. Increases in phenylethylamine urinary excretion correlate positively with improvement in depression (16).

  The effect of phenylethylamine on the dopaminergic nigrostriatal system of rats was described in a study. The rotational behavioral response to the i.v. injection of phenylethylamine was quantified in animals with a unilateral 6-hydroxydopamine lesion of the nigrostriatal dopamine system. After phenylethylamine injection all animals (16/16) induced rotations ipsilateral to the side of the brain lesion. The dose-response curve showed that at doses as low as 1.75 mg/kg ipsilateral turns increase, with a dose-related rotational response between 1.75 mg/kg and 11.66 mg/kg, no differences being found at doses between 11.66 and 29.16 mg/kg. Rotations began a few seconds after phenylethylamine injection. The highest response was found 30-60 s after the injection. The duration of the response was dose-related (4 min for the 3.5 mg/kg doses). It was concluded that at low doses, phenylethylamine stimulates the release of dopamine from the cytoplasmic pool and behaves as a dopamine receptor agonist with a very rapid and brief action (17).

  The effects of phenylethylamine on striatal acetylcholine release in freely moving rats using in vivo microdialysis was studied. Phenylethylamine at 12.5 mg/kg, i.p. did not affect acetylcholine release in the striatum, whereas 25 and 50 mg/kg, i.p.

induced an increase in acetylcholine release in the striatum at 15-45 min. The extracellular acetylcholine level in the striatum was significantly decreased by local application of phenylethylamine (10 and 100 µM) in the striatum via a microdialysis probe. It was concluded that systemic administration of phenylethylamine increases acetylcholine release, whereas locally applied phenylethylamine decreases striatal acetylcholine release in freely moving rats. The dopaminergic system, through the dopamine D-2 receptor, seems to be involved in the locally applied phenylethylamine-induced decrease in acetylcholine in the striatum (18).

The cerebrovascular actions of phenylethylamine, an amine that has been implicated in the pathogenesis of migraine, were investigated in 16 anesthetized baboons. The influence of monoaminergic blocking agents and of a specific inhibitor of monoamine oxidase upon the cerebral circulatory and metabolic actions of phenylethylamine were examined. The reductions in cerebral blood flow (28 percent) and cerebral oxygen consumption (31 percent) that accompany the intracarotid administration of phenylethylamine (24.2 µg/kg body weight/min) were unaffected by the prior administration of either phenoxybenzamine (1.5 mg/kg bodyweight,iv) or pimozide (0.5 mg/kg body weight, iv). The administration of phenoxybenzamine and pimozide per se did not significantly disturb cerebral blood flow or oxygen consumption. The ability of migraine patients to oxidatively deaminate phenylethylamine is reduced at the time of their attacks. The administration of the monoamine oxidase type B inhibitor, deprenyl (1 mg/kg body weight, iv), did not effect significant changes in cerebral blood flow or cerebral oxygen consumption. However, following deprenyl, the administration of phenylethylamine (4.8 µg/kg body weight/min), a concentration which was without effect in normal animals, significantly reduced cerebral blood flow (19).

The effects of phenylethylamine (6.25, 12.5, and 25.0 mg/kg body weight, i.p.) on spontaneous motor activity were examined in rats before (novel situation) and after they had experience of the test environment (familiar situation), in an undrugged state. In a novel cage, 12.5 mg/kg phenylethylamine stimulated rearing and locomotion. A dose of 25.0 mg/kg phenylethylamine also increased rearing and produced stereotyped head movements, but did not increase locomotion, in a novel environment. In a familiar cage, both 12.5 and 25.0 mg/kg phenylethylamine stimulated locomotion and sniffing, whereas rearing was unaffected by phenylethylamine treatment under these conditions. These data provide a striking instance of a qualitative change in the behavioural response to a psychostimulant compound which is associated with the relative familiarity of the animal with the test environment. In addition, the results show that phenylethylamine induces stereotypy at high doses and increases locomotor activity at moderate doses, which is a further illustration of the similarity in the unconditioned behavioural effects of phenylethylamine and amphetamine (20).

- **autonomic system**: The autonomic effects of phenylethylamine may be largely mediated by catecholamine release from sympathetic nerve endings. The central effects appear to be mediated in part by release of catecholamines and serotonin and in part by direct stimulation of specific receptors. The peripheral sympathomimetic effects of phenylethylamine is prevented by catecholamine

depletion by reserpine or by blockade of the amine pump by imipramine (16).

Other

Critical assessment
Phenylethylamine is classified as a neuromodulator of dopaminergic and possibly serotonergic and noradrenergic synapses. Phenylethylamine produced a bronchoconstriction of isolated perfused lungs of guinea-pig. No data are available on phenylethylamine inhalation effects in human. Therefore it is unknown whether the phenylethylamine dose in cigarette will exert a bronchoconstrictory effect. Phenylethylamine exerts both positive inotropic and vasoconstrictory effects in dogs. Phenylethylamine tended to produce an initial tachycardia followed by a bradycardia in dogs. Phenylethylamine has amphetamine-like effects in rats including symphatomimetic effects, increasing nonspecific motoractivity, exploratory behavior, steoreotypic behavior, electrophysiological alerting, reinforcement of complex sesquences behavior and anorectic effects. It is suggested that endogenous phenylethylamine may contribute to the antidepressant, stimulant, or euphoriant effects of several drugs. Phenylethylamine exerts its CNS effect at high doses or when the MAO is inhibited. Based on the current CNS data, it is unknown whether the phenylethylamine dose in cigarette is enough to exert any CNS effect.

Conclusion
Not enough data are available on inhalation effects of phenylethylamine on the pulmonary system in human. Therefore, it is unknown whether the phenylethylamine dose in cigarettes (estimated 12.1 mg/day/25 cigarettes) will affect the pulmonary system. The (longterm) effects of phenylethylamine or its pyrolysis/combustion products on the pulmonary system are also unknown and need further study.

PHARMACOKINETICS
Absorption
In-vitro studies with perfused lungs of rats and rabbits have shown that a large portion of the exposed phenylethylamine (95 %) is transported rapidly through the pulmonary endothelium. No data were available on the absorption through the alveoli epithelium (21, 22).

Bioavailability
No data are available on bioavailability through the gastro-intestinal and pulmonary system. Although a high portion of phenylethylamine is absorbed through the pulmonary endothelium (95 %), it is rapidly neutralised by the pulmonary monoamine oxidase enzymes and consequently the bioavailability through the mentioned system is reduced. Intake of MAO-inhibitors will increase the bioavailability of phenylethylamine (21-23).

Distribution
Phenylethylamine is heterogenously distributed in various brain regions of human. Total tissue levels are low (< 10 ng/g tissue), compared with other biogenic amines (which range from 100 to 5000 ng/g tissue), probably because of poor storage and rapid turnover rate (half-life 5 – 10 min) (16). When radioactively labelled ^{14}C-

phenylethylamine was injected intravenously in rat, radioactivity was measured in all tissues, including the brain. Its clearance from these tissues and from brain regions was very fast (24). Phenylethylamine has been found also in various mouse tissues: the highest concentrations were found in the small intestine, followed by the blood and liver. Concentrations of approximately of 5 ng/g wet weight were detected in brain tissue, which increased after inhibition of monoamine oxidase by pargyline (25). Phenylethylamine is highly lipid-soluble and readily crosses the blood-brain barrier. Blood-borne phenylethylamine is accumulated by the brain against a concentration gradient. Brain and peripheral phenylethylamine are in dynamic equilibrium (16, 26).

Metabolism
In the nervous tissue, phenylethylamine is synthesized by decarboxylation of phenylalanine, a reaction that is catalyzed by the enzyme aromatic L-amino acid decarboxylase. Phenylethylamine is metabolized by MAO, primarily by type-B (and to a small extent MAO-A), and aldehyde dehydrogenase to phenylacetic acid, which is the major metabolite of phenylethylamine in the brain. The regional distribution of phenylacetic acid in the brain coincides with that of phenylethylamine. Exogenous phenylethylamine in humans is primarily metabolized to phenylacetic acid. Approximately 10% of brain phenylethylamine is also metabolized to phenylethanolamine by dopamine-ß-hydroxylase. Phenylethanolamine is present in human brain and animal brain and may function as a cotransmitter in norepinephrine synapses (11, 16).

Monoamine oxidase is responsible for the pulmonary metabolism of phenylethylamine. In a study the effects of treatment of rats with the tricyclic antidepressant desmethylimipramine (DMI) on the disposition of phenylethylamine in isolated perfused rat lungs was investigated. DMI accumulation in the lung reached a plateau after 6 days of treatment with mean values of 1.1, 6.1, and 315 nmol/lung at dose levels of 0.67, 6.7, and 33 mumol/kg/day, respectively. During a 10-min perfusion at a concentration of 10^{-6} M phenylethylamine was rapidly taken up and extensively metabolized by lungs from control animals. Phenylethylamine clearance in perfused lung was decreased in a dose-related manner by DMI treatment with a corresponding decrease in its metabolism. In efflux experiments, unmetabolized phenylethylamine was only found in the perfusate from lungs of DMI-treated rats. It was concluded that phenylethylamine clearance after DMI treatment results almost entirely from inhibition of pulmonary MAO (21).

Inactivation of phenylethylamine was studied in a preparation of rabbit lung perfused with Krebs physiological medium at 37 ºC. Percentage removal was high with phenylethylamine (95%). Inactivation of phenylethylamine could be accounted for by metabolic degradation to deaminated products, which appeared in lung effluent within 90 s of the beginning of amine perfusion. When intrapulmonary metabolism of phenylethylamine was inhibited by simultaneous perfusion with semicarbazide (10 mM) and pargyline (10 μM), the removal rate was unaltered, establishing that uptake of the amine from the vascular space is not dependent on metabolism at least for 4 min infusions (22).

Excretion
Phenylethylamine is excreted in the urine. Oral ingestion of phenylethylamine

increased the urinary excretion of phenylacetic acid and mandelic acid (27-29).

Kinetic parameters
Phenylethylamine crosses the blood-brain barrier easily and its concentration in the brain after peripheral injection peaks within 5 minutes and returns to normal level within 30 min. The turnover of endogenous phenylethylamine in the brain is high with a half-life of 0.4 min (11).

Critical assessment
In-vitro studies have shown that phenylethylamine is rapidly absorbed by the pulmonary endothelial tissue and is also rapidly inactivated by pulmonary MAO. When radioactively labelled ^{14}C-phenylethylamine was injected intravenously in rat, radioactivity was measured in all tissues, including the brain. Phenylethylamine crosses the blood-brain barrier easily and its concentration in the brain after peripheral injection peaks within 5 minutes and returns to normal level within 30 min. The turnover of endogenous phenylethylamine in the brain is high with a half-life of 0.4 min. Phenylethylamine is metabolized by MAO, primarily by type-B (and to a small extent MAO-A), and aldehyde dehydrogenase to phenylacetic acid, which is the major metabolite of phenylethylamine in the brain. Based on *in-vitro* kinetic data of phenylethylamine, the pulmonary MAO will reduce the phenylethylamine intake through cigarette smoking.

Conclusion
There are no in-vivo pharmacokinetic data available on respiratory intake of phenylethylamine. Based on the *in-vitro* data, probably pulmonary MAO will reduce the bioavailability of phenylethylamine through cigarette smoking.

TOXICOLOGY
Acute toxicity
Human
The effect of 5 mg phenylethylamine in apple juice on 27 healthy volunteers was studied using a randomized placebo-controlled double-blind procedure. Phenylethylamine produced symptoms like headache, dizziness and discomfort in some volunteers (30).

Animal
LD_{50} oral mouse 400 mg/kg (1)
LDLo oral rat 800 mg/kg (1)
LD_{50} subcutaneous mouse 320 mg/kg (1)
LD_{50} intravenous mouse 100 mg/kg (1)
LDLo intraperitoneal rat 100 mg/kg (1)

In one study, stereotyped sniffing behaviour together with forepaw padding -defined as the phenylethylamine syndrome- was induced by MAO-B inhibitors in rats injected with 30 mg/kg i.p. phenylethylamine. The comparison of the abilities of the MAO-B inhibitors to induce the syndrome and to inhibit MAO-B in rat brain homogenates indicated that at least 75% of MAO-B activity in rat brain had to be inhibited to induce the phenylethylamine syndrome. A good correlation was found between the abilities of MAO-B inhibitors to induce the behavioral syndrome and to

increase levels of phenylethylamine in rat brain (31).

In another study, male Swiss mice were treated systemically with phenylethylamine (25-150 mg/kg), and observed in isolation or in groups of five. Phenylethylamine at a dose of 25 mg/kg depressed activity and caused sedation, but at 50 mg/kg produced a brief stimulation of activity. At higher dose levels (75-150 mg/kg bw) the compound induced a biphasic stimulation of activity which was associated with the development of two distinct groups of stereotyped activities. Group testing significantly antagonized early phase stereotypy (forepaw padding, headweaving, compulsive grooming) but had no effect on, or potentiated, late phase stereotypy (rearing, licking). In addition grouped mice were more active and hyperreactive than isolated mice were (32).

Local tolerance
Human
No data are available.

Animal
No data are available.

Repeated dose toxicity
Subacute
The behavioural consequences of daily phenylethylamine administration for a period of 6 weeks have been examined. Rats showed signs of serotonin behavioral syndrome (forepaw padding, headweaving, splayed hindlimbs) after a single i.p. injection of phenylethylamine 50 mg/kg or 7 daily injections of 25 mg/kg. The syndrome reached peak intensity after 3 weeks treatment. These data provide strong evidence for an effect of phenylethylamine on brain serotonin systems (33).

Semichronic
No data are available.

Chronic
No data are available.

Carcinogenicity
Human
No data are available.

Animal
No data are available.

Reproduction toxicology
Human
No data available.

Animal
In-vitro studies with mouse embryos showed that phenylethylamine concentrations of 121 and 1210 mg/l were lethal (24 hr) and induced neural tube closure defects in 67% of the embryos at 12 mg/l (34).

Mutagenicity
Human
No data available.

Animal
No data available.

Other

Critical assessment
Phenylethylamine (5 mg) produced symptoms like headache, dizziness and discomfort in some volunteers (n = 27). The LD50 value in rats varies between 100 mg/kg (i.p.) to 800 mg/kg (oral). No data are available on the inhalation toxicological effect of phenylethylamine. Therefore, no conclusion can be drawn whether the phenylethylamine dose in one cigarette (0.49 mg/cigarette) will have significant systemic and local toxicological effects.

Conclusion
No data are available on inhalation toxicological effects of phenylethylamine. The long-term effect of this compound via the respiratory system needs to be studied.

INTERACTIONS
Chemical
A reaction of phenylethylamine and other biogenic amines such as serotonin, dopamine, histamine, tyramine and tryptamine with components of cigarette smoke was observed. Both formaldehyde and cyanide, which are known to be present in cigarette smoke, were involved in the reaction with the primary amines. The reaction was time dependent and was enhanced by an increase in temperature or by incubation under alkaline conditions. Cyanomethyl adduct formation was increased when smoke from cigarettes with higher tar and nicotine content was used. When the amines were incubated with human saliva obtained after cigarette smoking, cyanomethylamine products were readily detected (35). When the amine substrates phenylethylamine, p-tyramine and serotonin were incubated with the cigarette smoke solution, lipophilic adducts were formed non-enzymatically. These mixtures exhibit considerable MAO inhibitory activity. The inhibition of MAO by cigarette smoke may well be related to the low platelet MAO activity found in cigarette smokers (36).

In vivo
The safety, pharmacokinetics, and pharmacodynamics of single oral doses up to 48 mg and daily (for 28 days) doses up to 24 mg mofegiline were investigated in healthy male volunteers. Mofegiline rapidly and markedly inhibited platelet monoamine oxidase B (MAOB) activity, which returned to baseline within 14 days. Urinary excretion of phenylethylamine increased proportionately with doses up to 24 mg (37).

The cerebrovascular actions of phenylethylamine, an amine that has been implicated in the pathogenesis of migraine, were investigated in 16 anesthetized baboons. The influence of monoaminergic blocking agents and of a specific inhibitor of monoamine

oxidase upon the cerebral circulatory and metabolic actions of phenylethylamine were examined. The reductions in cerebral blood flow (28 percent) and cerebral oxygen consumption (31 percent) that accompany the intracarotid administration of phenylethylamine (0.25 mg/kg/min) were unaffected by the prior administration of either phenoxybenzamine (1.5 mg/kg, iv) or pimozide (0.5 mg/kg, iv). The administration of phenoxybenzamine and pimozide per se did not significantly disturb cerebral blood flow or oxygen consumption. The ability of migraine patients to oxidatively deaminate phenylethylamine is reduced at the time of their attacks. In the present experiments, the administration of the monoamine oxidase type B inhibitor, deprenyl (1 mg/kg, iv), did not effect significant changes in cerebral blood flow or cerebral oxygen consumption. However, following deprenyl, the administration of phenylethylamine (5 µg/kg/min), a concentration which was without effect in normal animals, significantly reduced cerebral blood flow (19).

Monoamine oxidase (MAO) is responsible for the pulmonary metabolism of phenylethylamine. The effects of treatment of rats with the tricyclic antidepressant desmethylimipramine (DMI) on the disposition phenylethylamine in isolated perfused rat lungs was investigated. During a 10-min perfusion at a concentration of 10^{-6} M phenylethylamine were rapidly taken up and extensively metabolized by lungs from control animals. Phenylethylamine clearance in perfused lung was decreased in a dose-related manner by DMI treatment with a corresponding decrease in its metabolism. In efflux experiments, unmetabolized phenylethylamine was only found in the perfusate from lungs of DMI-treated rats. It was concluded that phenylethylamine clearance after DMI results almost entirely from inhibition of pulmonary MAO. The data also suggest that there may be two discrete pools of MAO in lung, one of which is relatively unaffected by DMI (21).

Critical assessment
Chemical
Phenylethylamine can react with aldehydes and cyanides. Adducts formed with other cigarette components have MAO inhibitory properties.

In vivo
Phenylethylamine shows an interaction with monoamine oxidase inhibitors (MAOI). The MAO-I increases the phenylethylamine level in the body. It is plausible that phenylethylamine availability from cigarette smoking will be increased when MAO is inhibited.

Conclusion
Chemical
Phenylethylamine can react with aldehydes and cyanides in cigarettes and the formed adducts can inhibit MAO.

In vivo
MAO is responsible for the metabolism of phenylethylamine. Therefore, MAO-I increases phenylethylamine level in the body.

DEPENDENCY
Phenylethylamine is an endogenous brain amine, which has been characterised as an endogenous amphetamine. The rewarding properties of the structurally similar drug amphetamine in humans and other species indicate a possible role for endogenous

phenylethylamine in neural processes underlying reward or reinforcement. Evidence for reinforcing properties of phenylethylamine in the drug self-administration and place preference paradigms have been investigated (38).

The reinforcement properties of phenylethylamine compared to amphetamine or cocaine were investigated in dogs. The relative potencies of these compounds in maintaining self-administration behaviour during the 4-hr session was d-amphetamine greater than cocaine greater than or equal to phenylethylamine. It was concluded that phenylethylamine can function as a reinforcer or may play a physiological role in the reinforcement process (39). Furthermore, it was shown that in MAOI-B treated squirrel monkeys, phenylethylamine (0.3 – 1.0 mg/kg) affected the discriminative-stimulus and reinforcing-stimulus compared with amphetamine (0.3 mg/kg) (40).

Effects of smoking cessation
No data available.

Critical assessment
Phenylethylamine has reinforcing properties qualitatively comparable to amphetamine. Whether phenylethylamine in cigarette plays a role in the reinforcing effect of cigarette smoking is unknown.

Conclusion
Phenylethylamine has reinforcing properties.

COMMERCIAL USE
No data available.

BENEFICIAL EFFECTS
In depressed subjects treated with an MAOI, phenylethylamine markedly improves mood (because phenylethylamine is rapidly metabolized by MAO, phenylethylamine alone produces no noticeable effects). The addition of 10 to 30 mg/day of phenylethylamine to current treatment with amitryptiline plus phenelzine terminated the episode of depression in 2 of the 3 inpatients with major depressive disorder who had not achieved any significant recovery with tricyclic antidepressants, MAOI, or their combination (16).

Critical assessment
Phenylethylamine could be used to treat depression disorder in human. Whether phenylethylamine in cigarette plays a role to the possible anti-depressive effect of cigarette smoking is unknown.

Conclusion
Phenylethylamine has anti-depressive properties.

SUMMARY AND FINAL CONCLUSION
Phenylethylamine is a natural tobacco component and is also added to tobacco as a component of cocoa powder, which is used as a flavouring agent. The estimated

phenylethylamine amount in dried tobacco plant is 70 – 485 µg/g dry weight. The average amount of phenylethylamine in cocoa varies between 0.22 – 22 µg/g.

The estimated natural phenylethylamine amount from tobacco plant in cigarette is at least 2200 times higher than phenylethylamine from added cocoa. Therefore, it is debatable whether phenylethylamine should be considered as an additive to tobacco. The daily potential intake of phenylethylamine (12.1 mg/25 cigarettes/day) from cigarettes (from tobacco plant and added cocoa) is higher than phenylethylamine intake from other sources such as chocolate, sausage and cheese (0.5 – 4 mg/day). Assuming similar bioavailability and no loss by combustion, the plasma concentration reached after ingestion of phenylethylamine from chocolate sources or other food sources is expected to be lower than after exposure from cigarettes. Also the different route of application via smoking as compared with other sources should be taken into account. Therefore, the systemic and the local effect of smoking related exposure to phenylethylamine might be a point of concern. Since nothing is known about the pyrolysis/combustion products of phenylethylamine, this may also be a point of concern.

Phenylethylamine is classified as a neuromodulator of dopaminergic and possibly serotonergic and noradrenergic synapses. At the molecular physiological level, phenylethylamine potentiates transmission by postsynaptic and possibly presynaptic action. Phenylethylamine produced a bronchoconstriction of isolated perfused lungs of guinea-pig. No data are available on phenylethylamine inhalation effects in human. Therefore, it is unknown whether the phenylethylamine dose in cigarette will exert bronchoconstrictory effects.
Phenylethylamine exerts both positive inotropic and vasoconstrictory effects in dogs. Phenylethylamine tended to produce an initial tachycardia followed by a bradycardia in dogs. Phenylethylamine has amphetamine-like effects in rats including symphatomimetic effects, increasing nonspecific motoractivity, exploratory behavior, stereotypic behavior, electrophysiological alerting, reinforcement of complex behavior and anorectic effects. It is suggested that endogenous phenylethylamine may contribute to the antidepressant, stimulant, or euphoriant effects of several drugs. Phenylethylamine exerts its CNS effect at high doses or when the MAO is inhibited. Based on the current CNS data, it is unknown whether the phenylethylamine dose in cigarette is enough to exert any CNS effect.

In vitro studies have shown that phenylethylamine is rapidly absorbed by the pulmonary endothelial tissue and is also rapidly inactivated by pulmonary MAO. Phenylethylamine is distributed throughout the body. Phenylethylamine crosses the blood-brain barrier easily and its concentration in the brain after peripheral injection peaks within 5 minutes and returns to normal level within 30 min. The turnover of endogenous phenylethylamine in the brain is high with a half-life of 0.4 min. Phenylethylamine is metabolized by MAO, primarily by type-B (and to a small extent MAO-A), and aldehyde dehydrogenase to phenylacetic acid, which is the major metabolite of phenylethylamine in the brain. There are no in vivo pharmacokinetic data available on respiratory intake of phenylethylamine. Based on the in-vitro kinetic data of phenylethylamine, the pulmonary MAO will reduce the phenylethylamine intake through cigarette smoking.

Phenylethylamine (5 mg) produced symptoms like headache, dizziness and discomfort in some volunteers (n = 27). The LD50 value in rats varies between 100 mg/kg (i.p.) to 800 mg/kg (oral). No data are available on the inhalation toxicological effect of phenylethylamine. Therefore, no conclusion can be drawn whether the phenylethylamine dose in one cigarette (0.49 mg/cigarette) will exert significant systemic and local toxicological effects.

Phenylethylamine reacts with aldehydes and cyanides in cigarettes and forms adducts with those cigarette components. These adducts have MAOI properties. MAO metabolises phenylethylamine in the pulmonary system. Therefore, MAOI increase the phenylethylamine level in the body. It is plausible that phenylethylamine availability from cigarette smoking will be increased when MAO is inhibited.

Phenylethylamine has reinforcing properties qualitatively comparable to amphetamine. Whether phenylethylamine in cigarette plays a role in the reinforcing effect of cigarette smoking is unknown.

Phenylethylamine could be used to treat depression disorder in human. Whether phenylethylamine in cigarette plays a role in the possible anti-depressive effect of cigarette smoking is unknown.

Based on the metabolisation by MAO, it seems that pulmonary MAO will reduce the bioavailability of phenylethylamine through cigarette smoking. However, since no data are available on pharmacodynamic, pharmacokinetic and toxicological effects of phenylethylamine exposure through inhalation, the shortterm and longterm effects of exposure to phenylethylamine through smoking on the respiratory system cannot be established. Furthermore, its additive effects on other biogenic amines present in cigarette smoke are also not known and have to be studied.

More studies are needed on:
- the determination of pyrolysis/combustion products of phenylethylamine in cigarette smoke;
- the local (respiratory system) effects of long-term use of phenylethylamine alone and its pyrolysis/combustion products via inhalation;
- the local (respiratory system) effects of long-term use of phenylethylamine in combination with other biogenic amines via inhalation.
- Biavailability of phenylethylamine via respiratory exposure

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3.9　　Octopamine

GENERAL
IUPAC systematic name: Benzyl alcohol, alpha-(aminomethyl)-p-hydroxy-; octopamine (1)
Synonyms: alpha-(Aminomethyl)-p-hydroxybenzyl alcohol; Analet; Benzenemethanol, alpha-(aminomethyl)-4-hydroxy-; 1-(p-Hydroxyphenyl)-2-aminoethanol; p-Hydroxyphenylethanolamine; ND 50; Norden; Norfen; Norphen; Norsympathol; Norsynephrine; Octapamine; Paraoxyphenyl aminoethanol (1)
Molecular formula: $C_8H_{11}NO_2$ (2)

Molecular structure
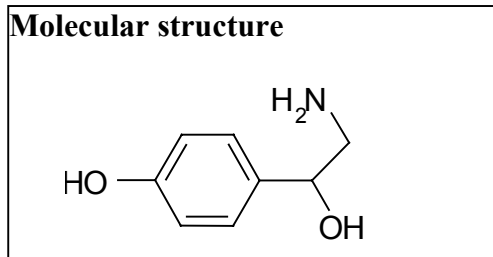
Molecular weight: 153.18 g/mol (2)
Alifatic: ethylgroup (2)
Aromatic: phenyl group (2)
N containing: amine group (2)
Halogen containing: no
CAS registry no.: 104-14-3 (2)
Storage:
R/S classification: no data available
dangercode (transport): no data available
Properties:
➢ melting point: crystals of the D-form molecule change at about 160 ºC to a compound which melts above 250 ºC (3)
➢ boiling point: no data available
➢ density: no data available
➢ refractive index: -37.4º in water of the D-form at 25 ºC (3)
➢ solubility: 1 g/ml (2)
➢ substance description: no data available
  • color: no data available
  • liquid/gas/powder: crystals (3)
  • odor/taste: no data available
➢ volatility: no data available
➢ pK_a: 8.81 (2)
➢ PA: no data available
➢ flammability: no data available
  • FP = no data available
  • FL Limits = no data available
  • IT = no data available
➢ decomposition temperature: no data available
➢ stability: no data available
➢ vapour pressure/ vapour tension (20 °C): $2.5 \ 10^{-5}$ mmHg ($3.3 \ 10^{-3}$ Pa) at 25ºC (2)

➢ vapour pressure (50 °C): no data available
➢ relative density: no data available
➢ octanol water partition coefficient, log P, log K_{OW}: -0.90 (2)
➢ conversion factor: not relevant

Critical assessment

Octopamine is counted as a pharmaceutical substance (4). It is a metabolite of tyramine (β-hydroxylated tyramine), and as such it contains an additional chemical functional group compared to tyramine, namely an alifatic alcohol group. Hence, it is likely that octopamine will be more polar than tyramine, and most probably it wll be more solvable in water.

Most probably, the tyramine-like structure of the molecule dominates it chemical character, especially the presence of the amine group and the phenolic hydroxyl. group. The dominance of the amine group is reflected in its product form, namely as its hydrochloride salt.

Conclusion

Octopamine is a biogenic amine, being the phenol analog of noradrenaline. Octopamine is closely related to tyramine, it namely is β-hydroxylated tyramine. Octopamine is presented as product commonly, it is in salt form (hydrochloride).

FUNCTION IN TOBACCO
No data available.

AMOUNT IN TOBACCO PRODUCTS
Octopamine is a component of cocoa (5), which is used as a flavouring agent in tobacco products. No data are available on the octopamine level in cocoa and in tobacco products.

AMOUNT IN SMOKE
No data are available on octopamine level in smoke.
• **main stream**
• **side stream**

SOURCE
Octopamine is a component of cocoa (5), which is used as a flavouring agent.

ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE
Octopamine is a natural component in cocoa (5) and in citrus fruit (6). The environmental level and human exposure is unknown.

COMBUSTION PRODUCTS
No data available.

CONSENSUS REPORTS
No data available.

STANDARDS AND RECOMMENDATIONS
ADI: No data available.

TWA$_{NL}$ = MAC: No data available.
TWA$_D$ =MAK: No data available.
TWA$_{USA}$: No data available.
STEL$_{NL}$: No data available.
STEL$_{USA}$: No data available.
LTEL: No data available.
TLV-C: No data available.
TLV-CARCINOGENICITY: No data available.
MAK-REPRODUCTION: No data available.

Others:

Reference value:
The plasma octopamine levels were measured in a population of 33 normal individuals ranging in age from 19 to 94 years. Significantly higher plasma octopamine levels were found in the age group 70-94 years. Excluding those individuals over the age of 70 years, the range of values was 0 to 0.68 ng per ml, with a mean value of 0.23 ng per ml (n = 25) (7). Serum octopamine levels in health control subjects were 1.75 ± 0.19 ng/ml (8).

CLASS
EG Carc. Cat.: No data available.
IARC-category: No data available.
CEC: No data available.

Critical assessment
The octopamine level in cocoa or in cigarette smoke is unknown. Also the environmental level and data on human exposure are not available. Furthermore, data on combustion products of octopamine are not available. Octopamine is a natural compound in the human body with a mean plasma value of 0.23 ng/ml.

Conclusion
No data are available to evaluate the octopamine exposure through cigarette smoking.

PHARMACODYNAMICS
Mechanism of action
Octopamine receptors in vertebrates are not found, although specific octopamine receptors have been cloned in invertebrates. Octopamine is known to exert adrenergic effects in mammals. It has been shown that octopamine can stimulate α_2 - adrenoceptors (ARs) in Chinese hamster ovary cells transfected with human α_2-ARs (9). Octopamine has about 1/100 th the α-adrenergic activity of noradrenaline in rats (10). Octopamine stimulates lipolysis through ß$_3$ -rather than ß$_1$ -or ß$_2$-AR activation in white adipocytes from different mammalian species. Octopamine is fully lipolytic in garden dormouse and Siberian hamster while tyramine was ineffective. Although being around one hundred-fold less potent than noradrenaline, octopamine was slightly more potent in these hibernators known for their high sensitivity to ß$_3$-AR agonists than in rat and markedly more active than in human adipocytes known for their limited responses to ß$_3$-AR agonists. Octopamine reduced insulin-dependent glucose transport in rat fat cells, a response also observed with noradrenaline and

selective $β_3$-AR agonists but not with $β_1$-or $β_2$-agonists. Human adipocytes, which endogenously express a high level of $α_2$-ARs, exhibited a clear $α_2$ -adrenergic antilipolytic response to adrenaline but not to octopamine. In Syrian hamster adipocytes, which also possess $α_2$-ARs, octopamine induced only a weak antilipolysis. Octopamine is a substrate of fat cell amine oxidases, with an apparent affinity similar to that of noradrenaline. Thus, octopamine could be considered as an endogenous selective $β_3$-AR agonist (11).

Octopamine stimulates adenylate cyclase. Via experimentation it was suggested that octopamine acts on intestinal dopamine D_1-receptor sites to produce relaxation of rabbit jejunum through an increase of cAMP (cyclic adenosine monophosphate) (12). Recently, a family of related mammalian 15 G protein-coupled receptors was identified of which two members (TA_1- and TA_2-receptors) have been shown to specifically bind and/or be activated by trace amines, such as phenylethylamine and tryptamine. However, these receptors display low affinity for octopamine (13).

Pulmonary system
- **breathing frequency**: no data available.
- **tidal volume**: no data available.
- **lung compliance**: no data available.
- **airway resistance**: Noradrenaline is 6000 fold more potent than octopamine to activate $β_1$-adrenergic receptors in guinea-pig atria and trachea. Octopamine had no detectable activity in concentrations as high as 10^{-4} M on the $β_2$-adrenoreceptor of the isolated trachea. If octopamine is co-released with noradrenaline in amounts proportional to their concentration, their activities at these structures are too low to be physiologically significant (14).

Cardiovascular system
- **blood pressure**: Effects of octopamine on sinus rate and atrial contractility were investigated using the isolated atrium preparation of dog. When octopamine, dopamine or noradrenaline was administered into the cannulated sinus node artery, positive chronotropic and inotropic responses were dose-related. The DR_{50} values (dose ratio at 50% maximum response) of octopamine, dopamine and noradrenaline were roughly 30-100: 30:1, respectively. The duration of action of octopamine was longest. The positive chronotropic and inotropic responses to octopamine are mainly due to tyramine-like action (15).
The perfusion of octopamine in pig produces an increase of cardiac output and decreases the pulmonary vascular resistances. The changes in the lung circulation are exerted by the direct action of this drug on nervous control of vascular walls (octopamine dose is not mentioned in the abstract) (16).
Octopamine injected in lateral ventricle of conscious spontaneously hypertensive rats decreased systolic blood pressure (SBP). The administration of pargyline, a MAO inhibitor, which increased brain octopamine, resulted in a reduction of systolic blood pressure. Octopamine hypotension was not antagonized by selective antagonists of post-synaptic α-adrenoceptors, indicating that octopamine may be involved in central blood pressure regulation (octopamine dose was not mentioned in the abstract) (17). Experiments on rat mesenteric arterioles, metarterioles and aortae demonstrate that octopamine is between 60 and 15,000 times less potent than noradrenaline on rat arterioles and metarterioles and is incapable of eliciting more than 40% occlusion of these terminal vessels. It is suggested that such data support the concept that octopamine, could serve as a

false adrenergic neurotransmitter agent and thus account for part or all of the hypotensive action of monoamine oxidase inhibitors like pargyline (18).

- **heart rate**: no data available.

Renal system

- **diuresis**: Octopamine was administered in doses ranging between 25-200 µg/min (1.6-20 µg/kg/min) both i.v. and into one renal artery of anaesthetized dogs. Octopamine was hypertensive in doses of 100 µg/min and more and this change was associated with a significant decrement in glomerular filtration rate (GFR) and renal perfusion. This amine also exerted a direct tubular effect since it decreased excretion of sodium and water and occurred in the absence of blood pressure or renal perfusional changes when given i.v.. When given into one renal artery octopamine produced only an ipsilateral antidiuresis and antinatriuresis, in the absence of any change to GFR or renal perfusion. Lithium clearances suggest that octopamine acts beyond the proximal tubule in altering the tubular reabsorption of salt and water. Because octopamine was found to increase blood pressure in the presence of a hypertensive infusion of noradrenaline, it is likely that this amine exerts a primary pharmacological effect rather than liberating noradrenaline from nerve terminals (19). An infusion of octopamine (220 µg/kg/min) in rats was associated with an increase in mean arterial pressure, urinary volume, urinary Na and K output and their filtration fractions. Contrary to the experiments on dogs, the glomerular filtration rate and renal plasma flow were not affected in rats. A sudden and marked decrease in mean blood pressure and diuresis was observed after stopping octopamine infusion (20).
- **saluresis**: see diuresis.

Nervous system

- **central nervous system**: Administration of octopamine by intracerebroventricular (i.c.v.) or intrathecal (i.t.) routes, but not orally, produced antinociception in the acetylcholine-induced abdominal constriction test (ED50 = 24.8 and 3.6 µg, respectively). Likewise, i.c.v. and i.t., but not peripheral (up to 200 mg/kg s.c.), administration increased latency in the 48 ºC hot-plate test (ED50 = 11.5 µg i.c.v. and 0.2 µg i.t.). These actions were relatively long-lasting and not blocked by naloxone. Antinociception following i.c.v. administration was abolished in reserpinized mice or by pretreatment with i.t. phentolamine (2 µg). These results suggest a moderate antinociceptive action of octopamine involving non-opioid, reserpine-sensitive, central pathways (21).

  Octopamine (50-250 µg) given intracerebroventricularly (icv) antagonized the head twitch response induced in the rat by 5-hydroxytryptophan or 5-methoxytryptamine, and hyperthermia induced by quipazine (serotonin agonist) in rats kept at high ambient temperature. Octopamine significantly depressed the cerebral level of serotonin, and reduced the concentration of 5-hydroxyindoleacetic acid. Octopamine depressed the serotonin turnover rate. These results indicate that octopamine given icv to rats antagonizes the central serotonergic system (22).

  In a study, the behavioral and neurochemical effects of intraventricular infusions of octopamine (3,200 µg), tryptophan (800 µg), and octopamine plus tryptophan delivered over 6 hours was studied in rats after performing a portacaval

anastomosis or a sham operation. After each infusion, each animal was rated for neurologic depression with a 17 point test battery. Although overt coma was not induced, octopamine infusions severely depressed neurologic function. Concentrations of noradrenaline, dopamine, and serotonin in the brain were significantly decreased after the infusion of octopamine. Levels of noradrenaline in the brain were significantly correlated with neurologic status and greater depletion of noradrenaline was associated with greater neurologic depression. It was thus demonstrated that infusing large amounts of the trace amine octopamine depresses behavior in the rat and this depression is most closely associated with depletion of stores of noradrenaline in the brain (23). The behavioral effects of octopamine (50, 100 and 250 µg, icv) was studied in rats. Octopamine significantly increased locomotor activity in all doses tested. Biochemical studies showed that octopamine decreased the cerebral concentration of GABA and reduced activity of glutamate decarboxylase in rats brain. Significant changes in concentrations of NA and DA in brain of rats pretreated with octopamine were found (24).

Octopamine (50-250 µg icv) activates both noradrenergic and dopaminergic system of the rat. In rats pretreated with reserpine the stimulatory action of octopamine was not inhibited, but even enhanced. Only selective destruction of dopamine containing neurons (6-hydroxydopamine, 200 microgram ivc, given 1 hr after desipramine, 25 mg/kg ip) prevents octopamine-induced hyperactivity. Octopamine depressed the noradrenaline level in the rat brain and increased utilization of the amine, but did not affect the level and utilization of dopamine (25). Intracerebroventricular administration of octopamine had opposite effects on locomotor activity depending on whether or not the rats were subjected to uncontrollable electric shocks. In unshocked rats, octopamine produced a large decrease in locomotor activity, but when the rats were subjected to unsignalled and uncontrollable electric shocks, a significant increase in locomotor activity resulted. The latter effect was observed either when the shocks were applied during the measurement of locomotor activity or when they were applied the day before (conditioned suppression paradigm). These results support the hypothesis of a neuromodulation of central noradrenergic transmission by octopamine (26). Octopamine (100, 250 and 500 µg in rat, icv) exerted a stimulating effect on the central nervous system in rats, which was evidenced by increased spontaneous and basal motor activity, increased exploratory activity in the free-field test, and also increased motor activity in reserpinised rats pretreated with nialamide. Octopamine decreased the body temperature and prolonged the duration of hexobarbital-induced sleep, and increased amphetamine-induced hyperactivity. Locomotor agitation after octopamine injection was inhibited by phenoxybenzamine and yohimbine in a dose of 10 mg/kg i.p. (27).

- **autonomic system**: Octopamine is localized within sympathetic nerve endings (28). The effect of octopamine on intestinal smooth muscle of rabbit isolated jejunum has been studied. Octopamine induced a dose-dependent decrease of muscle tone. Direct stimulation of adenylate cyclase by octopamine was demonstrated using radioimmunoassay of cAMP. Via experimentation it was suggested that octopamine acts on intestinal dopamine D_1-receptor sites to produce relaxation of rabbit jejunum through an increase of cAMP (12) (octopamine dose was not mentioned in the abstract).

Other

Critical assessment
Octopamine is known to exert adrenergic effects in mammals, although specific
receptors have been cloned only in invertebrates. Octopamine stimulates α_2 and β_3-
adrenergic receptors in rats. Furthermore, it affected the cAMP level in the cell via
the D_1-receptor. No data are available on the pulmonary effects of inhaled
octopamine. Because octopamine is much less potent than noradrenaline on the α and
β-adrenergic receptors, it is likely that a large amount of octopamine needs to be
inhaled to affect the pulmonary system. Octopamine has positive chronotropic and
inotropic effectson dog heart, but is significantly less potent than noradrenaline.
Octopamine has a central hypotensive effect, and a peripheral hypertensive or
hypotensive effect in rats depending on the α or β-adrenergic effect. Octopamine does
affect the central catechol amine level and thus affects the CNS.

Conclusion
No data are available on inhaled octopamine effect on the pulmonary system. Based
on the in vitro data, octopamine is less potent than noradrenaline and therefore a large
amount of octopamine needs to be inhaled to affect the pulmonary system.

PHARMACOKINETICS
Absorption
The enteric absorption is complete. However, metabolic enzymes in the gut of human
are responsible for a significant 'first pass effect' (29).

Bioavailability
The urinary excretion of the unchanged drug and its metabolites has been compared
after intravenous and oral administration of ^{3}H-octopamine to eight patients. Identical
amounts of ^{3}H-activity (80% of the dose) were excreted after the two routes of
dosing. Significant differences were found in the fraction of free urinary octopamine,
which amounted to 10.5% of the dose after infusion and 0.58% after oral
administration (29). These differences indicate that the bioavailability through oral
exposure is significantly lower than through i.v. exposure.

Distribution
The physiologically more active m-octopamine has been found in association with p-
octopamine in 10 organs of the rat. m-Octopamine is present in concentrations equal
to those of p-octopamine in heart, spleen, and liver and in concentrations from 30 to
60% of p-octopamine in adrenals, vas deferens, brain, kidney, large intestine, bladder,
and lungs. In vivo inhibition of monoamine oxidase (MAO) markedly increased the
concentrations of both m- and p-octopamine in all organs examined. Both amines
were virtually absent from all organs except the adrenals following chemical
sympathectomy with 6-hydroxydopamine, thereby establishing that m- and p-
octopamine are localized within sympathic nerve endings (28).
^{3}H-octopamine was found to be accumulated in human platelets, achieving a
maximum concentration gradient of 30:1 (30).
The measured concentration (ng/g wet tissue) of octopamine in rat brain was as
follows: whole brain (less cerebellum) (0.6); hypothalamus (3.2); striatum (0.5) and
cortex (0.6). Administration of pargyline (MAO-B inhibitor) resulted in an increase

(around ten-fold) in octopamine concentration in all the above brain regions (31).

Metabolism

MAO-A metabolises octopamine. In vivo inhibition of this enzyme in rats, reduced the deamination of octopamine in liver, lung and brain significantly (32). The N-methyltransferase seems to be also a metabolic pathway for octopamine in mammalian tissues (33). When octopamine was injected intraperitoneally into rats four metabolites were excreted in the urine: (i) unconjugated hydroxymandelic acid (OHMA) (16%), (ii) unconjugated hydroxyphenylglycol (OHPG) (4.5%), (iii) an acid-hydrolysable conjugate of OHPG (28%) and (iv) unconjugated octopamine (10%). Adult rats excreted OHMA (1.0 μg/day) but OHPG and octopamine could not be detected in urine. After the administration of a monoamine oxidase inhibitor, unconjugated octopamine (0.3 μ/day) was excreted in urine but OHPG could not be detected (34).

The only metabolic pathways for octopamine are deamination and conjugation. Following oral administration the percentage of conjugates was considerably higher than after intravenous infusion. This metabolic pattern appears typical of all phenylalkylamines with a hydroxyl group in the meta position. Ring hydroxylation to catecholamines was not observed. The enzymes mainly responsible for conjugation after oral administration are located in the gut wall. The resulting 'first pass effect', i.e. metabolism prior to the access to the central compartment, can account for the diminished pharmacodynamic effect after dosing by this route (29).

Pulmonary mitochondrial monoamine oxidase (MAO) activity was examined in preparations from rat, rabbit and guinea-pig. The oxidation of octopamine was greater in guinea-pig lung mitochondria than in rat or rabbit preparations (35).

Inactivation of octopamine was studied in a preparation of rabbit lung perfused with Krebs physiological medium at 37 ℃. Inactivation or removal of octopamine was calculated as the difference between the concentration of octopamine in the perfusion medium and the effluent, collected separately from each lung. 35 % of octopamine was inactivated by MAO. The deaminated metabolic products appeared in lung effluent within 90 sec beginning amine perfusion (36). Considering the presence of MAO in human lung tissue, it is likely that in situ elimination will occur in humans after inhalation.

Excretion

The urinary excretions of free and total octopamine were 5.7 ± 2.8 and 34.8 ± 16.6 ng/mg of creatinine, respectively, in normal human subjects (37).

Kinetic parameters

No data available.

Critical assessment

No data are available on pulmonary absorption of octopamine and on pulmonary bioavailability in human. The bioavailability through oral exposure is lower than through i.v. exposure in human, due to metabolization in the gut. In vitro studies with rabbit lung showed that 35 % of octopamine was inactivated by the pulmonary MAO. Considering the presence of MAO in human lung tissue, it is likely that in situ elimination will occur in humans after inhalation. Octopamine is widely distributed in the body. It is accumulated in the platelets. Mainly MAO-A metabolises octopamine. Because, octopamine is deaminated by MAO, it is likely that the octopamine turnover

is high in the body. Octopamine is excreted in conjugated and unconjugated form.

Conclusion
There are no in-vivo pharmacokinetic data available on respiratory intake of octopamine in human. Based on in-vivo metabolism data, probably pulmonary MAO will reduce the bioavailability of octopamine through cigarette smoking.

TOXICOLOGY
Acute toxicity
Human
No data available.

Animal
rat oral LD_{50}: 1240 mg/kg (1)
rat ipr. LD_{50}: 1350 mg/kg (1)
rat scu LD_{50}: 350 mg/kg (1)
mouse oral LD_{50}: 4200 mg/kg (1)
mouse ipr. LD_{50}: 600 mg/kg (1)
mouse scu. LD_{50}: 2070 mg/kg (1)
mouse iv. LD_{50}: 75 mg/kg (1)
guinea pig iv.LDLo: 200 mg/kg (1)

Octopamine administered to rats in doses of 50, 100 and 250 μg into the cerebral ventricles exerted a stimulating effect on the dopaminergic structures in the rat brain. In doses of 100 and 250 μg octopamine also had an anticataleptic effect (38).

Local tolerance
Human
No data available.

Animal
No data available.

Repeated dose toxicity
Subacute
No data available.

Semichronic
No data available.

Chronic
No data available.

Carcinogenicity
Human
No data available.

Animal
No data available.

Reproduction toxicology
Human
No data available.

Animal
No data available.

Mutagenicity
Human
No data available.

Animal
No data available.

Other

Critical assessment
No human toxicological data are available on octopamine inhalation. Mainly animal LD$_{50}$ data are available.

Conclusion
No data are available on octopamine toxicological effects in human.

INTERACTIONS
Chemical
Octopamine undergoes self-condensation between 155 ºC and 190 ºC in which the two amine groups yield 2,5-diaryl-piperazine derivative, with loss of two molecules of water (39).

In vivo
The effect of octopamine (0.158 – 15.8 µM) on the twitch responses of the prostatic portion of the rat vas deferens to electrical stimulation (0.025 Hz) was affected by inhibitor (praglyline) of monoamine oxidase (MAO) activity and antagonists of α$_1$- and α$_2$-adrenoceptors (corynanthine and yohimbine), respectively. Pretreatment with reserpine (5 mg/kg, 24 h; 2.5 mg/kg, 2 h before the experiment) largely prevented the effects of p-octopamine, but the amine still modified the twitch responses to field stimulation. Cocaine (10 µM) did not antagonize, but rather enhanced the inhibitory effects of p-octopamine in tissues with normal contents of noradrenaline (40).
The MAO-inhibitors and α-adrenergic antagonist seems to affect octopamine turnover in the mammalian brain (41, 42).

Octopamine (50 and 250 µg ivc) potentiated the tremorine (10 mg/kg ip) induced hypothermia in the rat. This effect was partially antagonized by atropine (10 mg/kg ip). Octopamine significantly prolonged the duration of pilocarpine (100 mg/kg iv) induced catalepsy in rats (43).

Critical assessment

Chemical

Octopamine undergoes selfcondensation between 155 and 190 ºC by the amine group.

In vivo

Octopamine level in the body is affected by MAO inhibitors and α-adrenergic antagonists.

Conclusion

Chemical

The amine group in octopamine seems to be reactive.

In vivo

Octopamine level in the body is affected by MAO inhibitors and α-adrenergic antagonists.

DEPENDENCY

No data available.

Effects of smoking cessation

No data available.

Critical assessment

Can not be made due to lack of data.

Conclusion

Can not be made due to lack of data.

COMMERCIAL USE

Octopamine is a sympathomimetic with predominantly α-adrenergic activity. It has been used as a oral treatment of hypotensive states (44).

BENEFICIAL EFFECTS

No data available.

Critical assessment

Can not be made due to lack of data.

Conclusion

Can not be made due to lack of data.

SUMMARY AND FINAL CONCLUSION

Octopamine is a component of cocoa, which is used as a flavouring agent in tobacco products. The octopamine level in cocoa or in cigarette smoke is unknown. Also the environmental level and data on human exposure are not available. Furthermore, data on combustion products of octopamine are not available. Octopamine is a natural compound in the human body with a mean plasma value of 0.23 ng/ml.

Octopamine stimulates α_2 and β_3-adrenergic receptors in rats. Furthermore, it affected the cAMP level in the cell via the D_1-receptor. Not enough data are available on pulmonary effects of octopamine. Because octopamine is much less potent than noradrenaline on the α and β-adrenergic receptors, it is likely that a large amount of octopamine needs be inhaled to affect the pulmonary system.

No data are available on pulmonary absorption of octopamine and on pulmonary bioavailability in human. In vitro studies with rabbit lung showed that 35 % of octopamine was inactivated by the pulmonary MAO. Considering the presence of MAO in human lung tissue, it is likely that in situ elimination will occur in humans after inhalation. Octopamine is widely distributed in the body. It is accumulated in the platelets. Mainly MAO-A metabolises octopamine. Octopamine is excreted in conjugated and unconjugated form. No data are available on the kinetic parameters of octopamine. Because, octopamine is deaminated by MAO, it is likely that the octopamine turnover in the body is high and thus also in the pulmonary tissue.

No human toxicological data are available on octopamine inhalation. Mainly animal LD50 data are available.

Octopamine level in the body is affected by MAO inhibitors and α-adrenergic antagonists.

Based on the octopamine metabolisation by MAO, it seems that pulmonary MAO will reduce the bioavailability of octopamine through cigarette smoking. However, since no data are available on pharmacodynamic, pharmacokinetic and toxicological effects of octopamine exposure through inhalation, the shortterm and longterm effects of exposure to octopamine through smoking on the respiratory system cannot be established. Furthermore, its additive effects on other biogenic amines present in cigarette smoke are also not known and have to be studied.

More studies are needed on:
-   the determination of octopamine level in cocoa and cigarette smoke
-   the determination of pyrolysis/combustion products of octopamine in cigarette smoke;
-   the local (respiratory system) effects of long-term use of octopamine alone and its pyrolysis/combustion products via inhalation;
-   the local (respiratory system) effects of long-term use of octopamine in combination with other biogenic amines via inhalation.

Date this sheet was genereted
Based on literature available in april 2002.

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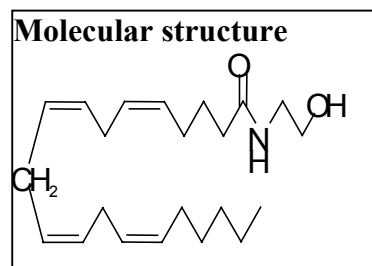
3.10 Anandamide

GENERAL
IUPAC systematic name: no data available
Synonyms: Arachidonoyl ethanolamide; N-Arachidonoyl-2-hydroxyethylamide; Arachidonylethanolamide; 5,8,11,14-Eicosatetraenoylethanolamide; N-(2-Hydroxyethyl)anachidonamide; N-(2-Hydroxyethyl)-5,8,11,14-eicosatetraenamide (all-Z)- (1)
Molecular formula: $C_{22}H_{37}NO_2$ (2)

Molecular structure

Molecular weight: 347.5 g/mol (2)
Alifatic: yes
Aromatic: no
N containing: yes
Halogen containing: no
CAS registry no.: 94421-68-8 (2)
Storage:
R/S classification: S 24/25 (2)
dangercode (transport): no data available
Properties:
➢ melting point: no data available
➢ boiling point: no data available
➢ density: 0.92 g/ml (2)
➢ refractive index: no data available
➢ solubility: soluble in ethanol (2)
➢ substance description:
  • color: light yellow (2)
  • liquid/gas/powder: liquid, oil (2)
  • odor/taste: no data available
➢ volatility: no data available
➢ pK_a: no data available
➢ PA: no data available
➢ flammability:
  • FP = no data available
  • FL Limits = no data available
  • IT = no data available
➢ decomposition temperature: no data available
➢ stability: no data available
➢ vapour pressure/ vapour tension (20 °C): no data available
➢ vapour pressure (50 °C): no data available
➢ relative density: no data available

➢ octanol water partition coefficient, log P, log K_{OW}: no data available
➢ conversion factor: not relevant

Critical assessment

The compound is a bioactive amide of a fatty acid, which was isolated and then its structure determined by NMR and several MS-techniques and confirmed by synthesis. Values for classical physical properties could not be found in literature. Mass spectrometric behavior reveals that anandamide easily undergoes thermal dehydration upon energy supply (3).

Striking structural features are: the aliphatic hydroxylgroup, the amide bond and the multi-unsaturated carbon chain.

Conclusion

'Pure'anandamide looks like an oil, will lose water upon heating and possesses some active chemical sites such as an hydroxyl group, an amide group and four unsaturated carbon-carbon bonds.

FUNCTION IN TOBACCO
No data available.

AMOUNT IN TOBACCO PRODUCTS
A source of anandamide in cigarettes is cocoa powder. A typical casing concentration of cocoa powder for cigarette tobacco is 1% (4).
The amount of anandamide found in cocoa powder is around 0.05 µg/g (5).
Assuming one cigarette weights approximately 1 g, the anandamide amount from cocoa powder in one cigarette is estimated to be ± 0.5 ng.

AMOUNT IN SMOKE
- **main stream:** no data available.
- **side stream:** no data available.

SOURCE
Anandamide is added to tobacco as a component of cocoa powder, which is used as flavouring agent (4).

ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE
Anandamide was found in small quantities in human milk (± 3 µg/l) as an endogenous products (6).
The environmental exposure to anandamide is unknown.

COMBUSTION PRODUCTS
No data available.

CONSENSUS REPORTS
No data available.

STANDARDS AND RECOMMENDATIONS
ADI: No data available.
TWA$_{NL}$ = MAC: No data available.

TWA$_D$ =MAK: No data available.
TWA$_{USA}$: No data available.
STEL$_{NL}$: No data available.
STEL$_{USA}$: No data available.
LTEL: No data available.
TLV-C: No data available.
TLV-CARCINOGENICITY: No data available.
MAK-REPRODUCTION: No data available.

Others:

Reference value:
Endocannabinoid-like compounds, such as anandamide were determined in human brain. Anandamide level was 56 ± 17 ng/mg protein, which represented 7.7% of total endocannabinoid-like compounds (7). No data are available on blood anandamide level of normal human subjects. However, some data are available on blood anandamide level of pregnant women by IVF-embryo transfer. The anandamide blood level was higher in women who failed to achieve an ongoing pregnancy than in those who became pregnant (1.4 ± 0.8 ng/ml and 0.3 ± 0.3 ng/ml, respectively) (8). Anandamide was determined in plasma prepared from rat blood collected either by cardiac puncture or by decapitation. After cardiac puncture, anandamide level was 1.1 ± 0.2 ng/ml (mean $\pm$ sem, n = 9). By contrast, after decapitation anandamide was dramatically elevated (50 ± 4.5 ng/ml) (9). Anandamide level, measured in deproteinated rat blood plasma, was 1.8 ng/ml (10).

CLASS
EG Carc. Cat.: No data available.
IARC-category: No data available.
CEC: No data available.

Critical assessment
On the assumption that anandamide is not degraded during tobacco processing and cigarette combustion, the exposure level of anandamide through cigarette smoking is 12.5 ng/day (at smoking 25 cigarettes per day). Due to lack of data, the exposure through cigarette smoking can not be compared with environmental anandamide exposure. By comparing the anandamide exposure through cigarette smoking with the endogenous anandamide level in human brain and blood (56 ng/mg protein and 1.4 µg/l, respectively), it can be concluded that anandamide level in cigarette is significantly lower than the endogenous pool.
No data are available on the pyrolysis/combustion products of anandamide.

Conclusion
The anandamide exposure through cigarette smoking is significantly lower than the endogenous anandamide level in human subjects. However, no conclusion can be drawn on the local anandamide exposure on the respiratory system and this might be a point of concern.

PHARMACODYNAMICS
Mechanism of action
Cannabinoid receptors, the molecular targets of the active principle of cannabis Δ^9-tetrahydrocannabinol, are activated by a small family of naturally occuring lipids that include anandamide (11). Two cannabinoid receptors have been identified to date; CB_1 and CB_2. These are G-protein coupled receptors (12).
The CB_1 receptor and its splice variant CB_{1A}, are found predominantly in the brain with highest densities in the hippocampus, cerebellum and striatum. Considerably lower expression is found in peripheral tissue including lung, testis, uterus and vascular tissue. The CB_2 receptor is found predominantly in the spleen and in haemopoietic cells and has only 44% overall nucleotide sequence identity with the CB_1 receptor. Following agonist binding, the CB_1 receptor mediates inhibition of adenylate cyclase, inhibition of N- and P/Q-type calcium channels, stimulation of potassium channels, and activation of mitogen-activated protein kinase. The CB_2 receptor mediates inhibition of adenylate cyclase and activation of mitogen-activated protein kinase. Anandamide is released from neurons upon depolarization through a mechanism that requires calcium-dependent cleavage from a phospholipid precursor in neuronal membranes. The release of anandamide is followed by rapid uptake into the plasma and hydrolysis by fatty-acid amidohydrolase.The psychoactive cannabinoids increase the activity of dopaminergic neurons in the ventral tegmental area-mesolimbic pathway (13). Other effects of anandamide that are not mediated via cannabinoid receptors include inhibition of L-type Ca^{2+} channels, stimulation of vanilloid receptors (VR_1), transient changes in intracellular Ca^{2+}, and disruption of gap junction function. Activation of VR_1 receptors by anandamide causes release of calcitonin-gene-related-peptide (14).

Pulmonary system
- **breathing frequency**: see airway resistance.
- **tidal volume**: see airway resistance.
- **lung compliance**: see airway resistance.
- **airway resistance**: Anandamide was tested for bronchodilator activities. Conscious guinea pigs were given cumulative i.v. doses of anandamide (1.0, 3.0, and 10.0 mg/kg) to assess its effect on dynamic compliance (C-dyn), total pulmonary resistance (R-L), tidal volume (V-T) and breathing frequency (f). Anandamide did not significantly affect C-dyn, R-L, V-T and f. These results suggest that in vivo anandamide has minimal direct airway smooth muscle-related actions (15). Calignano et al. (1990) postulated that activation of CB_1-receptors by locally released anandamide may participate in the control of bronchial contractility. How anadamide exerts such a control may depend, however, on the state of the bronchial muscle. When the bronchospasm was induced by capsaicin (intratracheal, ± 67 % of the maximal bronchoconstriction) in anaesthitized guinea-pigs , then anandamide produced a dose-dependent (0.5 –5mg/kg, i.v.) attenuation of the induced-bronchospasm (eliminated the bronchospasm at 5 mg/kg). Anandamide (5 mg/kg, i.v.) had no direct bronchomotor action (11.8 % of maximal bronchoconstriction). After vagotomy, systemic application of anadamide produced a dose-dependent bronchoconstriction in guinea-pigs (the highest dose, 5 mg/kg i.v., exerted ± 55% of the maximal bronchoconstriction) (16). Another study showed that sensory nerves innervating blood vessels play a role in the local and systemic regulation of the cardiovascular and respiratory (CVR) systems. The CVR reflexes evoked by administration of anandamide (75 -

750 µg/kg) into the hindlimb vasculature of anaesthetized rats was investigated. Anandamide caused a rapid dose-dependent reflex fall in blood pressure and an increase in ventilation when injected intra-arterially into the hindlimb. Vagotomy or carotid sinus sectioning had no significant effect on anandamide induced responses. Thus the endogenous cannabinoid, anandamide evoked CVR reflexes when injected intra-arterially into the rat hindlimb. These responses appear to be mediated as a reflex via VR_1 located on sensory nerve endings within the hindlimb vasculature (17).

In vitro studies (18, 19) with isolated guinea-pig bronchi, showed some effects of anandamide on the bronchus. Anandamide produced a modest contractile response in isolated guinea-pig bronchus compared with the vanilloid receptor agonist capsaicin. It seems that the anandamide induced contractile response in guinea-pig isolated bronchus is dependent upon the activation of vanilloid receptors on airway sensory nerves. The cannabinoid receptors do not appear to play a role in this regard (anandamide dose was not mentioned in the abstract) (18). In another study, it was shown that anandamide did not contract the guinea-pig bronchus significantly at concentrations up to 100 µM. The contractile effect to 100 µM anandamide was $40.53 \pm 7.04\%$ (19).

Cardiovascular system
- **blood pressure**: see heart rate.
- **heart rate**: Anandamide induces marked cardiovascular effects in rats. It elicits a triphasic response: an immediate transient bradycardia and hypertension (phase I) is followed by a brief pressor response (phase II) and then a more prolonged decrease in blood pressure (phase III). The former (phase I) is mediated by VR_1 receptor and the latter (phase III) is due to cannabinoid CB_1 receptor activation. Mechanisms underlying the phase II effect are unknown (20). A study of the phase I cardiovascular effects of anandamide on rats showed that the systemic (i.v.) ED_{50} value (anandamide dose decreasing the heart rate and the blood pressure by 150 beats/min and 20 mmHg respectively) was 2.6 mg/kg body weight (bw) (21). The above described cardiovascular effects by anandamide were confirmed by another study. At doses between 3 and 30 mg/kg, time-dependent cardiovascular changes were observed. An immediate bradycardia exceeding 50% was seen within 10-15 s of administration and lasted up to 11 minutes following the highest dose. In contrast the change in mean arterial pressure was biphasic: an immediate 20 % decrease in mean arterial pressure followed by a significant increase in blood pressure that lasted about 13 min after the highest dose (22).

Renal system
- **diuresis**: no data available.
- **saluresis**: no data available.

Nervous system
- **central nervous system**: The endocannabinoid anandamide is involved in modulating appetitive behaviour. Pre-satiated rats received an intrahypothalamic injection of anandamide (50 ng/0.5 µl) followed by measurement of food intake at 3 h post injection. Administration of anandamide induced significant hyperphagia. The intrahypothalamic anandamide initiates appetite by stimulation of CB_1 receptors (23). In another study, pre-satiated male rats (n=18), received

subcutaneous injections of anandamide (0.5, 1.0, 5.0, 10.0 mg/kg) before 3-h, nocturnal food intake tests. All doses of anandamide induced significant overeating, with 1.0 mg/kg being most potent (24).

The possible role of the endocannabinoid anandamide on modulating the behavioral and neurochemical consequences of semi-starvation was described in a study. The effect of very low dose anandamide (1 µg/kg) administration on food intake, cognitive function and catecholaminergic and serotonergic pathways in two murine brain areas concerned with appetite (hypothalamus) and learning (hippocampus), and the peripheral corticosterone response to the stress of 40% diet restriction was studied. Anandamide-treated mice consumed 44% more food (P<0.05) during 1 week of 2.5-h feeding each day. In the hypothalamus, there were significantly increased concentrations of noradrenaline (P<0.01), dopamine (P<0.05) and serotonine (5-HT) (P<0.001). In the hippocampus, anandamide increased significantly norepinephrine and dopamine, but decreased 5-HT (all at P<0.001). Diet restriction was accompanied in both areas by a significant decrease in all neurotransmitter concentrations that were partially restored by anandamide for dopamine and 5-HT, but not for noradrenaline. In animals on diet restriction, anandamide significantly improved impaired maze performance. Noradrenaline turnover and plasma corticosterone levels were also raised significantly by anandamide (25).

- **autonomic system**: Cannabinoid inhibition of sympathetic innervation of the peripheral vasculature is due to CB_1-receptor mediated inhibition of noradrenaline release from sympathetic nerve terminals (26). Treatment of isolated human atria with anandamide reduces the release of ^{3}H-noradrenaline in response to electrical stimulation (27).

Other

Anandamide has anti-hyperalgesic properties in models of somatic and visceral inflammation. In the turpentine-inflamed rat urinary bladder a reversal of the inflammation-associated viscero-visceral hyperreflexia (wh) was observed when anandamide was administered 135 min after the induction of inflammation. Anandamide attenuated the *wh* response in a dose related manner, with a threshold of 25 mg/kg (i.a). (28).

Intracerebroventricular administration to mice of anandamide induced dose-related antinociception in the 55 ºC warm-water tail-flick test (29).

The antinociceptive effects of anandamide were investigated in 12 adult rhesus monkeys (Macaca mulatta). The antinociceptive effects were indicated by the latencies to remove the tail from a 50 ºC water bath. Anandamide (10 mg/kg i.m.) produced a significant antinociception (30).

The effect of anandamide on upper gastrointestinal motility in mice was investigated. Anandamide (0.5-20 mg/kg, i.p.), dose-dependently delayed gastrointestinal motility (31).

Critical assessment

Two cannabinoid (CB_1 and CB_2) and the vanilloid receptors are activated by anandamide. It is suggested that anandamide may control the bronchus tone. Andamide attenuates bronchospasm induced by capsaicin, but also induces bronchoconstriction (± 5 mg/kg, i.v.) in guinea-pigs. The effective anandamide dose exerting the bronchial effects seems to be significantly higher than the anandamide dose in cigarette smoke (12.5 ng/25 cigarettes). This cigarette anandamide dose is

also significantly lower than the dose needed to affect the cardiovascular system (± 2.6 mg/kg bw) and central nervous system (0.001 mg/kg – 10 mg/kg). However, in all the mentioned studies anandamide was administered by other systems than the pulmonary system.

Conclusion
Anandamide level in cigarette smoke seems to be insufficient to exert any systemic pharmacological effects. However, no data on pulmonary exposure of anandamide are available and therefore, the local anandamide effects are unknown.

PHARMACOKINETICS
Absorption
No data are available on absorption through the pulmonary system. An *in vitro* study with celllines (neuroblastoma, glioma and laryngeal carcinoma cells) showed that cellular uptake of anandamide is governed by a concentration gradient of unbound anandamide e.g. facilitated diffusion-mediated transport (32).

Bioavailability
No data are available on the anandamide bioavailability through the pulmonary system.
Di Marzo et al (6) suggested that 1.6 – 5% of orally administered anandamide enter the bloodstream, probably due to extensive metabolism in the gastrointestinal tract by enzyme fatty acid amide hydrolyse.

Distribution
Anandamide was found in human hippocampus and parahippocampal cortex, striatum, and cerebellum, which are the brain areas known to express high levels of CB_1-receptors. Significant levels of anandamide were also found in the thalamus which expresses low levels of CB_1-receptors and in the spleen which expresses high levels of the CB_2-receptor. Small amounts of anandamide were detected in the heart. Only trace quantities were detected in pooled serum, plasma and cerebrospinal fluid (CSF). The distribution of anandamide in brain and spleen supports its potential role as an endogenous agonist in central and peripheral tissues, The low levels found in serum, plasma, and CSF suggest that it is metabolized in tissues where it is synthesized and that its action is probably not hormonal in nature (33).
Male mice were administered 50 mg/kg ^{3}H-anandamide (i.v.). At 1, 5, 15 and 30 min after administration, the animals were sacrificed and the distribution of radio activity in various tissues was determined. The radio activity was detectable in brain by 1 min after injection. At 1 min after injection, the rank order of radioactivity per milligram or microliter of tissue was adrenal > lung > kidney > plasma > heart > liver > diaphragm > brain > fat (34).

Metabolism
Anandamide is hydrolysed by fatty-acid amide hydrolase (FAAH) to free arachidonic acid and ethanolamine. FAAH is an endoplasmic reticular integral membrane-bound enzyme. FAAH is widely distributed in the brain. Outside the brain, high FAAH levels are found in pancreas, kidney and in smaller extent in the liver (11, 35).

Excretion
No data available.

Kinetic parameters
Male mice were administered 50 mg/kg ^{3}H-anandamide (i.v.). At 1, 5, 15 and 30 min after administration, the animals were sacrificed and various tissues were removed, solubilized and counted to determine the distribution of the radioactivity. Also, the anadamide were determined in the samples from brain, adrenal gland and in plasma.The radio activity was detectable in brain 1 min after injection. Although the 1 and 5 min metabolic profiles of brain radio activity showed that anandamide was clearly present, most anandamide had already been transformed to arachidonic acid and other polar metabolites, and there were almost no detectable brain levels of anandamide at 15 and 30 min in plasma and adrenal gland. It is suggested tha anandamide quickly reaches the brain and that the rapid metabolism of anandamide plays a key role in the time course of the pharmacological activity of this naturally occurring cannabinoid receptor ligand (34).

Critical assessment
The oral data indicate a low bioavailabilty of anandamide. The *in vivo* studies seems to indicate that anandamide is endogenously distributed widely in the human body. Anandamide is also extensively metabolized as indicated by the half life ($t_{1/2} < 5$ min). There are no data available on pharmacokinetics in animals and humans from respiratory studies.

Conclusion
There are no in-vivo pharmacokinetic data available on respiratory exposure of anandamide. The rapid elimination of anandamide indicates that pulmonary anandamide exposure will not exert any systemic effects.

TOXICOLOGY
Acute toxicity
Human
No data available.

Animal
TDLo i.p. rat:140 µg/kg (1)
TDLo s.c. mouse: 100 mg/kg (1)
High doses of injected anandamide (10 – 100 mg/kg of body weight) cause typical *in vivo* cannabimimetic inhibitory effects (decreased motor activity, rearing activity, ring catalepsy, hypothermia, analgesia and agonistic behaviour) and inhibition of leukocyte phagocytosis. In contrast, low doses of injected anandamide (0.01 mg/kg) stimulated activities in open field and ring, increase aggresive behaviour and stimulated phagocytosis (36).
The acute anandamide effects were studied in unanaesthetized freely behaving rats. Intravenous anandamide caused dose-related decreases in locomotor behaviour, a pronounced hyper-reflexia, and a moderate antinociceptive state. At doses between 3 and 30 mg/kg, a dose-dependent hypothermia and profound, time-dependent cardiovascular changes were also observed.The exerted behavioural and physiological effects were similar to those seen following natural cannabinoids (22).

Local tolerance
Human

No data available.

Animal
No data available.

Repeated dose toxicity
Subacute
Subacute treatment of rats with anandamide (20 mg/kg i.p. for 15 days) resulted in behavioral tolerance without any change in cannabinoid receptor binding in the brain regions studied (striatum, cortex, hippocampus, and cerebellum), suggesting that receptor down-regulation was not involved in the development of anandamide behavioral tolerance (37).

Semichronic
No data available.

Chronic
No data available.

Carcinogenicity
Human
No data available.

Animal
No data available.

Reproduction toxicology
Human
The anandamide-degrading enzyme, fatty acid amide hydrolase (FAAH), had significantly lower activity (46 ± 17 versus 161 ± 74 pmol/min per mg protein) and protein content (0.10 ± 0.03 versus 0.23 ± 0.06 units) in lymphocytes of IVF-embryo transfer patients who failed to achieve an ongoing pregnancy than in those who became pregnant, and this was paralleled by a significant increase in blood anandamide (1.4 ± 0.8 ng/ml and 0.3 ± 0.3 ng/ml respectively). Taken together with the reported negative effects of anandamide on embryo implantation, it seems that low FAAH activity and subsequent increased anandamide levels in blood might be one of the causes of implantation failure or pregnancy loss (8).

Animal
The behavioural response to anandamide was examined in developing mice from day 13 into adulthood. It was observed that depression of ambulation in an open field and the analgetic response to anandamide were not fully developed until adulthood. In a separate set of experiments, five daily injections of anandamide (sc., 20 mg/kg) was administered during the last trimester of pregnancy. No effects on birth weight, litter size, sex ratio and eye opening were detected after maternal anandamide treatment. Further, no effects on open field performance of the offspring were observed until 4 weeks of age. However, from 40 days of age, a number of differences between the prenatal anandamide and control offspring were detected. Thus, the offspring from anandamide -treated dams showed impaired responsiveness to a challenge with anandamide expressed as a lack of immobility in the ring test for catalepsy,

hypothermia and analgesia. On the other hand, without challenge, they exhibited a spontaneous decrease in open field activity, catalepsy, hypothermia and a hypoalgetic tendency. These data suggest that exposure to excessive amounts of anandamide during gestation alters the functioning of the anandamide-CB receptor system (38).

Cannabinoids cause increase in the number of stillbirths and delay of delivery. The effect of anandamide on prostaglandin secretion in pregnant rats was investigated. Anandamide i.p. was injected with a daily dose of 0.02 mg/kg b.w. over the third week of gestation. Anandamide caused a delay of pregnancy and lowered serum prostaglandin (PG)F 1alpha and PGF 2alpha. There were increased number of stillbirths in anandamide treated dams. It was postulated that the delay of pregnancy and the augmentation of stillbirth is due to the low PG level (39).

The anandamide was investigated on the postnatal development of the hypothalamo-pituitary axis (HPA) when administered during the third week of gestation. Rat pups were killed every fifth day from delivery to the 20th postnatal day; gonads, pituitary, and rest of body were weighed, and samples were collected for analysis of gonadotropin releasing hormone in the hypothalamus and luteinizing hormone, follicle stimulating hormone, prolactin, and growth hormone in the pituitaries and sera. Anandamide caused predominantly inhibitory effects on the measured parameters. The inhibition was most pronounced immediately following delivery, whereas at the end of the investigated period (20th postnatal day) no differences were observed (40) (no data on anandamide dose were mentioned in the abstract).

Mutagenicity
Human
No data available.

Animal
No data available.

Other

Critical assessment
The TDlo was 140 µg/kg i.p. for rats and 100 mg/kg s.c. for mice. The anandamide dose in cigarettes (12.5 ng/25 cigarettes) compared with the animal TDlo, seems to indicate that the anandamide level in cigarettes is insufficient to exert any systemic toxicological effects. Because no data are available on the inhalation toxicological effect of anandamide, the local pulmonary toxicological effect is unknown.

Conclusion
No data are available on inhalation toxicological effects of anandamide. The long-term effect of this compound via the respiratory system needs to be studied.

INTERACTIONS
Chemical
No data available.

In vivo

Lipopolysaccharide (LPS) increases the levels of the endogenous cannabinoid anandamide level in rat macrophages. LPS enhances the levels of anandamide (fourfold over controls) also in human lymphocytes. LPS inhibits the activity of the anandamide -degrading enzyme fatty acid amide hydrolase (FAAH) in these cells, by downregulating the gene expression at transcriptional level (41) (the anandamide dose was not mentioned in the abstract).

In the absence of indomethacin, anandamide did not contract the guinea-pig bronchus at concentrations up to 100 µM. In the presence of indomethacin (10 µM), anandamide induced concentration-related contractions with a potency (pEC50 (negatively log of EC50)) value of 5.18 ± 0.11. The vanilloid receptor antagonist, capsazepine (10 µm), significantly attenuated the contractile effect of anandamide. The response to 100 pM anandamide being 40.53 ± 7.04% in the presence of vehicle and 1.57 ± 8.93% in the presence of 10 µM capsazepine. The contractile actions of anandamide was markedly enhanced by the peptidase inhibitor thiorphan. The lipoxygenase inhibitors 5,8,11,14-eicosatetraynoic acid (etya) and 5,8,11 eicosatriynoic acid (eti) reduced the effect of 100 µM anandamide from 34.7 ± 1.9% (vehicle) to 7.7 ± 5% (etya, 10 pM) and from 41.85 ± 4.25% (n=6) (vehicle) to 10.31 ± 3.54 (n=6) (eti, 20 µM) (19).

The ability of a series of homologues and analogues of palmitoylethanolamide to inhibit the uptake and fatty acid amidohydrolase (FAAH)-catalysed hydrolysis of [H-3]-anandamide ([H-3]-AEA) has been investigated. Palmitoylethanolamide and homologues with chain lengths from 12 - 18 carbon atoms inhibited rat ^{3}H-anandamide metabolism with pec(50) values of around 5. Homologues with chain lengths less than or equal to eight carbon atoms gave <20% inhibition at 100 µM anandamide. R-palmitoyt-(2-methyl)ethanotamide, palmitoylisopropylamide and olcoylethanolamide inhibited ^{3}H-anandamide metabolism with pec(50) values of 5.39 (competitive inhibition), 4.89 (mixed type inhibition) and 5.33 (mixed type inhibition), respectively. Most of the compounds had little effect upon the uptake of ^{3}H-anandamide into C6 and /or RBL-2H3 cells. However. palmitoylcyclohexamide (100 µM) and palmitoylisopropylamide (30 and 100 µM) produced more inhibition of ^{3}H-anandamide uptake than expected to result from inhibition of ^{3}H-anandamide metabolism alone. In intact C6 cells, palmitoylisopropylamide and oleoylethanolamide inhibited formation of ^{3}H-ethanolamine from ^{3}H-anandamide to a similar extent as AM 404, whereas palmitoylethanolamide, palmitoylcyclohexamide and R-palmitoyl-(2-methyl)ethanotamide were less effective. Palmitoylisopropylamide may prove useful as a template for design of compounds that reduce the cellular accumulation and metabolism of anandamide without affecting either CB_1 or CB_2 receptors (42).

Palmitoylethanolamide (PEA) has been shown to act in synergy with anandamide. PEA potently enhances the anti-proliferative effects of anandamide on human breast cancer cells (HBCCs), in part by inhibiting the expression of fatty acid amide hydrolase (FAAH), the major enzyme catalysing anandamide degradation. PEA (1-10 µM) enhanced in a dose-related manner the inhibitory effect of anandamide on both basal and nerve growth factor (NGF)-induced HBCC proliferation, without inducing any cytostatic effect by itself. PEA (5 µM) decreased the IC50 values for anandamide inhibitory effects by 3-6-fold. The effect of PEA was due in part to inhibition of anandamide degradation, since treatment of MCF-7 cells with 5 µM PEA caused a similar to 30-40 % down-regulation of FAAH expression and activity (43).

Critical assessment
Chemical
No data available for assessment.

In vivo
The anandamide level is affected either by inhibition of the anandamide degrading enzym or by inhibition of the anandamide transport through the cellmembrane.

Conclusion
Chemical
No data available for conclusion.

In vivo
The anandamide level is affected either by inhibition of the anandamide degrading enzyme or by inhibition of the anandamide transport through the cellmembrane.

DEPENDENCY
Cannabinoids have a long history of consumption for recreational and medical reasons. In humans, psychoactive cannabinoids produce euphoria, enhancement of sensory perception, tachycardia, antinociception, difficulties in concentration and impairment of memory. The cognitive deficiencies seem to persist after withdrawal. The psychoactive cannabinoids increase the activity of dopaminergic neurons in the ventral tegmental area-mesolimbic pathway. Since these dopaminergic circuits are known to play a pivotal role in mediating the reinforcing (rewarding) effects of the most drugs of abuse, the enhanced dopaminergic drive elicited by the cannabinoids is thought to underlie the reinforcing and abuse properties of marijuana.Thus, cannabinoids share a final common neuronal action with other major drugs of abuse such as morphine, ethanol and nicotine in producing facilitation of the mesolimbic dopamine system (13). There is evidence that cannabinoids cause tolerance and physical dependence in humans and animals. The question is whether the endogenous ligand for the cannabinoid receptor, anandamide, induces also behavioral tolerance and physical dependence in rats. Rats were injected with anandamide (20 mg/kg i.v.) daily for 2 weeks. To assess tolerance, on days 1, 8 and 15 of treatment, rats were observed and behavior was tested. Two common methods were employed to assess physical dependence: interruption of anandamide dosing and vehicle substitution or administration of a cannabinoid CB_1 receptor antagonist (3 mg/kg i.v.). Full or partial tolerance developed to the classical behavioral effects elicited by the cannabinoids: hypothermia, catalepsy, hypomotility, decrease in stereotypic activity (rearing and grooming) and hindlimb splaying. No tolerance to anandamide was observed for reduced defecation. An abstinence syndrome appeared after abrupt cessation of cannabinoid intake and after withdrawal precipitated by CB_1 receptor antagonist; the withdrawal signs were scratching, licking and biting, eating of feces, ptosis, arched back, wet dog shakes, head shakes, myoclonic spasms, writhing, forepaw fluttering, teeth chattering and piloerection. These findings indicate that the endogenous cannabinoid ligand, administered exogenously, induces both tolerance and physical dependence in rats (44).

However, other studies (45, 46) indicate that anandamide has no addictive properties. The recent discovery of anandamide, an endogenous ligand for cannabinoid receptors, and the synthesis of SR141716A, a cannabinoid antagonist selective for

brain cannabinoid CB_1-receptors, have provided new tools to explore the mechanisms underlying cannabis abuse and dependence. Drug discrimination is the animal model with the most predictive validity and specificity for investigation of the psychoactive effects of cannabinoids related to their abuse potential. However, attempts to train animals to discriminate anandamide (or SR141716A) have so far been unsuccessful (45). The physical dependence on THC [Delta(9)-tetrahydrocannabinol] was demonstrated by using SR 141716A, a cannabinoid antagonist. This demonstration prompted to determine whether anandamide, an endogenous cannabinoid agonist, would also produce physical dependence. A low-dose regimen (10, 20, 40 and 40) or a high-dose regimen (25, 50, 100 and 100) expressed as mg/kg/24 hr was infused i.p. on a continuous basis, from days 1 through 4, respectively. During the infusion, especially at the high-dose regimen, the rats became immobile and developed eyelid ptosis. Abrupt discontinuation of anandamide did not elicit rebound behavioral activity. Neither arachidonic acid, a precursor and metabolite of anandamide (50, 100, 200 and 200 mg/kg/24 hr on days 1 through 4, respectively), nor 2-Me-F-AN [2-methylarachidonyl-(2'-fluoroethyl)-amide], a metabolically stable analog of anandamide (5, 10, 20 and 20 mg/kg/24 hr for 4 days, respectively), had remarkable effects. Notably, groups pretreated with anandamide or 2-Rne-F-AN and challenged with SR 141716A did not show significantly elevated behavioral scores when compared with SR 141716A controls. On the other hand, nearly all groups receiving SR 141716A showed significant activation of these behaviors compared with vehicle controls, which suggests that this cannabinoid antagonist itself was activating behavior. It was concluded that anandamide has little if any capacity for physical dependence (46).

Effects of smoking cessation
No data available.

Critical assessment
The psychoactive cannabinoids have physical dependence properties, but there is inconclusive evidence that endogenous cannabinoids such as anandamide, have physical dependence properties. The anandamide level in cigarettes (12.5 ng/25 cigarettes) seems to be clearly insufficient to have any dependency properties, compared with the anandamide dose used to investigate the dependency properties of anandamide (10 – 100 mg/kg).

Conclusion
Anandamide through cigarette smoking does not seem to have physical dependence properties.

COMMERCIAL USE
No data available.

BENEFICIAL EFFECTS
Anandamide potently and selectively inhibits the proliferation of human breast cancer cells in vitro. Anandamide dose-dependently inhibited the proliferation of MCF-7 and EFM-19 cells with IC50 (inhibitory concentration) values between 0.5 and 1.5 µM and 83-92% maximal inhibition at 5-10 µM. The proliferation of several other

nonmammary tumoral cell lines was not affected by anandamide. The anti-proliferative effect of anandamide was not due to toxicity or to apoptosis of cells but was accompanied by a reduction of cells in the s phase of the cell cycle. Anandamide cytostatic effect was inhibited by the selective CB_1 receptor antagonist SR 141716A (47).

Critical assessment
The beneficial effect of anandamide was investigated *in vitro* and can not be related to any beneficial effect of anandamide in cigarettes.

Conclusion
No conclusion can be drawn on beneficial effects of anandamide in cigarettes.

SUMMARY AND FINAL CONCLUSION
Anandamide does not have a function in cigarette. A source of anandamide in cigarettes is cocoa powder, which is used as flavor enhancer. A typical casing concentration of cocoa powder for cigarette tobacco is 1%. The amount of anandamide found in cocoa powder is around 0.05 µg/g.
Assuming one cigarette weights approximately 1 g, the anandamide amount from cocoa powder in one cigarette is estimated to be ± 0.5 ng.
On the assumption that anandamide is not degraded during tobacco processing and cigarette combustion, the exposure level of anandamide through cigarette smoking is 12.5 ng/day (at smoking 25 cigarettes per day). The exposure through cigarette smoking can not be compared with environmental anandamide exposure, due to lack of data. However, by comparing the anandamide exposure through cigarette smoking with the endogenous anandamide level in human brain and blood (56 ng/mg protein and 1.4 ng/ml, respectively), it can be concluded that anandamide level in cigarettes is significantly lower than the endogenous pool.
No data are available on the pyrolysis/combustion products of anandamide.

Two cannabinoid (CB_1 and CB_2) and the vanilloid receptors are activated by anandamide. It has been suggested that anandamide may control the bronchus tone. Anandamide attenuates bronchospasm induced by capsaicin, but also induces bronchoconstriction (± 5 mg/kg, i.v.) in guinea-pigs. The effective anandamide dose exerting the bronchial effects seems to be significantly higher than the anandamide dose in cigarette smoke (12.5 ng/25 cigarettes). This cigarette anandamide dose is also significantly lower than the dose needed to affect the cardiovascular system (± 2.6 mg/kg bw) and central nervous system (0.001 mg/kg – 10 mg/kg). However, in all the mentioned studies anandamide was administered by other routes than directly to the pulmonary system and therefore, it is not known whether anandamide pulmonary exposure will exert any respiratory effects.

The oral data indicate a low bioavailabilty of anandamide and an extensive metabolism in the gastrointestinal tract. Anandamide is widely distributed in the human body. Anandamide is also extensively metabolized as indicated by the half life ($t_{1/2}$ < 5 min). There are no data on pharmacokinetics in animals and humans from respiratory studies.

The TDlo was 140 µg/kg i.p. for rats and 100 mg/kg s.c. for mice. The anandamide

dose in cigarettes (12.5 ng/25 cigarettes) compared with the animal TDlo, seems to indicate that the anandamide level in cigarettes is insufficient to exert any acute systemic toxicological effects. Because, no data are available on the inhalation toxicological effect of anandamide, the local pulmonary toxicological effect is unknown.

There are no data available on the chemical interaction of anandamide. The anandamide level is affected either by inhibition of the anandamide degrading enzym or by inhibition of the anandamide transport through the cell membrane.

The psychoactive cannabinoids have physical dependence properties, but there is inconclusive evidence that endogenous cannabinoids such as anandamide, have physical dependence properties. The anandamide level in cigarettes (12.5 ng/25 cigarettes) seems to be clearly insufficient to have any dependency properties, compared with the anandamide dose used to investigate the dependency properties of anandamide (10 – 100 mg/kg).

The anandamide level in cigarettes seems to be insufficient to exert any systemic pharmacological and toxicological effects. Since no data are available on pharmacodynamic, pharmacokinetic and toxicological effects of anandamide exposure through inhalation, the shortterm and longterm effects of exposure to anandamide through smoking on the respiratory system cannot be established.
More studies are needed on:
- the determination of anandamide level in cigarette smoke
- the determination of pyrolysis/combustion products of anandamide in cigarette smoke
- the local (respiratory system) effects of long-term use of anandamide alone and its pyrolysis/combustion products via inhalation.

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4.   General overview and discussion

4.1   Exposure levels

Cocoa level in cigarettes ranges between 1% (w/w) and 3 % (w/w) (1)(2). Ten psychoactive compounds of cocoa were discussed in this review. The final exposure level of these compounds via cigarette smoking depends on the cocoa level in cigarette, the cigarette processing and combustion during smoking. In the next table the average 'potential' daily intake of these compounds by smoking 25 cigarettes/day are shown.

Table 1: Potential exposure levels of psychoactive compounds through cigarette smoking or food intake

Compound	Daily intake by smoking 25 cigarettes/day[a]	Estimated daily intake via food	Plasma reference value
Theobromine	4.75 mg	38.3 mg	not applicable
Caffeine	0.5 mg	200 –300 mg	not applicable
Serotonin	$15 \cdot 10^{-3}$ mg	15 µg – 15 mg	$0.79 \cdot 10^{-3}$ mg/l
Histamine	$0.33 \cdot 10^{-3}$ mg	< 2.6 mg	$0.48 – 0.53 \cdot 10^{-3}$ mg/l
Tryptophan	0.75 mg	250 – 900 mg	9.8 mg/l
Tryptamine	$0.2 \cdot 10^{-3}$ mg from cocoa 1 mg from tobacco	0.15 - 0.8 mg	$0.1–1.5 \cdot 10^{-6}$ mg/g[b]
Tyramine	$4 \cdot 10^{-3}$ mg from cocoa 10 mg from tobacco	0.2 – 10 mg	$1.3 – 4.0 \cdot 10^{-3}$ mg/l
Phenylethylamine	$5.5 \cdot 10^{-3}$ mg from cocoa 12.1 mg from tobacco	< 4 mg	$1.13 \cdot 10^{-3}$ mg/l
Octopamine	unknown	unknown	$0.23 \cdot 10^{-3}$ mg/l
Anandamide	$12.5 \cdot 10^{-6}$ mg	$0.9 \cdot 10^{-3}$ mg	$1.4 \cdot 10^{-3}$ mg/l

a   = based on a cocoa level of 1 % (w/w) in cigarettes
b   = brain level in ng/g wet weight

The expression 'potential' level of the compounds is used, because the assumption is made that 100 % of the compounds in cigarettes originating from cocoa is transferred to cigarette smoke. However, we acknowledge that level of the compounds in cigarette may decrease due to tobacco processing, storing and combustion. For ease of comparison between the exposure levels of the compounds via cigarette smoking and via food intake, it is assumed that 100 % of the compounds in cigarettes originating from cocoa is transferred to cigarette smoke. In reality, the level of exposure to these compounds via cigarette smoking will be significantly lower.

The exposure to theobromine, caffeine, serotonin, histamine, tryptophan and anandamide via food intake is significantly higher than exposure to these compounds via cigarette smoking. The exposure of other compounds, such as tryptamine, tyramine, phenylethylamine via cigarette smoking is higher or at least comparable with the exposure via food intake. The relatively higher exposure to these compounds via cigarette smoking is attributed to the natural occurrence of these compounds in tobacco rather than by addition of cocoa to cigarettes. Therefore, it is unlikely that cocoa will affect the cigarette smoking addiction via those compounds. All the compounds investigated occur naturally in the human body, except for theobromine and caffeine. However, the daily intake of theobromine and caffeine via food surpassed the exposure via smoking significantly.

The exposure to tyramine and phenylethylamine via smoking and to a lesser extent to tryptamine seems to be relatively high compared with the exposure level via food and also when compared with the plasma reference level.

Although the exposure for most of the compounds through food intake is higher than through smoking, the effect of exposure through smoking must not be neglected, because the exposure route is different. The exposure through food intake will exert systemic effects and will be subjected to first-pass effect whereas the exposure through smoking will probably exert only local effects and is not subjected to first-pass effect. Besides, during smoking the compounds are subjected to combustion resulting in compounds with different pharmacological properties. Therefore, it is reasonable to investigate whether the psychoactive cocoa compounds and their combustion products may increase the addictive properties of cigarettes.

4.2   Effects

4.2.1   Theobromine

Compared with other methylxanthines such as caffeine or theophylline, the action of theobromine on the central nervous system is considered weak. The central nervous effects of theobromine on human volunteers were investigated in a study, by looking at the subjective effects of theobromine. In that study, Mumford et al. (1994) (3) found that four of their seven volunteers could discriminate theobromine from placebo at an oral dose of 560 mg. The discriminative parameters were changes in mood and behaviour and having motivation to work. Theobromine is assumed to have bronchodilatory effects, thereby increasing the absorption of nicotine. However, the bronchodilatory properties are very weak compared to the bronchodilatory effect of theophylline. A dose of 15 mg theophylline aerosol induced a significant decrease of the airway resistance. This decrease was not observed immediately or within 30 min of theophylline administration. Comparing this information with the amount of theobromine in cigarettes (0.19 mg/cigarette), it can be concluded that the theobromine level in cigarettes is not enough to exert any bronchodilatory effects. Furthermore, the role of theobromine in cocoa craving has been reviewed in the literature and the conclusion was that this agent is not responsible for the craving qualities of chocolate. Based on the evidence discussed above, it can be concluded that theobromine will not affect the cigarette smoking addiction (4, 5).

Because no data were available on the combustion products of theobromine, their effect on the cigarette smoking addiction could not be evaluated. Furthermore, the long-term effect on the respiratory system of theobromine is unknown in combination with other methylxanthines or with its combustion products.

4.2.2   Caffeine

The physiological effects of caffeine have been extensively investigated. Caffeine is known as a psychostimulant. Caffeine seems to have low addictive properties. Daily caffeine consumption through coffee intake is significantly higher (± 400 times) than the caffeine intake through smoking. Due to the high oral bioavailability, it seems that the caffeine dose (0.02 mg/cigarette) in one cigarette is negligible to exert any effect. Mumford et al. (1994) (3) found subjective effects of caffeine between 10 – 45 min after oral administration of 72 mg caffeine.

The bronchodilatory effect of caffeine is interesting because it may increase the bioavailability of nicotine. The bronchodilatory property of caffeine is equipotent or less compared with the methylxanthine theophylline. This means that the caffeine amount (0.02

mg/cigarette) in one cigarette is not high enough to exert any bronchodilatory effect. In view of the low dose of caffeine in cigarettes, the absence of bronchodilatory effects and the low addictive properties of caffeine, it seems unlikely that caffeine plays a role in addiction to cigarette smoking. Because no data were available on the combustion products of caffeine, their effect on the cigarette smoking addiction could not be evaluated. Furthermore, the long-term effect on the respiratory system of caffeine is unknown in combination with other methylxanthines or with its the combustion products.

4.2.3   Serotonin
Serotonin is a neurotransmitter of both the central and the peripheral nervous systems and plays an important role in regulation of mood and behaviour (6). The serotonin intake through smoking (0.6 µg/cigarette) will not exert any systemic effect due to the large endogenous pool (10   mg) and also due to rapid metabolisation by MAO. Therefore, pulmonary intake may probably exert only local effects. Serotonin has bronchoconstrictory effect in animals. In normal human subjects the bronchoconstrictory effect was not observed. It can be concluded that serotonin in cigarette is not likely to increase the addiction to cigarette smoking.

4.2.4   Histamine
Histamine has a bronchoconstrictory effect, which means that it may decrease the bioavailablity of nicotine. Histamine is used for diagnostic purposes in asthmatics. Histamine has a bronchoconstrictory effect with a cut-off point for PD_{20} between $0.7 - 1.2$ mg in normal human subjects. These values for histamine are significantly higher than the histamine dose in one cigarette ($\pm$ 13 ng/cigarette). Thus histamine from added cocoa in cigarettes will probably not exert any bronchoconstrictory effect.

4.2.5   Tryptophan
Tryptophan is an essential amino acid and is a precursor for a variety of active compounds including serotonin, melatonin and tryptamine. The level of these active compounds will not be affected by tryptophan exposure through smoking, because the daily intake ($250 - 900$ mg/day) of tryptophan through food surpassed the exposure through smoking (0.75 mg/day) significantly and there is a large endogenous tryptophan pool present. There are no data available on the respiratory effects of tryptophan through pulmonary exposure.
Tryptophan contains reactive groups and forms reaction products with other compounds during combustion, such as beta-carbolines. Beta-carbolines are known inhibitors of MAO. There are indications that cigarette smoke contains MAO-I constituents and smokers have decreased MAO activity (7, 8). Others argue that one smokes for anti-depression properties. The prevalence of cigarette smoking is significantly higher by depressive persons than persons who are not (9). It can be concluded that the MAO-I properties of cigarette smoke may contribute to tobacco dependency.
It can be concluded that it is unlikely that tryptophan has any addictive properties but its reaction products formed during combustion may contribute to the addiction to cigarette smoking.

4.2.6   Phenylethylamine
Phenylethylamine is a natural compound of the tobacco plant and cocoa. The estimated phenylethylamine level in cigarettes originating from tobacco (12.1 mg/ 25 cigarettes) is about 2200 times higher than from added cocoa (5.5 µg/25 cigarettes). Therefore, it is

unlikely that cocoa will affect the addiction to cigarette smoking through the psychoactive compound phenylethylamine. Because phenylethylamine exposure through smoking is relatively high compared with the exposure level through food, it is interesting to investigate the contribution of phenylethylamine to the addiction to cigarette smoking.

Derivatives of phenylethylamine are stimulant and hallucinogenic substances such as amphetamine, mescaline and some neurotransmitters such as dopamine, adrenaline and noradrenaline. Phenylethylamine is classified as a neuromodulator of dopaminergic and possibly serotinergic and noradrenergic synapses. Phenylethylamine has biphasic effect on guinea pig isolated lung. After an initial relaxation at low concentration (10^{-7} – 10^{-5} M) it induces contraction at higher concentration (10^{-4} – 10^{-3} M). When phenylethylamine was perfused in guinea-pig lung, the pulmonary MAO inactivated 95 % of phenylethylamine, indicating a rapid metabolisation by MAO. It is unclear how these results can be extrapolated to the effect of phenylethylamine in cigarette smoke on the bioavailability of nicotine in the pulmonary system. Phenylethylamine has reinforcing properties comparable to amphetamine. Whether phenylethylamine in cigarettes plays a role to the reinforcing effect of cigarette smoking is unknown. Phenylethylamine, like tryptophan, contains reactive groups and forms reaction products during combustion, which have MAO-I properties. Therefore, phenylethylamine may play a role in the cigarette smoking addiction through the MAO-I effects of its reaction products.

It can be concluded that phenylethylamine level from added cocoa to cigarette is insufficient to exert any physical effect. However, the phenylethylamine level originating from tobacco may increase the addiction to cigarette smoking by its reinforcing properties or by the MAO-I properties of its reaction products.

4.2.7   Tryptamine

Tryptamine occurs naturally in tobacco plant and in cocoa. The estimated tryptamine level in cigarettes originating from tobacco is at least 5000 times higher than from added cocoa. Therefore, it is unlikely that cocoa will affect the addiction to cigarette smoking through its psychoactive compound tryptamine. Because the tryptamine level is relatively high compared with the exposure level through food, it is interesting to investigate the contribution of tryptamine to the addiction to cigarette smoking. There are not enough data on the pulmonary effects of tryptamine through smoking. Although tryptamine does affect the serotonin activity in the brain, it is unknown whether tryptamine plays a role in the tobacco dependency process. Furthermore, tryptamine is a substrate for MAO and will be metabolised rapidly by absorption through the pulmonary system. Tryptamine, like tryptophan and phenylethylamine, contains reactive groups and forms reaction products during combustion, such as beta-carbolines, which has MAO-I properties. Therefore, tryptamine may play a role in the cigarette smoking addiction through the MAO-I effects of its reaction products.

4.2.8   Tyramine

Tyramine is a natural compound of tobacco plant and cocoa. The estimated tyramine level in cigarettes originating from tobacco is at least 2700 times higher than from added cocoa. Therefore, it is unlikely that cocoa will affect the addiction to cigarette smoking through the psychoactive compound tyramine. Because tyramine level in cigarettes (10 mg/25 cigarette) is relatively high compared with the exposure level through food (< 10 mg/day), it is interesting to investigate the contribution of tyramine to cigarette smoking addiction.

Tyramine is an indirectly acting sympathomimetic substance. Tyramine releases noradrenaline from the sympathetic nervous system and leads to physiological reactions, such as increased blood pressure. Any direct effect of tyramine on the addiction to cigarette

smoking is unknown. Tyramine, like tryptophan and tryptamine, contains reactive groups and forms reaction products during combustion, which have MAO-I properties. Therefore, tyramine may play a role in the cigarette smoking addiction through the MAO-I effects of its reaction products.

4.2.9   Octopamine

The level of octopamine in cocoa is unknown and therefore the level in cigarettes could not be calculated.

Octopamine is an endogenous compound in the human body and is metabolised by MAO. When octopamine was perfused in guinea-pig lung, the pulmonary MAO inactivated 35 % of octopamine. The activity data of octopamine and noradrenaline on ß-adrenoreceptors indicate that the activity of octopamine is too low to have any significant physiological effect on the respiratory system. No data are available on possible dependency properties of octopamine.

4.2.10   Anandamide

Anandamide activates cannabinoid receptors in humans. Anandamide seems to control the tonus of the bronchus but compared with the dosis in cigarettes (12.5 ng/25 cigarettes) large doses ($\pm$ 5 mg/kg i.v.) are needed to affect the respiratory system. Therefore, it seems that anandamide will not affect the nicotine bioavailability through a bronchodilatory effect.

Due to recent discovery of anandamide in cocoa, it was suggested that anandamide may attribute to the craving quality of cocoa. However, others have calculated that large quantities of cocoa have to be ingested in order to show cannabimimetic effects (e.g. 25 kg chocolate has to be ingested) (10). It is obvious that the anandamide quantity present in cigarette will not induce such an effect.

Although, it is tempting to link anandamide with craving and the endogenous cannabinoid system, it seems unlikely that anandamide will contribute to the addiction to cigarette smoking.

4.3   Combined effects

In this review we discussed the ten best known pharmacologically active constituents found in cocoa and their effect on the addiction to cigarette smoking. The effect on the addiction to cigarette smoking was evaluated by considering the effect on the pulmonary bioavailability of nicotine and the addictive properties of those compounds.

The body is exposed to the psychoactive compounds via food and drinks or is synthesized by the body itself. The exposure to the psychoactive cocoa compounds via cigarette smoking is negligible compared with the exposure to the psychoactive compounds via food and drinks or compared with the endogenous production of those compounds. Furthermore, the compounds, especially the bioamines, are degraded rapidly when consumed. Some compounds do have addictive properties or affect the activity of compounds in the brain. However, based on the evidence discussed above, it is unlikely that the psychoactive compounds in tobacco originating cocoa exert any systemic pharmacological effects or increase the addiction to cigarette smoking.

Compounds, such as phenylethylamine, tryptamine and tyramine, are naturally occurring in tobacco. The intake of those compounds through cigarette smoking is comparable to or higher than the intake through food. The relatively higher exposure to these compounds via cigarette smoking compared with exposure via food and drinks is mainly attributed by the natural occurrence of these compounds in tobacco rather than by addition of cocoa to cigarettes. Therefore, it is unlikely that cocoa will affect the cigarette smoking addiction via

those compounds. It is not clear, whether the level of the naturally occurring psychoactive compounds in tobacco is high enough to play a role in the addiction to cigarette smoking.

The psychoactive compounds may affect the bioavailability of nicotine by acting on the respiratory system or increasing the permeability through the lung epithelium or increasing the smoke pH.

Several compounds affect the airway resistance in various ways and may have different effects on the nicotine absorption. For example, theobromine and caffeine have similar chemical structures (methylxanthines) and have bronchodilatory effects. Other compounds, such as histamine, have a bronchoconstrictory effect. The evidence in this report indicates that the level of the psychoactive compounds in cigarettes originating from cocoa is too low to exert a net bronchoactive effect.

The local effects of the compounds on the lung epithelium are unknown and therefore can not be evaluated. Furthermore, the effect of these compounds on the smoke pH is also unknown. Because most of the compounds have base properties due to the presence of a primary amine group, those compounds may increase the pH of smoke (tar). However, the level of these compounds in tar is probably negligible compared to the other pH controlling compounds (ammonia) in tar and therefore it is assumed that these compounds will not affect the smoke pH.

An interesting feature is the MAO-I properties of the combustion products of some compounds. The MAO-I properties of the combustion products may attribute to the MAO-I quality of cigarette smoking and may explain a part of the addiction to cigarette smoking.

The discussion in this report was based on short-term exposure to the psychoactive compounds. However, the long-term effect on the respiratory system of these compounds is unknown in combination with other compounds or with its combustion products.

5.   Conclusions and further considerations

Based on the available evidence presented in this study, it can be concluded that the level of the psychoactive cocoa compounds in cigarettes is negligible to exert any local or systemic effects via cigarette smoking. However, the long-term local and systemic effects are not known of these compounds or their combustion products. The combustion products of some psychoactive compounds have MAO-I properties and those combustion products may contribute to the addiction to cigarette smoking.

Although this study discussed the properties of the psychoactive compounds in relation to addiction to cigarette smoking extensively, some topics remained open. For example, the flavour properties of cocoa seem to be a more important parameter for the addiction to chocolate eating than the psychoactive compounds (4). Therefore, the flavour enhancing effect of cocoa in cigarettes may attribute to cigarette smoking addiction. Other topics on the addiction to cigarette smoking which need further investigation are the MAO-I properties of the combustion products, the combined effect of the psychoactive compounds on the nicotine bioavailability via cigarette smoking and the effect of long-term exposure to cocoa on cigarette smoking addiction.

5.1. References

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List of abbreviations

- **CAS registry no.**: Chemical Abstracts Service Registry Number is a numeric designation assigned by the American Chemical Societys Chemical Abstracts Service and uniquely identifies a specific chemical compound. This entry allows one to conclusively identify a material regardless of the name or naming system used.
- **R**: Risk phrases: Warnings on the label about the harmful propertie(s) of the substance.
- **S**: Safety phrases: Directions on the label about the necessary safety precautions to handle the substance. See appendix 1.
- **PA**: proton affinity in the gas phase, kcal/mol
- **FP**: Flash point in °C, which is the minimum temperature at which the vapor pressure of a liquid is sufficient to form an ignitable mixture with air near the surface of the liquid.
- **FL Limits**: Flammable limits (often called explosive limits) in %, which specify the range of concentration of the vapor in air (in percent by volume) for which a flame can propagate. Below the lower flammable limit, the gas mixture is too lean to burn; above the upper flammable limit, the mixture is too rich. Values refer to ambient temperature and pressure and are dependent on the precise test conditions.
- **IT**: Ignition temperature (sometimes called autoignition temperature) in °C, which is the minimum remperature required for self-sustained combustion in the absence of an external ignition source.
- **ADI**: Acceptable Daily Intake.
- **TWA**: Time Weighed Average.
- **MAC**: Maximum Acceptable Concentration.
- **STEL**: Short-term exposure limit for airborne contaminants, which should not be exceeded for more than 15 min. A 'C' following a value indicates a ceiling limit which should not be exceeded even for very brief periods because of acute toxic effects of the substance.
- **LTEL**: Long-Term Exposure Limit (8 hours exposure). Exposure limit: maximum concentration of a chemical agent as time-weighed average of a reference period (8 h/day; 40 h/week) above which no employee may be exposed.
- **TLV-C**: Treshold Limit Value.
- **MAK-reproduction**: Classification of substances on foetal harm according to the German MAK-Werte-Liste.
  - ➢ A = The substance is clearly able to cause foetal harm.
  - ➢ B = Possible risk on foetal harm.
  - ➢ C = In compliance with MAK-value, risk of foetal harm is not to be feared.
  - ➢ D = Foetal toxicity stil unclear. Based on the available information, classification in group A-C is not possible (yet).
- **IARC-category**:
  - ➢ Group 1: The agent is carcinogenic to humans.
  - ➢ Group 2A: The agent is probably carcinogenic to humans.
  - ➢ Group 2B: The agent is possibly carcinogenic to humans.
  - ➢ Group 3: The agent is not classifiable as to its carcinogenicity to humans.
  - ➢ Group 4: The agent is probably not carcinogenic to humans.
- **CEC**:
  - ➢ C = corrosive
  - ➢ E = explosive
  - ➢ F = highly flammable
  - ➢ F+ = extremely flammable

➢ O   = oxidising
➢ T   = toxic
➢ T+   = very toxic
➢ Xi   = irritant
➢ Xn   = harmful

RISK AND SAFETY CLASSIFICATION
Risk classification
- R1   Explosive when dry
- R2   Risk of explosion by shock, friction, fire or other sources of ignition
- R3   Extreme risk of explosion by shock, friction, fire or other source of ignition
- R4   Forms very sensitive explosive metallic compounds
- R5   Heating may cause an explosion
- R6   Explosive with or without contact with air
- R7   May cause fire
- R8   Contact with combustible material may cause fire
- R9   Explosive when mixed with combustible material
- R10 Flammable
- R11 Highly flammable
- R12 Extremely flammable

- R14 Reacts violently with water
- R15 Contact with water liberates extremely flammable gases
- R16 Explosive when mixed with oxidising substances
- R17 Spontaneously flammable in air
- R18 In use, may form flammable/explosive vapour-air mixture
- R19 May form explosive peroxides
- R20 Harmful by inhalation
- R21 Harmful in contact with skin
- R22 Harmful if swallowed
- R23 Toxic by inhalation
- R24 Toxic in contact with skin
- R25 Toxic if swallowed
- R26 Very toxic by inhalation
- R27 Very toxic in contact with skin
- R28   Very toxic if swallowed
- R29   Contact with water liberates toxic gas
- R30 Can become highly flammable in use
- R31   Contact with acids liberates toxic gas
- R32   Contact with acids liberates very toxic gas
- R33   Danger of cumulative effects
- R34 Causes burns
- R35 Causes severe burns
- R36   Irritating to eyes
- R37   Irritating to respiratory system
- R38 Irritating to skin
- R39 Danger of very serious irreversible effects

- R40 Limited evidence of a carcinogenic effect
- R41 Risk of serious damage to eyes
- R42 May cause sensitisation by inhalation
- R43 May cause sensitisation by skin contact
- R44 Risk of explosion if heated under confinement
- R45 May cause cancer
- R46 May cause heritable genetic damage

- R48 Danger of serious damage to health by prolonged exposure
- R49 May cause cancer by inhalation
- R50 Very toxic to aquatic organisms
- R51 Toxic to aquatic organisms
- R52 Harmful to aquatic organisms
- R53 May cause long-term adverse effects in the aquatic environment
- R54 Toxic to flora
- R55 Toxic to fauna
- R56 Toxic to soil organisms
- R57 Toxic to bees
- R58 May cause long-term adverse effects in the environment
- R59 Dangerous for the ozone layer.
- R60 May impair fertility
- R61 May cause harm to the unborn child
- R62 Possible risk of impaired fertility
- R63 Possible risk of harm to the unborn child.
- R64 May cause harm to breastfed babies
- R65 Harmful: may cause lung damage if swallowed
- R66 Repeated exposure may cause skin dryness or cracking
- R67 Vapours may cause drowsiness and dizziness
- R68 Possible risk of irreversible effects

Safety classification
- S1 Keep locked up
- S2 Keep out of the reach of children
- S3 Keep in a cool place
- S4 Keep away from living quarters
- S5 Keep contents under ... (appropriate liquid to be specified by the manufacturer)
- S6 Keep under ... (inert gas to be specified by the manufacturer)
- S7 Keep container tightly closed
- S8 Keep container dry
- S9 Keep container in a well-ventilated place

- S12 Do not keep the container sealed
- S13 Keep away from food, drink and animal feedingstuffs
- S14 Keep away from ... (incompatible materials to be indicated by the manufacturer)
- S15 Keep away from heat
- S16 Keep away from sources of ignition - No smoking
- S17 Keep away from combustible material

- S18   Handle and open container with care

- S20   When using do not eat or drink
- S21   When using do not smoke
- S22   Do not breathe dust
- S23   Do not breathe gas/fumes/vapour/spray (appropriate wording to be specified by the manufacturer)
- S24   Avoid contact with skin
- S25   Avoid contact with eyes
- S26   In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
- S27   Take off immediately all contaminated clothing.
- S28   After contact with skin, wash immediately with plenty of ... (to be specified by the manufacturer).
- S29   Do not empty into drains
- S30   Never add water to this product

- S33   Take precautionary measures against static discharges

- S35   This material and its container must be disposed of in a safe way.
- S36   Wear suitable protective clothing
- S37   Wear suitable gloves
- S38   In case of insufficient ventilation, wear suitable respiratory equipment
- S39   Wear eye/face protection
- S40   To clean the floor and all objects contaminated by this material use ... (to be specified by the manufacturer)
- S41   In case of fire and/or explosion do not breathe fumes
- S42   During fumigation/spraying wear suitable respiratory equipment (appropriate wording to be specified by the manufacturer)
- S43   In case of fire use ... (indicate in the space the precise type of fire-fighting equipment. If water increases the risk add: Never use water).

- S45   In case of accident or if you feel unwell seek medical advice immediately (show the label where possible).
- S46   If swallowed, seek medical advice immediately and show this container or label.
- S47   Keep at temperature not exceeding ... °C (to be specified by the manufacturer).
- S48   Keep wetted with .... (appropriate material to be specified by the manufacturer).
- S49   Keep only in the original container.
- S50   Do not mix with ... (to be specified by the manufacturer)
- S51   Use only in well-ventilated areas
- S52   Not recommended for interior use on large surface areas
- S53   Avoid exposure - Obtain special instructions before use

- S56   Dispose of this material and its container to hazardous or special waste collection point.
- S57   Use appropriate containment to avoid environmental contamination

- S59   Refer to manufacturer for information on recovery/recycling

- S60   This material and its container must be disposed of as hazardous waste
- S61   Avoid release to the environment. Refer to special instructions/Safety data sheet
- S62   If swallowed, do not induce vomiting: seek medical advice immediately and show this container or label.
- S63   In case of accident by inhalation: remove casualty to fresh air and keep at rest.
- S64   If swallowed, rinse mouth with water, (only if the person is conscious).