Scientific Committee on Emerging and Newly Identified Health Risks

SCENIHR

Addictiveness and Attractiveness of Tobacco Additives

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ABSTRACT

The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) has been asked to evaluate the role of tobacco additives in the addictiveness and attractiveness of tobacco products.

The criteria for dependence established in humans indicate that tobacco has a high addictive potential, but it remains difficult to assess the addictiveness of individual additives. In animal studies the addictive potency of the final tobacco product cannot be assessed. The reinforcing potency of drugs is measured after intravenous injections and suggests that the addictive potential of pure nicotine is weak. The currently used methods to define addictiveness of nicotine and additives are thus not considered adequate.

In humans, the positive correlation between tobacco consumption and dependence suggests that individuals with high nicotine levels in their blood are more dependent. In animal studies using self-administration, an inverted U-shaped dose-response curve has generally been revealed suggesting that the addictiveness of nicotine is not directly linear with the dose. There is however substantial variation in the response to nicotine in both animals and humans, and genetic factors probably play an important role.

No tobacco additives, which are addictive by themselves, have so far been identified. However, sugars, which are present in high quantities in most tobacco products, give rise to acetaldehyde in tobacco smoke. Acetaldehyde given intravenously is addictive and enhances the addictiveness of nicotine in experimental animals. Additives that facilitate deeper inhalation (e.g. menthol) or inhibit the metabolism of nicotine may enhance the addictiveness of nicotine indirectly. Substances such as ammonia that increase the pH of the tobacco and the smoke, result in higher amounts of uncharged nicotine. However, it is uncertain if more nicotine is absorbed with higher smoke pH. For smokeless tobacco it seems that an increased pH enhances nicotine absorption in the mouth.

The methods used to quantify the addictive potency of additives have limitations because of technical challenges in experimentally manipulating the presence or absence of an additive in a tobacco product. Such experiments require large technical and financial resources. In addition, there are ethical issues if testing in humans is considered. Due to these limitations, the available methodologies are not considered adequate.

A number of technical characteristics of cigarettes (ventilation, packing, geometry) influence the content of different substances in the smoke and the size of smoke particles. Many smokers compensate for a lower dose of nicotine by increasing puff volume and frequency, and by deeper inhalation. The particle size of the smoke aerosol does not seem to substantially influence the exposure to nicotine.

Attractiveness is defined as the stimulation to use a product. The attractiveness of tobacco products may be increased by a number of additives but is also influenced by external factors such as marketing, price etc. Animal models do not currently exist for the assessment of attractiveness. In humans, the attractiveness of individual tobacco products may be compared in panel studies, surveys, and by experimental measures. Another method is to experimentally adjust tobacco products to exclude or include individual additives and test responses to them. However, this type of research is difficult nowadays due to ethical considerations that will usually preclude human testing.

The use of fruit and candy flavours seems to favour smoking initiation in young people. Menthol also attracts a number of smokers (in particular African Americans). Some additives decrease the harshness and increase the smoothness of the smoke. Certain additives yield a full and white smoke and other additives reduce the lingering odour of the smoke in order to favour the acceptability of smoking to people around.

Additives considered attractive may in principle lead to brand preference or a higher consumption of tobacco products. However, it remains difficult to distinguish the direct effects of these additives from indirect effects such as the marketing towards specific groups.

Keywords: addictiveness, additives, attractiveness, cigarettes, cigars, nicotine, SCENIHR, smokeless tobacco, smoking, target groups, tobacco, waterpipe

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EXECUTIVE SUMMARY

The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) has been asked to evaluate the role of tobacco additives in the addictiveness and attractiveness of tobacco products. A summary of the answers are presented below.

1. Criteria which will define whether an additive or a combination of additives increases the addictive potency of the final tobacco product

In human studies there are clinical criteria for dependence, laboratory measures of self-administration, as well as preference studies. These criteria indicate that tobacco in humans has a high addictive potential, but they have limitations when assessing the addictiveness of individual additives in the final tobacco product. There is no widely-agreed universal standard for human studies and as a result various possible endpoints exist. In addicted individuals a modified regulation of neural networks exists, and the potential to induce such modifications should be the criteria used to define the addictive potency of a product.

In animal studies the reinforcing potency of a drug is used as a criterion for the addictive potential. However, self-administration studies indicate that pure nicotine could have a weak addictive potential. An evaluation of the role of additives has not yet been done in animals.

2. Methods currently used for assessing the addictive potency of a substance

Many different methods are used in humans, but there is a lack of consistency between them. Human studies have limitations in design (e.g. the use of conditioned cues, and the need to work with smokers). Furthermore, ethical issues may arise when testing substances in humans.

There is currently no animal model to assess the addictive potency of the final tobacco product; however, pure nicotine has been studied extensively. The experimental animal models are mainly based on self-administration in rodents, usually rats. The evaluation of addictiveness is based on the re-inforcing properties of the drug. However, there is no consensus on the predictive validity for the addictiveness of tobacco products in humans. In animal studies pure nicotine is injected intravenously and shows only a weak addictive potential whereas in humans, tobacco is used differently (e.g. inhalation, oral consumption) and is highly addictive. No method currently used to define addictive potency of a compound can therefore be considered as adequate.

3. Dose-dependency of development of nicotine addictiveness

In humans, there are little data available on pure nicotine use. However, tobacco consumption (e.g. number of cigarettes smoked per day) is positively correlated with dependence. This suggests that individuals who maintain higher nicotine levels in their blood are more dependent than individuals who maintain low levels.

In animal studies, an inverted U-shaped dose-response curve has generally been revealed suggesting that the addictiveness of nicotine is not directly linear with the dose. As mentioned before, pure nicotine is only weakly addictive in animal studies.

There is substantial variation in the response to nicotine and its addictive potential in both animals and humans, and genetic factors probably play an important role.

4. Additives in tobacco products that are addictive by themselves

No tobacco additives, which are addictive by themselves, have so far been identified. However, sugars which are added in high quantities to most tobacco products, give rise to acetaldehyde in tobacco smoke and acetaldehyde given intravenously is self-administered by animals and thus may be considered addictive.

Experiments using denicotinised cigarettes show that besides nicotine, other factors in cigarette smoke probably play an important role in craving and reinforcement. Although these unknown factors do not have pharmacologic effects similar to nicotine and are probably not addictive, they definitely play a role in smoking behaviour

5. Additives that enhance the addictiveness of nicotine

Sugars or their derivatives produce numerous substances upon heating. One of these is acetaldehyde, which enhances the addictiveness of nicotine when injected into experimental animals, probably by inhibiting monoamine oxidase (MAO) in the brain. Smokers have decreased levels of MAO in the brain. However, there is no proof that acetaldehyde in the smoke contributes significantly to blood levels of acetaldehyde. On the other hand, acetaldehyde generates in the smoke the compounds harman and norharman which may also inhibit MAO.

Additives that facilitate deeper inhalation (e.g. menthol) may enhance the addictiveness of nicotine indirectly. Other substances may enhance the addictiveness of nicotine by inhibiting its metabolism. Substances such as ammonia that increase the pH of the tobacco (and the smoke) result in higher amounts of uncharged nicotine that is more easily absorbed by the cells. However, due to the high buffer capacity of the lining fluid in the lungs it is uncertain if more nicotine is absorbed with higher smoke pH. For smokeless tobacco it has been shown that more nicotine is absorbed in the mouth when the pH of the product is increased.

6. Methods to quantify the potency of additives in enhancing the addictiveness of nicotine

The methods used to quantify the potency of additives in enhancing the addictiveness of nicotine or tobacco products are described above. The limitations of these methods arise from technical challenges in experimentally manipulating the presence or absence of an additive in the tobacco products used in these experiments. Such experiments have probably been carried out by the tobacco industry for some additives, especially sugars and their derivatives, but they require large technical and financial resources. In addition, there are ethical issues if testing in humans is considered. Because of these limitations, the available methodologies are not considered adequate.

7. Technical characteristics that enhance the addictive potential of tobacco products

A number of technical characteristics of cigarettes influence the content of different substances in the smoke and the size of smoke particles. The so-called TNCO values (tar, nicotine and carbon monoxide (CO)) are determined by, amongst other things, ventilation (paper, filter), the packing of the tobacco and the geometry of the cigarettes. Many smokers compensate for a lower dose of nicotine by increasing puff volume and frequency, and by deeper inhalation. Based on the limited publicly available information, it seems that exposure to nicotine cannot be substantially increased by altering the particle size of the smoke aerosol.

8. Criteria for considering an additive or a combination of additives as attractive

The criterion for attractiveness is the stimulation to use the product. Attractiveness of additives refers to factors such as taste, smell and other sensory attributes. In addition, a number of external factors (e.g. ease of use, flexibility of the dosing system, cost etc.) contribute to the attractiveness of the product.

The attractiveness of tobacco products may be increased by a number of additives that create a specific taste/flavour in order to attract certain target groups. An attractive effect may be obtained by changing the appearance of the product and the smoke, decreasing the harshness of the smoke, and inducing a pleasant experience of smoking.

In order to make smoking more acceptable to other people nearby, some additives reduce lingering odour or side-stream smoke visibility.

9. Methods currently used for assessing attractiveness

Animal models do not currently exist for the assessment of attractiveness.

In humans, the attractiveness of individual tobacco products may be compared with other tobacco products by panel studies and surveys, and by experimental measures. When examining what is known about the additive content of these products, judgements can be made as to the role of individual additives in the overall attractiveness of the product.

Another method is to experimentally adjust tobacco products to include or exclude individual additives and test responses to them. In addition, the quantity of the additive can be varied to assess dose response and whether there is a threshold below which any impact is not observed.

However, this type of research is difficult nowadays due to ethical considerations that will usually preclude human testing of different tobacco products, particularly among non-users or children. The methods currently used are thus not adequate.

10. Additives that increase attractiveness of tobacco products

Numerous additives are used in order to increase the attractiveness of tobacco products but it is very difficult to identify the role of individual additives in enhancing attractiveness.

Various sugars constitute a large proportion of additives, and the sweetness of the product is an important characteristic. The use of fruit and candy flavours in high amounts seems to favour smoking initiation by young people. Menthol also attracts a number of smokers (in particular African Americans) maybe due to its action on sensory nerve endings, resulting in a cooling effect.

Some additives decrease the harshness and increase the smoothness of the smoke. The harshness depends partly on the tar/nicotine ratio, but may also be decreased by additives such as propylene glycol and glycyrrhizin, a substance in liquorice.

Certain additives yield a full and white smoke (e.g. magnesium oxide, magnesium carbonate, sodium acetate, sodium citrate, calcium carbonate). Other additives reduce the lingering odour of the smoke in order to favour the acceptability of smoking to people around (e.g. acetylpyrazine, anethole, limonene, vanillin, and benzaldehyde).

In several countries there is a growing trend of using "natural" tobacco products advertised as containing no additives.

11. Association between additives and tobacco consumption – target groups

Additives considered attractive may in principle lead to brand preference or a higher consumption of tobacco products although it is difficult to distinguish the direct effects of these additives from indirect effects such as marketing towards specific groups. In the USA, the consumption of menthol cigarettes is relatively high among African Americans. Cigarettes with certain flavours (e.g. fruit, candy) appear to be developed to target young people.

Additives and design characteristics may modify consumption patterns. However, in spite of the many additives commonly used, tobacco products overtly marketed as containing additives (e.g. menthol cigarettes) command a relatively small market share in EU countries and there is presently a trend in several countries to use products labelled "without additives".

It is notable that waterpipe smoking is becoming increasingly popular in some EU countries (and elsewhere), potentially due to the flavoured tobaccos used and the mild/cool smoke that may facilitate the inhalation of large volumes into the lungs. Smokeless tobacco products have gained increased interest from the industry because they may be used in places where smoking is prohibited.

1. BACKGROUND

Some 72-92% of adult cigarette smokers meet the criteria for dependence[1]. While nicotine is recognised as an addictive substance in the tobacco leaf, the risk of addiction to pure nicotine products is very low compared to cigarettes[1]. Currently, it is being discussed in the public health community whether lowering the levels of nicotine in tobacco products would make people less addicted and accordingly reduce the consumption of tobacco products.

Tobacco additives were hardly used before 1970, but today they represent up to 10% of the cigarette weight. By altering the taste and smell of cigarettes the products are made more attractive and the smoke more palatable which leads to an increase of smoking initiation. At present, the role of additives in enhancing the addictiveness of tobacco products is not clear.

In order to make tobacco products more attractive, design features are introduced, e.g. package design and cigarette form. In addition, these features are used to undermine the effect of the maximum limits set by the Tobacco Products Directive 2001/37/EC on tar, nicotine, and carbon monoxide (CO) yields in cigarettes.

Legal background

Article 13 of the Tobacco Products Directive (2001/37/EC)[2] stipulates that Member States can keep or introduce, in accordance with the Treaty, more stringent rules concerning the manufacture, import, sale, and consumption of tobacco products which they deem necessary in order to protect public health. Member States may prohibit the use of ingredients which have the effect of increasing the addictive properties of tobacco products.

Article 12 of the Tobacco Products Directive invites the Commission to submit a proposal providing a common list of ingredients authorised for tobacco products, taking into account, *inter alia*, their addictiveness.

In its comments to the Green Paper *Towards a Europe free from tobacco smoke: policy options at EU level*[3], the European Parliament invited the Commission to propose, by 2008 if possible, an amendment to the Directive including an evaluation and authorisation procedure for tobacco additives and an immediate ban on all additives that are addiction-enhancing[4]. In its 2nd Report on the implementation of the Tobacco Products Directive[5] the Commission stresses the need for further work on the addictiveness of tobacco additives.

DG SANCO wishes to have a better understanding of the criteria based on which an additive can be considered (classified) as an addictive and/or attractive substance, the role of additives in tobacco products and the role of design features in the attractiveness and addictiveness of a tobacco product.

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[1] Henningfield JE, Zeller M. Could science-based regulation make tobacco products less addictive? Yale J Health Policy Law Ethics 2002; 3:127-38.

[2] http://eur-lex.europa.eu/pri/en/oj/dat/2001/l_194/l_19420010718en00260034.pdf

[3] http://ec.europa.eu/health/ph_determinants/life_style/Tobacco/Documents/gp_smoke_en.pdf
plus report on consultation:
http://ec.europa.eu/health/ph_determinants/life_style/Tobacco/Documents/smoke_free_frep_en.pdf

[4] http://www.europarl.europa.eu/sides/getDoc.do?pubRef=-//EP//NONSGML+REPORT+A6-2007-0336+0+DOC+PDF+V0//EN

[5] http://ec.europa.eu/health/ph_determinants/life_style/Tobacco/Documents/tobacco_products_en.pdf

2. TERMS OF REFERENCE

In the light of the most recent scientific information, the Scientific Committee is requested to answer the following questions:

1. Which are the criteria which will define whether an additive or a combination of additives increases the addictive potency of the final tobacco product?
2. What are the methods currently used for assessing the addictive potency of a substance and are they considered adequate?
3. Is the development of nicotine addictiveness dose-dependent?
4. Which additives are addictive themselves in tobacco products?
5. Which additives enhance the addictiveness of nicotine and how?
6. Which are the methods used to quantify the potency of additives in enhancing the addictiveness of nicotine and are they considered adequate?
7. Which technical characteristics enhance the addictive potential of tobacco products?
8. Which are the criteria based on which an additive or a combination of additives can be considered (classified) attractive?
9. What are the methods currently used for assessing attractiveness and are they considered adequate?
10. Which additives increase attractiveness of tobacco products?
11. What is the association between additives and tobacco consumption (independent of any addictive potential they might have)? Which additives are used to target specific groups?

3. SCIENTIFIC RATIONALE

3.1. Introduction

According to a report from WHO (2008), about 100 million people died in the 20th century from tobacco use. The number of deaths in 2007 due to tobacco related diseases was about 5.4 million and if current smoking patterns continue, more than 8 million deaths are expected to occur each year due to tobacco smoking by the year 2030. In the EU, about a third of the adult population are smokers. The number of deaths from smoking per year is currently about 500,000 in the EU and more than 1.5 million in the whole European region (WHO 2007a). The vast majority of smokers use cigarettes, while other ways of smoking are less frequent (e.g. cigars, pipes, waterpipes). Apart from smoking tobacco, other tobacco forms (i.e. smokeless tobacco) may also have deleterious public health effects (SCENIHR 2008). In addition, exposure to tobacco smoke in the environment, so-called "passive smoking" or "second-hand smoking", is an important cause of excess mortality and morbidity. Passive smokers have a significantly increased risk for several diseases such as lung cancer (IARC 2004), respiratory diseases (Jaakkola and Jaakkola 2002a, Jaakkola and Jaakkola 2002b) and cardiovascular diseases (Whincup et al. 2004).

The addictiveness of nicotine is enforced by substances in tobacco leaves that inhibit the action of monoamine oxidase (MAO) in the body (Berlin and Anthenelli 2001). Apart from naturally occurring substances in tobacco leaves, a number of ingredients in the final product may create or increase dependence. The tobacco industry has admitted the use of 599 different cigarette additives in the United States (US), which are claimed to improve taste and reduce harshness of the smoke (Rabinoff et al. 2007). Current US-style cigarettes contain about 10% of additives by weight; mainly sugars, humectants, cocoa and liquorice. Most other additives are used in small amounts. As discussed later in this opinion, cigars, pipe tobacco and smokeless tobacco generally contain fewer additives than cigarettes. Tobacco used in water pipes is characterised by a high content of water and various sugars.

Certain flavours (e.g. candy and fruit) have been used largely to make tobacco products more appealing to children (called "young adults" by the tobacco industry). In order to decrease the appeal of cigarettes to children, the US Food and Drug Administration (FDA) banned the use of a number of flavours as additives in cigarettes in September 2009 (http://www.fda.gov/TobaccoProducts/GuidanceComplianceRegulatoryInformation/FlavoredTobacco/default.htm). Menthol is not one of the banned additives, but is currently being evaluated by the Tobacco Products Scientific Advisory Committee of the FDA. In other parts of the world (e.g. Canada, Australia, New Zealand), legal measures on additives are established or are in preparation. In Europe, some countries, such as Germany, United Kingdom, Austria, Romania and France, use positive and/or negative lists which respectively allow or prohibit the use of specific compounds as tobacco additives, whereas other countries do not have such a regulation.

It is the purpose of the present opinion to examine the criteria for classifying tobacco additives as addictive or attractive, and to evaluate their role for the creation or maintenance of dependence on tobacco products. This would serve as the scientific basis for regulation of the use of additives in order to reduce the toxicity and the addictiveness of the final tobacco product. An important question is whether some additives are addictive by themselves or if they act by increasing the addictiveness of nicotine. The different methods of assessing addictiveness of an additive, alone or in combination with other substances, will be reviewed. In addition to the interactions between additives and constituents of tobacco, the burning of tobacco creates other complex chemical substances that may be toxic or favour addiction. An example of this is acetaldehyde, formed by the pyrolysis of various sugars in the tobacco (see section 3.8.1.4.). The technical characteristics of tobacco products, in particular of cigarettes, may also influence their addictive potential. A number of additives favour attractiveness of tobacco products, and may thus promote smoking initiation. In this context special attention will be paid to how additives may be used to target specific groups.

3.2. Methodology

A public call for information[6] was launched in November 2009, giving all stakeholders the opportunity to submit relevant scientific information concerning tobacco additives. The information asked for concerned: 1) details about the manufacturing process of tobacco products; and 2) methods applicable for assessment of attractiveness. A number of organisations and major tobacco companies responded. The information received has been evaluated carefully and was in many cases useful for writing the opinion. A particular problem in the area of tobacco products is that a number of studies relevant for this opinion have never been published but exist as internal documents of the tobacco industry. Some of the documents contain sensitive information showing health risks associated with smoking. In 1992, 60 documents were destroyed by Imperial Tobacco Canada in order to avoid exposure of the company to liability or embarrassment. Hammond et al. (2009a) have recently reviewed the contents of these documents that were recovered at the British American Tobacco headquarters in the United Kingdom and were released in 1998 through court disclosure in a trial in Minnesota. The author concludes that most of the studies that were carried out by researchers employed by the industry were scientifically valid. They gave evidence that cigarette smoke was carcinogenic and addictive. Since then, a great number of industry documents have become publicly available and can be found in two searchable databases, http://tobaccodocuments.org and http://legacy.library.ucsf.edu. The collections continue to be updated and currently contain more than 60 million pages in over 11 million documents.

Furthermore, a tobacco documents bibliography is also available which includes papers and publications based on documented research, broadly classified into several groups. Some examples of publications based on research of industry documents appearing under the heading of "Ingredients and Design" illustrate the tobacco industry research and development strategy on issues including: smoker preferences (Chaiton et al. 2005); smoking behaviour and product design (Hammond et al. 2006); targeting consumer groups with specific psychological needs (Cook et al. 2003); research on nicotine (Hurt and Robertson 1998); addictiveness (Scharfstein 1999, Slade et al. 1995, Stevenson and Proctor 2008, Vagg and Chapman 2005); manipulation/free base nicotine (Wayne et al. 2006, Wayne and Carpenter 2009); flavoured cigarettes (Lewis and Wackowski 2006); menthol (Kreslake et al 2008a, Wayne and Connolly 2004); youth targeting (Wayne and Connolly 2002); and particle size (Wayne et al. 2008a). Relevant publications are discussed in subsequent chapters of this opinion.

For the purpose of the present opinion, the health risks of tobacco products and additives have been investigated within different lines of evidence such as epidemiological studies, experimental studies in humans, experimental studies in animals, cell culture studies and in silico studies. To answer the questions in the Terms of Reference to this opinion, a weighted approach has been used, where data from all the available lines of evidence were integrated as appropriate. A more detailed description of how such weighting is performed is given in an earlier opinion of the SCENIHR (SCENIHR 2009). The primary sources for this opinion have been original scientific reports that are published in peer-reviewed scientific journals. In addition, the secondary sources used were the stakeholder information mentioned above and reports and opinions of other scientific committees as well as reports of various governmental bodies. In addition to the reports cited in the text and included in the list of references, various publications were noted but not considered appropriate for the purposes of developing the opinion.

[6] http://ec.europa.eu/health/ph_risk/committees/04_scenihr/scenihr_call_info_08_en.htm

3.3. Definitions

A number of terms related to tobacco products are explained below. For the list of abbreviations, see chapter 6. A full glossary can be found in chapter 8.

3.3.1. Technical characteristics

A wide variety of tobacco products are available worldwide such as cigarettes, cigars, pipe tobaccos, smokeless tobacco products etc. Each of these types is produced by using different tobaccos and additives and by using different manufacturing practices (Reviewed in IARC Monographs: 1985; 1986; 2004; and 2007).

Cigarette: The most common form of tobacco is the manufactured cigarette. Cigarettes are made from fine-cut tobacco leaves and are wrapped in paper or other non-tobacco material, filter–tipped or untipped, approximately 8 mm in diameter and 70-120 mm in length. Cigarettes are highly engineered, exquisitely designed "nicotine delivery devices". Design features encompass a wide range of design variables such as tobacco type and blend, chemical processing and additives, and in addition, physical features such as paper, filter and ventilation. It is also important to consider factors such as tobacco weight or density, and cigarette geometry (circumference and length). Cigarette additives have a range of purposes; e.g. to facilitate manufacture, increase shelf life, control burn rates, nicotine delivery, flavour and harshness/irritation etc. The physical design characteristics of the tobacco product interact with its chemical composition to influence its function and effect (WHO 2001). For example, the size of the cuttings of the tobacco in cigarettes and non-combusted and non-heated tobacco, and its level of acidity (measured as pH), interact to influence the release of nicotine from the product (Callicutt et al. 2006, Stevenson and Proctor 2008). Cigarette ventilation designs also modify free nicotine levels in the smoke. Similarly, the physical and chemical characteristics of cigarettes interact to alter the size distribution of the aerosol particles that convey nicotine and other chemicals, and thus influence absorption (WHO 2007b).

Roll your own (RYO) tobacco denotes any tobacco product which, because of its appearance, type, packaging, or labelling, is suitable for use and likely to be offered to, or purchased by, consumers as tobacco for making cigarettes. RYO cigarettes are cheaper substitutes for commercially manufactured brands and have gained popularity worldwide.

A cigar is a roll of tobacco wrapped in leaf tobacco or any other substance containing tobacco. There are four main types of cigars: little cigars, small cigars ("cigarillos"), regular cigars and premium cigars. Little cigars contain air-cured and fermented tobacco and are wrapped either in reconstituted tobacco or in cigarette paper that contains tobacco and/or tobacco extract. Some little cigars have cellulose acetate filter tips and are shaped like cigarettes. Cigarillos are small, narrow cigars with no cigarette paper or acetate filter. Regular and premium cigars are available in various shapes and sizes and are rolled to a tip at one end.

Pipe tobacco can be a blend of as many as 20-25 different tobaccos, or made of Burley varieties only. Some pipe tobaccos contain midrib tissues, and casings and sauces are frequently added.

A water pipe is one of the ancient forms of tobacco use. Cut or shredded tobacco is smouldered inside the head, which is covered by a perforated aluminium foil on which the glowing charcoal is placed. The smoke is drawn through a tube inside the water pipe, filtered through water in a container and reaches the smokers' mouth via a long flexible tube. A great variety of tobaccos, or mixture of tobaccos with additives, is used in such pipes.

Smokeless tobacco is consumed without burning the product, and can be used orally or nasally. It comes in two main forms: snuff (finely ground or cut tobacco leaves that can be dry or moist, loose or portion packed in sachets, and administered to the mouth, or the dry products to the nose or mouth); and chewing tobacco (loose leaf, in pouches of tobacco leaves, "plug" or "twist" form). According to the Tobacco Products Directive (2001/37/EC) chewing tobacco is not included in the definition of "tobacco for oral use", the sale of which is banned in all EU countries except Sweden. Swedish-type moist snuff (snus) consists of finely ground dry tobacco (Kentucky and Virginia tobacco), mixed with aromatic substances, salts (sodium chloride), water, humidifying agents and chemical buffering agents (sodium carbonate). The large variety of smokeless tobacco products available worldwide has been described in detail elsewhere (SCENIHR 2008).

Electronic cigarettes, or e-cigarettes, are battery-powered devices that vaporise nicotine, flavouring, and other chemicals into an inhalable vapour (Pauly et al. 2007). Chemical analyses have detected tobacco-associated chemicals that may be harmful to humans, including known human carcinogens (Kuehn 2009). E-cigarettes have been marketed recently for a range of uses, including, as a cessation aid and as an alternative to cigarettes in smoke-free zones. The different brands vary greatly in content of nicotine and other chemicals, but the health risks or efficacy as cessation aids have not yet been sufficiently documented (Bullen et al. 2010).

3.3.2. Contents, ingredients, and additives

According to the terminology used in the WHO Framework Convention and the recommendation by the Scientific Advisory Committee in 2003, the term "contents" is used synonymously with the term "ingredients". Consequently, it means all product components, the materials used to manufacture those components, residual substances from agricultural practices, storage and processing, substances that can migrate from packaging into the product, as well as what may be termed "additives" and "processing aids" in some countries and regions (WHO 2007b).

Based on the 2nd Report on the Application of the Tobacco Products Directive (EC 2007b), the current definition of "ingredients" in Article 2 (5) covers any substance or constituent used in the manufacture or preparation of a tobacco product and still present in the finished product even if in an altered form, including paper, filter, inks and adhesives. It does not cover the tobacco leaf itself or other natural or unprocessed tobacco plant parts.

For the purpose of this report, we consider that the WHO definition is the most useful, as some of the added ingredients (e.g. different forms of sugar) are already present in the tobacco leaves. Tobacco leaves may also in some cases contain various toxic substances such as cadmium or radioactive isotopes. The possible presence of residual substances from agricultural practices will not be addressed in this report.

In order to avoid misunderstandings, the present report uses the term additives for added ingredients or substances. Additives are defined as any substance that is added, except water, during the course of manufacture of a tobacco product, including preservatives, humectants, flavours, and processing aids.

Natural or clean cigarettes are being marketed as having no chemicals or additives and the filters are made from natural cellulose. However, smoke from these cigarettes still contains all the carcinogens and toxins that come from the tobacco itself (Malson et al. 2002, McDaniel and Malone 2007).

Herbal cigarettes, although they may not contain tobacco, yield tar and carbon monoxide when smoked, and are thus also dangerous to health (Chen et al. 2007a, Gan et al. 2009).

3.3.3. Addiction and addictiveness

Addiction is the commonly used term referring to what is technically known as "dependence" and is widely employed to connote severe substance dependence, as has been demonstrated to occur in tobacco users. Dependence has been defined by the WHO

Expert Committee on Drug Dependence (WHO 2003) and The ICD-10 Classification of Mental and Behavioural Disorders: Clinical Descriptions and Diagnostic Guidelines (WHO 1992).

Addictiveness refers to the pharmacological potential of a substance to cause addiction. Abuse liability of a drug is the likelihood that its use will result in addiction (dependence) and it can be assessed in laboratories by methods referred to as abuse liability testing. (Schuster and Henningfield 2003, Wayne and Henningfeld 2008b, WHO 2003).

The terms "dependence-causing" and "dependence potential" have been used as synonyms for "addictive" and "addictiveness", respectively. In addition to the neurobiological characteristics of the substance itself, dependence potential is related to the dose, speed of absorption, metabolism, and to physical and chemical features of the formulation (WHO 2007b).

3.3.4. Attractiveness

According to the WHO, the terms "attractiveness" or "consumer appeal" refer to factors such as taste, smell and other sensory attributes, ease of use, flexibility of the dosing system, cost, reputation or image, assumed risks and benefits, and other characteristics of a product designed to stimulate use (WHO 2007b). Physical product characteristics are often integrated with marketing (WHO 2007b). For example, a flavour such as "menthol", "mint", or "cherry", which is intended to appeal to a target population, may be incorporated into the product name or descriptors and marketed to reach out to that population (WHO 2007b). Attractiveness is also related to nicotine dosing characteristics, which is why smokeless tobacco product companies may include products ranging from lower dosing and slower onsetting "starter" products to higher dose maintenance products (FDA 1995, FDA 1996).

Although the risk of dependence on any substance is partially related to the attractiveness and/or ease of use of the delivery system, these features are not typically evaluated in dependence-potential testing but rather are generally described as factors affecting "consumer appeal" or "attractiveness". Addictiveness and attractiveness go hand in hand as the real world liability for abuse of and addiction to a tobacco product is to a large extent also related to the attractiveness of the tobacco product.

Attractiveness is powerfully determined by imagery and cultural associations that are cultivated by the tobacco industry and effects may therefore be indirect. Attractiveness is also influenced by product sensory characteristics using flavours, and product characteristics (as well as marketing) that are intended to reduce concerns or undesirable features (e.g. reduce concerns about cancer with "light" branding, and reduce noxious throat burn with various chemicals and "smoke smoothers") (Wayne and Henningfield 2008b).

3.4. Tobacco - manufacturing process

The manufacturing process for cigarettes has been described in several publications (Davis and Nielsen 2006, Hoffmann and Hoffmann 1997, IARC 2004, Wigand 2006). However, while the exact composition of each brand remains a trade secret, according to the Tobacco Products Directive (2001/37/EC) tobacco industries have to report the full list of additives in tobacco products, including the exact amount, to the competent authorities in the Member States.

Both the make-up of cigarettes and the composition of cigarette smoke have gradually changed in the last 50-60 years, including the use of a larger range of additives. The sales-weighted average "tar" and nicotine yields have declined. These changes have been primarily achieved by the introduction of filter tips, with and without perforation, selection of tobacco types and varieties, utilization of highly porous cigarette paper, and incorporation into the tobacco blend of reconstituted tobacco, opened and cut ribs, and "expanded tobacco" together with the use of a large number of additives/ingredients. At least four of the physical parameters of cigarettes have a decisive influence on smoke yields. These are the length of a cigarette, its circumference, the cut of the tobacco, and the packing density (Hoffmann and Hoffmann 1997). Agronomic factors such as production practices and soil characteristics, and environmental conditions such as rainfall, reportedly influence the accumulation of metals, including cadmium, beryllium, chromium, nickel and arsenic in the leaf.

Commercial tobacco products are predominantly produced from *Nicotiana tabacum*, while *Nicotiana rustica* is used on a limited commercial scale. Within the species *N. tabacum* one distinguishes four types: bright (Virginia), Burley, Maryland, and Turkish tobaccos. Bright tobacco is flue-cured by drying with artificial heat; Burley and Maryland tobaccos are air-cured; Turkish tobaccos are sun-cured. The properties of tobacco are based primarily on curing methods, locality of growth, position on the stalk from which the leaves have originated and factors such as colour quality and ripeness at harvest. Curing is the process for drying freshly harvested tobacco with partially or fully controlled temperature and moisture schedules. Freshly cured leaf is then threshed to separate stem from lamina, sometimes blended with other tobacco lamina and then re-dried to a uniform moisture level then packed into bales or hogsheads.

Virginia tobacco leaves contain a higher carbohydrate (e.g. sugars) level and lower nitrogen level than Burley leaves. The natural drying of the Burley leaves at relatively low temperatures allows plant respiration which continues to consume sugars during the process, leaving negligible sucrose and reducing sugars in the cured leaf. Burley leaves contain higher levels of nitrogen than Virginia leaves. The smoke of Virginia or flue-cured leaves is more aromatic and less alkaline than that of Burley tobacco, with a slight acidic taste resulting from the high levels of natural sugars. Burley tobacco produces a more alkaline smoke than flue-cured tobacco (Weeks 1999) and therefore imparts a bitter aroma and taste to cigarettes. Oriental leaves tend to have a low nitrogen content and moderate levels of carbohydrates, but fewer proteins, than the other varieties (Philip Morris 2010, Wolfe 1962).

A comprehensive integrated pest management programme is used to avoid insect infestation, e.g. chemical fumigation. The tobacco then undergoes aging and fermentation, usually for 1-3 years.

For the manufacture of cigarettes, specific tobacco blends utilizing desired tobacco types are prepared. Blending is the selection and thorough mixing of the tobacco-based components plus any associated casings, humectants and flavouring required for a particular product or brand. The tobacco based components may include the leaf lamina, cut and rolled stem, reconstituted sheet and expanded tobacco.

The tobaccos stored in bales are broken up, cut into specific dimensions, and combined with other blend components such as casing and top dressing, and adjustment of the moisture content. American blend cigarettes contain the four types of tobacco mentioned above plus reconstituted or homogenized sheet tobacco. This is made from tobacco dust, fines and particles, and leaf ribs and stems (IARC 2004). Reconstituted tobacco or homogenized sheet tobacco is a paper-like sheet approaching the thickness of tobacco laminae. It is made from tobacco dust, fines, and particles, and from ribs and stems; various additives may be incorporated. In the past, most of these "tobacco by-products" were wasted. The introduction of reconstituted tobacco or RECON, is the primary means by which ammonia chemistry and other chemicals are introduced into a cigarette. Expansion is a process which increases the shred filling power, e.g. puffed tobacco. Puffed, expanded, and freeze-dried tobaccos are modified preparations of cigarette tobacco and have up to twice the filling power, thus requiring less tobacco per cigarette. The principle applied here is to expand the tobacco cell walls by quick evaporation of water and other agents that readily volatilize.

Blending is carried out to achieve specific pH, taste, burning characteristics, and nicotine content. The pH strongly influences the concentration of free (i.e. non-protonated) nicotine in tobacco smoke, whereas the nitrate content influences the carcinogenic potential of smoke (IARC 2004).

Table 1 presents the classification of tobacco types based on curing methods and function.

Table 1 Classification of tobacco types based mainly on curing methods

Tobacco type	Characteristics / alternate names	Main use
Flue-cured	Leaves are yellow, blond, bright therefore also called Bright or Virginia	Cigarettes and also roll your own (RYO) cigarettes and pipe tobacco
Fire-cured	Light to dark brown cured over open fires (Kentucky)	RYO, chewing tobacco, cigars and smoking tobacco
Light air-cured	Burley (cured without supplementary heat) Maryland Perique	Mainly in cigarettes (also RYO, pipe tobacco and cigars) Cigarettes Pipe tobacco
Dark air-cured	Light to medium brown	Chewing tobacco and snuff, snus, dark cigarettes
Sun-cured	Oriental tobacco varieties Latakia	Turkish cigarettes (also RYO and pipe tobacco) Some pipe tobaccos
Cigar filler, **Cigar binder,** **Cigar wrapper**	Tobacco types for use as cigarfillers, binders and wrappers	Used for cigars

Ref: IARC Monograph 83 (2004), US Department of Agriculture (2001)

Two principal types of commercial cigarettes have traditionally been sold throughout the world: (i) American Blend cigarettes, which are made from a blend of Virginia, Burley and Oriental tobaccos, and (ii) Virginia cigarettes, which contain exclusively Virginia tobacco.

Casing refers to the sauce composed of a variety of ingredients such as humectants, sugars, cocoa, liquorice and fruit extracts (Hoffmann and Hoffmann 1997).

Casings are usually applied to tobacco strips or leaf early in the primary processing scheme to tone down or mute the strength or harshness of tobacco smoke, improve processibility of tobacco and add deep flavour notes to the smoke. Casings are traditionally added to US blended styles of product that contain significant proportions of Burley type tobacco blends. These casings are added to the Burley tobacco line through the means of the casing cylinder or Cased Leaf Dryer.

Ammonia technology has been used with US blended styles of products containing cased Burley tobacco. Ammonium salts could be added at the Cased Leaf Dryer (CLD) stage or with the manufactured reconstituted tobaccos.

There are no fixed rules as to where humectants, flavours and flavourings are added to the processed tobacco but generally the more volatile ingredients are added as late as possible during tobacco processing to prevent losses. Those tobacco blends that contain flavours and flavourings are usually held in a bin to allow for equilibration across the blend before it is passed to the making machine as the final blend. Top flavourings are generally applied to the total tobacco blend as one of the last steps in processing. They are usually carried in an alcohol base. They are used to improve quality of smoke, impart a pleasant pack aroma and side stream aroma. Menthol may be added at any of the following stages; spraying onto the final blend, through addition to the filter via a thread, or by application to the cigarette paper or the foil used to wrap the cigarettes. Due to the high level of volatility of menthol, different manufacturers have over the years developed a variety of methods for producing mentholated products that are as consistent as possible in terms of their finished product menthol levels (BAT 2010).

In cigarettes, flavours may be added to tobacco, cigarette paper, the filter, in a plastic pellet placed in the filter or the foil wrapper, in an attempt to enhance the tobacco flavour, mask unpleasant odour, and deliver a pleasant cigarette-pack aroma. Internal industry documents reveal additional flavour technologies such as flavour microencapsulation in the paper, carbon beads, and polymer-based flavour fibres inserted into the filter, flavoured tipping etc. (WHO 2007b).

As described above, the physical elements of the cigarette such as packing density, particle size distribution, rag cut per inch, colour appearance, resistance to draw, the appropriate paper, filter, tobacco type and the final tobacco blend, are carefully controlled (Wigand 2006). The final product is manufactured using high speed automated machines.

Over the years the tobacco industry has developed genetically modified (GM) tobacco plants with an aim, among others, to manipulate nicotine levels (Dunsby and Bero 2004). Reductions of nicotine levels have been in the range of 80-98%.

Philip Morris sought to use anti-sense biotechnology to disrupt enzymes involved in nicotine biosynthesis (US Patent 5684241). In 2003, Vector Tobacco began marketing a new cigarette that is produced from GM tobacco containing trace amounts of nicotine. The GM plant was produced by disrupting expression of the gene for quinolinate phosphoribosyl transferase, which encodes one of the rate-limiting enzymes in the nicotine biosynthetic pathway (Bonetta 2001). Vector Tobacco market Quest Cigarettes, which exist in three forms, ranging in nicotine content from 0.6 mg per cigarette to 0.05 mg per cigarette. They are marketed as a smoking cessation or reduction aid, with the manufacturer claiming that graded reduction of nicotine exposure through the gradual use of increasingly lower nicotine content cigarettes will lead to the eventual extinction of nicotine dependence and conditioned associations with related cues (Bonetta 2001).

Large scale field-trials have also been conducted despite consumer opposition and fear of tobacco growers that GM crops would be turned down by several countries.

3.4.1. Conclusions on manufacturing

Cigarettes, which are the predominant tobacco product, are highly engineered nicotine delivery devices that are mass produced by the major industries by integrated automation.

The properties of tobacco products depend on locality of growth, position of leaves on the stalk, ripeness and curing method. The different curing methods (drying procedures) determine the sugar content and colour of the tobacco leaves. During the manufacturing process of cigarettes, a number of substances are added at different stages for various reasons, such as providing consistency of the product, creating a unique brand, and promoting attractiveness.

3.5. Technical characteristics of cigarettes

Parts of cigarettes, like the paper and filter have technical features which affect the constitution of main-stream and side-stream smoke.

3.5.1. Introduction

Considering the natural origin of tobacco leaves, their content will, both qualitatively and quantitatively, depend on the season, local weather conditions and geographical origin. Consumers do not like to smoke a product that changes over time, i.e. smoking a constant product is preferred. In order to produce a constant product, i.e. to mask the batch to batch variation in taste, tobacco companies use a large variety of additives in the manufacture of tobacco products. In addition, the tobacco companies strongly prefer to maintain the same TNCO-values (tar, nicotine and carbon monoxide) of their products. To achieve consistency in TNCO values, tobacco producers change (amongst others) the ventilation of the products. The ventilation through the filter can be increased by punching more (or wider) ventilation holes. The ventilation of a cigarette can also be changed by using commercially available cigarette paper wraps with another grade of porosity.

Relevant technical characteristics of cigarettes are the following:

- Ventilation of the paper (paper porosity);
- Ventilation holes in the filter;
- Ventilation holes in the paper wrap;
- Packing of tobacco (dense or loose); and
- Geometry (length, diameter).

Ventilation

Large efforts have been made by the tobacco industry to investigate the effect of ventilation on the size distribution of the smoke aerosol. Depending on the size, the smoke particles enter and deposit at different levels of the airways (upper or lower airways). The purpose of this research was either to enhance the absorption of nicotine, to decrease the toxic potential of the product or to manipulate the taste of the smoke.

The main effect of ventilation is the dilution of the tobacco smoke. As such, the concentration of smoke components is reduced which not only leads to a lower dose of nicotine, but also to a lower concentration of other (toxic) components. It appears, however, that smokers compensate for the lower dose of nicotine per puff (due to increased ventilation) by increasing their puff volume, puff frequency, and deeper inhalation of the smoke (Jarvis et al. 2001, Scherer 1999). Many other smokers consciously or unconsciously block a part of the ventilation holes with their fingers so that more concentrated smoke is inhaled.

Another feature of ventilation is that it may affect the particle size and particle size distribution of the smoke aerosol, i.e. increasing the ventilation is supposed to decrease the mean particle size of the aerosol. It is difficult to assess whether an increase in ventilation indeed reduces the particle size, as only few studies are reported in publicly available literature.

3.5.2. Technical limitations

It is difficult to determine the size of the particles and their distribution in cigarette smoke, mainly because the half-life of the particles is very short (0.1-1 sec). Rapid ageing of the aerosol results in larger particles as they have time to coalesce, i.e. a secondary aerosol containing larger particles at the expense of smaller particles is rapidly formed (Harris and Kay 1959). Therefore, only sophisticated on-line sampling and detection allows a proper measurement of the particle distribution of the smoke aerosol. Obviously, these techniques require large financial resources and highly qualified technical personnel.

A number of variables other than ventilation may affect the particle size; moisture of the cigarette (relative humidity), puff volume, puff number (e.g. first or last puff), butt length, length of the cigarette, electrostatic charges, etc. Different unities are used in the studies to express the size of the particles (mean diameter, count median aerodynamic diameter, mass median aerodynamic diameter) which hampers quantitative comparison of the data. The aerosol is produced during burning, i.e. directly behind the burning cone at the tip of the cigarette the superheated vapour condenses and forms an aerosol; the longer the aerosol stays in the cigarette the larger the size of the particles.

Due to the number of different particle sizing methods, instrumentation and sampling and detection techniques applied, as well as differences in the cigarettes and smoking conditions, variable results are found and the results of different investigations are difficult to compare. Important limiting factors for many techniques are low time of resolution and the ageing of the smoke. Over time various methods have been developed to improve the accuracy of the measurements.

3.5.3. Smoke particles

Particle size may be relevant for the absorption of nicotine into the bloodstream.

Cigarette smoke particle size has generally been reported with MMD (mass median diameter) in the size range of 0.3-0.5 µm and CMD (count median diameter) in the range of 0.2-0.4 µm (Bernstein 2004, Wayne et al. 2008a). Particles larger than 1 µm are mostly trapped within the cigarette, whereas ultra-fine particles (less than 0.1 µm - nano-particle range) probably will adhere to the surface of the paper, tobacco and filter or coagulate into larger particles (Stratton et al. 2001), see section 3.5.4. Differences in particle size found in many studies were quite small and some internal tobacco documents concluded that the measurable influence of conventional design changes was insignificant (Philip Morris 1991, Wayne et al. 2008a). Of the four variables applied by Philip Morris to change the size of the particles (filler, filter, paper and ventilation) only ventilation had any significant effect (Cox et al. 1992). In addition, butt length and puff volume affect the size of the particles. There is a clear trend of decreased size of the particles at shorter butt lengths; the average size at 20 mm was 0.29 µm and at 55 mm it was 0.34 µm. Cox et al. (1992), taking all the variables mentioned above into account, reported deviations of about 10 to 30%. Surface mean diameter increased from 0.32 to 0.42 µm when the ventilation was increased from 0 to 60%. Based on their results, Cox et al. (1992) suggested that aerosol coagulation in the cigarette rod is the main mechanism for change in particle size.

Bernstein (2004) reviewed the available data of the tobacco smoke particulates which go back to 1950s. The main findings include:

- No difference in particle size between plain (non-filter) and filter cigarettes.
- Particle size depends on puff number (e.g. first vs. last puff).
- Relative humidity of the tobacco does not affect or only marginally affects particle size.
- Aged tobacco smoke contains larger particles than fresh smoke.

Over all the studies reviewed by Bernstein the size of the smoke particles range, roughly from 0.17 to 0.60 µm either expressed as CMD or MMD.

A study by McCusker et al. (1983) compares mass median aerodynamic diameter of ultra-low-tar, low-tar and medium-tar rated cigarettes (with and without filter). Particle size was less than 0.6 µm and not affected by the cigarette filters. Among the 10 brands tested ventilation ranged from 22 to 94%. The mass median aerodynamic diameter ranged from 0.36 µm to 0.56 µm, but did not correlate with ventilation efficiency. The number of particles was, however, reduced by 20–90% by applying the commercial filters and the particles were present in the higher puff numbers. Interestingly, blocking of the ventilation holes on the filters of ultra-low-tar cigarettes increased the particle concentration. This is explained by the longer residence time (longer transit time from cone to filter) of the newly formed particles in the cigarette rod.

As mentioned in section 3.5.2, only sophisticated on-line sampling and detection allows a proper assessment of the particle size and distribution. Moreover, the relevance of ultra-fine particles for nicotine absorption has only been taken seriously for the last two decades; therefore, most of the older studies did not focus on the presence of ultra-fine particles.

Recently, using on-line measurement of the particle size (range measured 5–1000 nm), Adam et al. (2009) reported that non ventilated cigarettes smoked under the intense regime, which includes blocking the ventilation holes resulted in count median diameter of 0.18 µm, whereas 70% ventilated cigarettes smoked under a milder standard smoking regime lead to a diameter of 0.28 µm. The particle size of mainstream smoke of Virginia cigarettes, smoked under a standard smoking regime, was 0.22 µm and 0.25 µm at 0 and 70% ventilation, respectively. For the intense smoking regime the respective particle sizes were 0.18 and 0.22 µm. Interestingly when the ventilation was increased from 0 to 70% the total number of particles decreased dramatically from 2.3×10^{12} to 0.3×10^{12}, and total mass of particles dropped from 17.2 to 2.3 mg (standard smoking regime). In another recent paper by Gowadia et al. (2009) the particle size (mass median aerodynamic diameter) was found to be approximately constant (0.9–1.0 µm) for three different puffing regimes. The smoke was collected in a conditioning chamber and the particle size distribution was determined by UV spectrometry.

Particle size of waterpipe smoke was shown to be somewhat smaller than that of cigarette smoke. Monn et al. (2007) reported waterpipe smoke particle median diameter in a full smoking set containing charcoal, tobacco and water, of 40 nm; the smoke of the heated tobacco alone ranged from 10 nm to 200 nm while the burning of charcoal was mostly responsible for the particles smaller than 50 nm. Fromme and colleagues found two phases of particle emission during a waterpipe session: when the charcoal was lit, the particle diameter was around 100 nm and during the smoking session it decreased to 17 nm (Fromme et al. 2009). Daher et al. (2010) found similar particle sizes to the Monn study in side-stream smoke from waterpipes, which was significantly smaller than particle sizes in side-stream smoke from cigarettes with a median diameter of 139 nm and a large number of particles smaller than 100 nm.

3.5.4. Deposition of particles

Although the size of the particle is an important factor for the deposition in the lung, the relationship between particle size and deposition in the lung is complex and factors other than size alone, such as respiration rate, depth of inhalation and flow rate, affect lung deposition (Sarangapani and Wexler 2000).

In figure 1 the relative deposition of particles (dependent on the aerodynamic diameter) in humans is depicted. Particles larger than 1 µm will mainly deposit in the extra-thoracic region. Smaller particles will deposit in different regions, but the general statement that smaller particles deposit deeper in the lung is not entirely true. Very small particles (a few nm) will mainly deposit in the extra-thoracic region. Peak alveolar deposition is around 30-20 nm and becomes less important at sizes less than 8-9 nm (ICRP 1994, Oberdörster et al. 2005). The question whether the ultra-fine particle size is relevant for mainstream tobacco smoke is unanswered. From a theoretical point of view removal of ultra-fine particles is to be expected due to adherence to the surface of the paper or to the tobacco and filter, or due to coagulation into larger particles (Stratton et al. 2001) (see section 3.5.3), however this needs to be confirmed experimentally.

Other points of concern in the inhalation of ultra-fine particles are the translocation of these particles: (1) from the lumen of the lung to the circulation; and (2) from the olfactory nerve endings in the nose to the brain. These two events have been described for several solid nanoparticles in the lungs of animals and humans (Kreyling et al. 2002, Nemmar et al. 2002,), and in the noses of rodents (Oberdörster et al. 2002). These phenomena have not been shown for tobacco smoke derived particles which are not solid nanoparticles (although combustion derived particles have been studied in the lung); therefore, only theoretical/hypothetical considerations can be made (which fall outside the scope of this opinion).

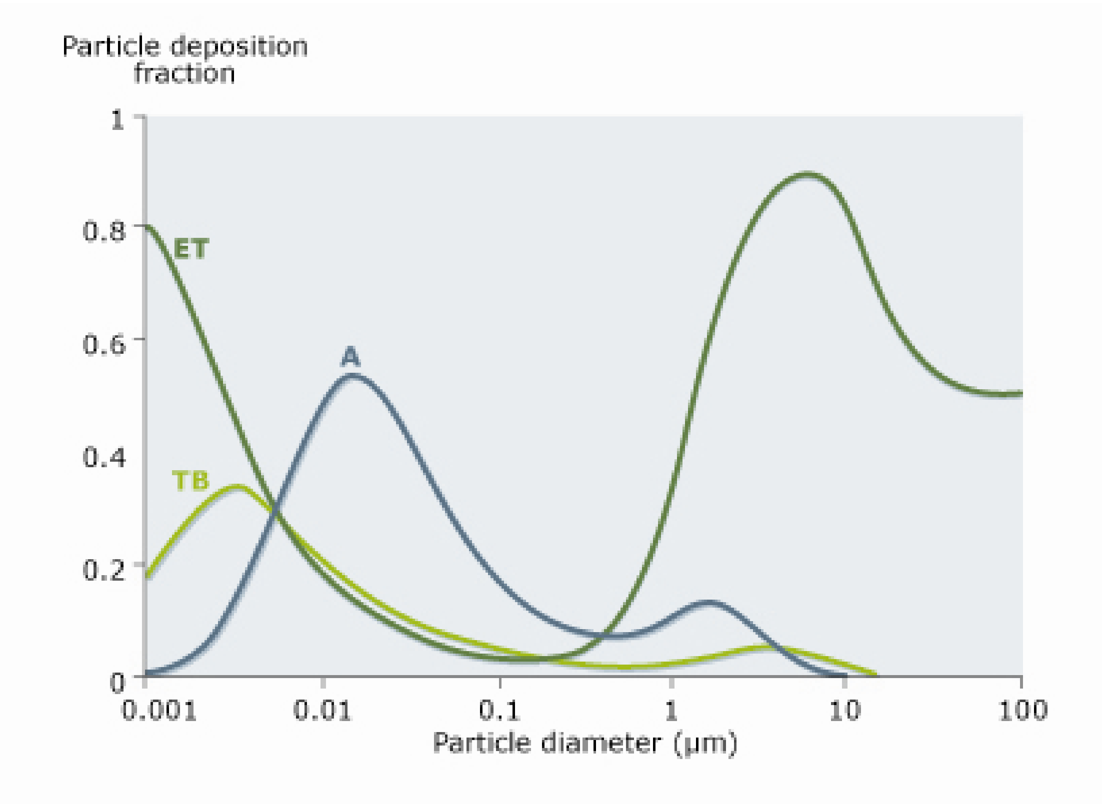

Figure 1 **Predicted deposition of inhaled particles during nose breathing. Fractional deposition in: extrathoracic (ET); trachea-bronchial (TB); and alveolar (A) regions (adapted from ICRP 1994).**

3.5.5. Light cigarettes as an example of cigarettes with high ventilation

The best known application of changing ventilation is the development of light cigarettes. Light cigarettes have been marketed as products with a lower health risk as they should deliver less tar and other toxic compounds in the smoke inhaled. As will be described in detail in section 3.10.1 many smokers of light cigarettes inhale the smoke deeper and increase the number of puffs, so the health risks are probably not lower than for smokers of regular cigarettes (Frost et al. 1995). Animal studies have shown that self-administration of a low dose of nicotine at a high frequency gives a more reinforcing effect as compared to self-administration of a higher dose at a low frequency (in this comparison total dose self-administered is the same) (Harris et al. 2008, Harris et al. 2009, O'Dell et al. 2007).

3.5.6. Conclusions on technical characteristics

A number of technical characteristics of cigarettes influence the content of different substances in the smoke and the size of smoke particles. The so-called TNCO values (tar, nicotine and CO) are determined by, amongst other things, ventilation (paper, filter), the packing of the tobacco and the geometry of the cigarettes. Smokers usually compensate for a lower dose of nicotine by increasing puff volume and frequency, and by deeper inhalation. Data obtained in animal studies suggest that cigarettes with high ventilation (often described as "light" or "low tar") may favour addiction to nicotine in the smokers of these products, because of an increased smoking frequency.

The particle size of smoke aerosol of commercial cigarettes is around 0.4 to 2 μm. A large fraction of ultrafine particles (<0.1 μm) probably adheres to the surface of the paper or the filter, or coagulates into larger particles, and will thus not be present in the smoke as such. The small smoke particles (submicron meter range) will enter the lower airways and alveoli, while larger particles (micron meter range) will be deposited increasingly in the upper airways.

Considering the manufacturing of cigarettes, the change of the technical characteristics of cigarettes may affect the mean particle size and, therefore, the distribution of the smoke aerosol. However, based on the limited publicly available information, it seems that exposure to nicotine cannot be substantially increased by altering the particle size of the smoke aerosol.

3.6. Nicotine

3.6.1. Pharmacological effects (incl. metabolism of nicotine)

3.6.1.1. Brief historical overview

Nicotine is the principal component alkaloid of tobacco, occurring throughout the plant (*Nicotiana tabacum*), especially in the leaves. The plant and the compound are named after Jean Nicot, a French ambassador to Portugal, who sent tobacco seeds to Paris in 1550. Crude nicotine was known by 1571, and the compound was obtained in purified form in 1828; the correct molecular formula was established in 1843, and the first laboratory synthesis was reported in 1904. It is one of the few liquid alkaloids; colourless and extremely toxic. Nicotine is commercially obtained from tobacco scraps; it has been used as an insecticide and as a veterinary vermifuge.

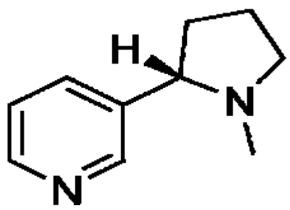

Figure 2 Structure of nicotine (CAS number 54-11-5)

3.6.1.2. General pharmacodynamic (physiological) effects

Nicotine administration induces a series of multifaceted effects which show great interindividual variability, i.e. the effects vary greatly from person to person. This is reflected in a non-linear and complex dose-response relationship ensuing from a summation of stimulatory and inhibitory actions in the central and peripheral nervous systems.

Low doses of nicotine, including those in the range of inhaled cigarette smoke (1-2 mg), produce stimulation of ganglionic neurotransmission (vegetative ganglia). This generates a complex response which results from a mix of sympathetic and parasympathetic actions. Thus, tachycardia and rise of blood pressure are to a large extent the consequence of sympathetic ganglia activation that induces an increased adrenaline release in the adrenal medulla (via splanchnic nerve stimulation). At the same time, the nicotine action on the carotid and aortic chemoreceptors and on the brain regulating centres modifies the cardiovascular effects determining the great variability observed in the final response. Therefore, the direct nicotine effects on heart rate and blood pressure are rapidly counterbalanced by the peripheral and central cardiovascular compensatory reflexes. Similarly, nicotine–induced activation of parasympathetic ganglia and cholinergic terminals causes an increase of the gastrointestinal peristalsis. In susceptible subjects, first doses may cause nausea, vomiting and related effects of hypercholinergic activation. Nicotine also increases blood glucose levels and the activity of exocrine glands. In the brain, nicotine is clearly a stimulant at low doses. It produces a pattern of alertness in the EEG, mediates fast synaptic transmission, and positively modulates a range of cognitive functions. As a result, it improves attention, learning, arousal, motor skill, facilitates memory functions and decreases irritability and anxiety, among other CNS functions (Balfour and Fagerström 1996, Benowitz 2008, Fattinger et al. 1997, Grybko et al. 2010).

An important pharmacological characteristic of nicotine is the rapid development of tolerance to its unwanted effects. Although there is a great individual variability, in many cases tolerance to the peripheral effects appears a few days after the first exposure (Benowitz 2008).

3.6.1.3. Toxicity effects

At high doses, after the initial stimulation, nicotine rapidly produces a ganglionic blockade due to the inhibition of transmission, which is a consequence of a persistent depolarisation of all autonomic ganglia. This depression of all autonomic ganglia results in bradycardia, hypotension, impairment of adrenaline release, etc. Similarly, a biphasic nicotine-induced action is also observed in the adrenal medulla (a discharge of catecholamines is evoked by small doses whilst their release is blocked by larger doses). It should be noted that most peripheral effects are influenced by compensatory reflexes. In the CNS large doses induce a generalised mental depression, tremors, nausea, and convulsions. The acute lethal dose of nicotine in an adult human is estimated to be about 60 mg (Benowitz 2008, García-Estrada and Fischman 1977, Solarino et al. 2010). This dose (less than 1 mg/kg) is derived from old reported cases of intoxication when nicotine was widely used as an insecticide (Grusz-Harday 1967, Lockhart 1939). In rats the LD_{50} is ~50 mg/kg and in mice ~3 mg/kg (Okamoto et al. 1994). Acute nicotine poisoning has occurred in children who accidentally ingest tobacco or are occupationally exposed to wet tobacco leaves. Children have played a role, and they continue to do so in many places, in agricultural production of tobacco, where absorption of nicotine from the plant is likely to happen. This nicotine-induced acute condition is known as green tobacco sickness. Clinical features are similar to those observed in adults (Gehlbach et al. 1974, McKnight and Spiller 2005).

Ingestion of tobacco products is a major reason for infant and child toxic exposures reported to poison control centres. The large majority (90%) of such accidental poisonings in the population involve children up to 6 years of age (Connolly et al. 2010). However, ingestion of cigarettes and cigarette butts by children aged ≤ 6 years resulted in minor toxic effects (CDC 1997).

Malizia et al. (1983) described four children who ingested two cigarettes each and developed salivation, vomiting, diarrhoea, tachypnoea, tachycardia, and hypotension within 30 minutes, and depressed respiration and cardiac arrhythmias within 40 minutes. Convulsions occurred within 60 minutes of ingestion. All recovered after gastric lavage with activated charcoal, intermittent positive pressure ventilation, and 5 mg diazepam intravenously for convulsions.

A prospective review of 51 cases of tobacco ingestion and five cases of nicotine resin chewing gum exposure was conducted to evaluate the incidence and degree of toxicity caused by these products in children. A dose-response relationship was observed for cigarette exposures. Nine of 10 children ingesting more than one cigarette or three cigarette butts developed signs or symptoms (Smolinske et al. 1988).

3.6.1.4. The nicotinic acetylcholine receptor

Nicotine acts on a class of cholinergic receptors which are ligand-gated ion channels (nicotine acetylcholine receptors: nAChR). These kinds of receptors are structurally similar to the ones operated by GABA, glycine, glutamate, 5-HT_3, etc. Nicotine binding to the nAChR opens the channel and increases its ionic permeability for monovalent cations (Na^+, K^+) and divalent cations (Ca^{2+}, Mg^{2+}), although with difficulty for the latter and depending on the subtype of nAChR. Neuronal nAChR embrace a conjunct of at least 20 homologous subtypes that mediate fast synaptic transmission throughout the central and peripheral nervous systems (Xiu et al. 2009).

Neuronal nAChR are pentamers of homomeric or heteromeric combinations of α (α_2 to α_{10}) and β (β_2 to β_4) subunits, which possess different pharmacological and biophysical properties and locations in the brain (Gotti et al. 2006).

The nAChRs in the CNS are localised both in postsynaptic and presynaptic neural membranes. Studies in recent years have shown that the primary site of nicotine action is presynaptic, and that nAChRs facilitate the release of neurotransmitters when localized in non-cholinergic terminals. In fact, nAChRs are present in the terminals of most of the neurotransmitter systems (GABAergic, glycinergic glutamatergic, dopaminergic, serotonergic, etc.). Likewise, nAChRs have been identified, in different densities, in most of the brain areas.

Nine individual subunits of nAChRs in the human brain have been identified and cloned, and they combine in various conformations to form individual receptor subunits. The structure of individual receptors and the subtype composition are not completely understood. Only a finite number of naturally occurring functional nAChR constructs have been identified (Luetje 2004).

The pentameric structure of the neuronal nAChR and the considerable molecular diversity of its subunits offer the possibility of a large number of nAChRs with different physiological properties. The stoichiometry of most nAChRs in the brain is still uncertain (Kuryatov et al. 2000).

For example, the neuronal nAChR subunits on presynaptic terminals of dopamine neurons projecting to the striatum have been fully defined (Luetje 2004), as has the complete subunit composition of four major presynaptic nAChR subtypes in the striatum (Salminen et al. 2004).

It should also be noted that chronic exposure to nicotine induces a marked increase in the density of nAChRs in most neurotransmitter systems and brain areas (Walsh et al. 2008).

3.6.1.5. Nicotine pharmacokinetics and metabolism

Nicotine as a weak base (pKa = 8.0) is rapidly absorbed across biological membranes with an environment at physiological pH (7.4) or slightly alkaline. This is the case for nicotine in cigarette smoke when it reaches the lung alveoli (Pankow et al. 2003). The average nicotine content of a cigarette (6-10 mg) delivers about 1 mg of nicotine (0.5-2 mg) systematically through the smoker's lungs (Henningfield et al. 1993). The pulmonary bioavailability (the amount absorbed from smoke) of inhaled nicotine is 80-90%. After inhalation it reaches high levels in the brain within 10-20 seconds, thus being equivalent to, or even faster than, an intravenous administration (Gourlay and Benowitz 1997, Hukkanen et al. 2005). In both cases the hepatic first-pass effect (metabolism) is avoided allowing higher levels of unmetabolised nicotine to be delivered to the brain. In addition, nicotine easily crosses the blood-brain barrier.

In contrast, the buccal and gastric bioavailability of nicotine is low (20-40%) due to the acidic environment at which nicotine is protonated and therefore poorly absorbed through local membranes. Better absorption is obtained in the intestinal mucosa because of its alkaline pH. Nonetheless, the liver first-pass metabolism contributes to the impairment of the oral bioavailability to a greater extent. The time of nicotine blood maximal concentration for oral administrations is about 60-90 min. Nonetheless, nicotine bioavailability through the skin is high (75-100%).

Nicotine is widely distributed in the body (liver, kidney, lungs, etc.; with adipose tissue showing the lowest affinity). Brain tissue exhibits a high affinity for nicotine. It has been reported that nAChR binding capacity for nicotine is increased in smokers compared to non smokers (Breese et al. 1997, Perry et al. 1999). This reflects the higher density of nAChRs in the brain of smokers (nicotine-induced up-regulation of nAChRs). However, the quantity of nicotine delivered from the tobacco product which reaches the brain is higher in non dependent smokers than in heavy smokers (Rose et al. 2010a).

The blood half-life ($t_{½}$) of nicotine after cigarette smoking or intravenous administration is about 2 hours ($t_{½}$ = 100-150 min). The disposition of nicotine shows a multiexponential elimination (Hukkanen et al. 2005). However cotinine, the main metabolite of nicotine, has a $t_{½} \approx 19$ hours. It was found recently that every puff of a cigarette induces a peak of nicotine in the arterial blood (Berridge et al. 2010) with a $t_{½}$ of 45 seconds, but that these peaks do not occur in the brain (Rose et al. 2010a). This finding rules out that the lack of efficacy of NRT (gums or patches) is due to a continuous delivery of nicotine. In the liver nicotine is mostly metabolized in the endoplasmic reticulum by the cytochrome P450 (CYP) system, mainly by CYP2A6 and CYP2B6. The major metabolite produced by CYP through nicotine oxidation is cotinine, which is further converted to cotinine glucuronide and other metabolites. It should be noted that CYP oxidative metabolism of nicotine to cotinine and its glucuronide conjugation are inhibited by menthol, a commonly used cigarette additive. The pathway of nicotine to cotinine represents around 70-80% of nicotine biotransformation in humans and, therefore, is commonly used as a quantitative biomarker of nicotine exposure as well as of CYP2A6 metabolic activity, which exhibits an important variation in function in humans (Benowitz 2008, Dempsey et al. 2004, Hukkanen et al. 2005, Hukkanen et al. 2010). Many other minor metabolites of nicotine are produced by CYP, glucuronidation, demethylation and other enzymatic pathways. These metabolites have no nicotinic activity, with the exception of nornicotine which is produced by N-demethylation of nicotine in humans and other mammals (besides being a major tobacco leaf alkaloid). Although nornicotine is a minor metabolite, it has been shown that after repeated nicotine administration it accumulates in the brain at pharmacologically relevant concentrations acting as agonist on nAChRs but with about 10-fold lower potency (Dwoskin et al. 2001, Hukkanen et al. 2005).

Renal excretion is the major route of elimination of nicotine and its metabolites (>90% of a dose). Unchanged nicotine accounts for about 10%, and nicotine glucuronide and nicotine N′-oxide for about 5% each, of the total nicotine-derived amount present in urine. Trans-3′-hydroxycotinine (35-40%) and its glucuronide (~10%) are the principal nicotine metabolites determined in urine, both after a single dose and in smokers; unchanged cotinine (10-15%), cotinine glucuronide (~15%) and cotinine N′-oxide (~4%) represent the rest of the cotinine metabolic pathway excreted. Small amounts of a large array of nicotine metabolites produced in the minor biotransformation pathways are also detected in urine. Nevertheless, the pattern of nicotine metabolites and their amounts are highly variable in humans due to the important polymorphism of CYPs and the other enzymatic pathways involved in the metabolic disposition of xenobiotics (Benowitz et al. 2006, Benowitz 2008, Hukkanen et al. 2005). It has been suggested that this genetic variation in xenobiotic metabolism, especially that of CYP2A6, has a role in smoking behaviour and nicotine dependence (Malaiyandi et al. 2005).

3.6.1.6. Conclusions on nicotine pharmacology

The main effect of nicotine (besides its action on the cholinergic system) is the presynaptic release in the brain of neurotransmitters such as acetylcholine, noradrenaline, dopamine, serotonin, glutamate, GABA and opioid peptides. This allows the possibility that many compounds may modify the action of nicotine on the presynaptic nicotine receptors, and consequently modify the activity of nicotine in the brain. There is substantial interindividual variability in the action and metabolism of nicotine and many aspects of its pharmacology are still not fully understood.

Nicotine metabolism may be modified by compounds inducing or inhibiting the activity of the cytochrome P450 system and other metabolic pathways, thus determining pharmacokinetic changes. While the half-life of nicotine in the arterial blood is short, nicotine levels in the brain remain at high levels much longer.

3.6.2. Addictive properties of nicotine

Nicotine exposure produces adaptive changes in the central nervous system (CNS) leading to an addictive process characterised by compulsive tobacco use, loss of control over tobacco consumption despite the harmful effects, the appearance of withdrawal symptoms upon the cessation of tobacco smoking, and relapse after periods of abstinence (McLellan et al. 2000). As in other addictive processes, the initiation of nicotine addiction has been related to its capacity to induce rewarding/reinforcing effects. However, the negative consequences of nicotine abstinence have a crucial motivational significance for maintenance and relapse of this addictive behaviour (Koob and Le Moal, 2008). The terms "reward" and "reinforcement" are often misused and confused. Reward describes stimuli that have appetitive (desirable) consequences and/or produce a hypothetical pleasurable internal state (hedonia). Reinforcement refers to the ability of a stimulus to promote behavioural responses in order to obtain (positive reinforcement) or to avoid (negative reinforcement) such a stimulus. A drug like nicotine that produces rewarding effects will also promote behavioural responses to obtain the drug, i.e. positive reinforcing effects. On the other hand, the effects induced by a drug can be associated with some particular neutral stimuli. After learning the association, this neutral stimulus becomes a conditioned stimulus associated with the drug that can also promote behavioural responses by itself. Several animal models of drug reward/reinforcement are based on these conditioning processes.

The neurobiology of nicotine addiction is a complex phenomenon in which various transmitter systems are involved (Berrendero et al. 2010). The experimental animal models that have been used to investigate nicotine addiction are mainly models of nicotine reward/reinforcement and have been useful to define the neurobiological substrate involved in this behavioural response that is crucial for the nicotine addictive process. New complex behavioural models that resemble the main diagnosis for drug addiction in humans have been developed more recently (Belin et al. 2008, Deroche-Gamonet et al. 2004, Vanderschuren and Everitt 2004). These models of addiction are extremely complex and have been validated only for cocaine addiction. Due to their complexity, these models have still not been used to investigate the neurobiology of drug addiction. Therefore, all the valuable information currently available about drug addiction, including nicotine addiction, is based on the results obtained in experimental models that evaluate drug rewarding/reinforcing effects (see section 3.9 for details about significance of the models).

3.6.2.1. Nicotinic acetylcholine receptors subunits and nicotine rewarding/reinforcing effects

The mesocorticolimbic system plays a crucial role in the rewarding/reinforcing properties of nicotine (Koob and Le Moal 2008). An important component of this system is the dopamine (DA) projection from the ventral tegmental area (VTA) to the frontal cortex and limbic structures, such as the nucleus accumbens (NAc). Nicotine administration increases DA activity in the NAc and other limbic structures (Di Chiara and Imperato 1988) by direct stimulation of nicotinic acetylcholine receptors subunits (nAChRs) within the VTA (Nisell et al. 1994). $\alpha_4\beta_2$ containing nAChRs located on DA cell bodies contribute decisively to the final activation of VTA DA neurons (Mansvelder and McGehee 2003). Indeed, the administration of selective $\alpha_4\beta_2$ antagonists block nicotine-self-administration in rodents (Grottick et al. 2000). In agreement, mice with the β_2 subunit knocked out do not self-administer nicotine (Picciotto et al. 1998). The specific location of nAChRs containing the β_2 subunit in the VTA plays a crucial role in the mediation of nicotine reinforcement as demonstrated by genetic studies in mice (Maskos et al. 2005). In addition, α_4 knockout mice fail to show nicotine-dependent enhancement of DA release in the NAc (Marubio et al. 2003), whereas a single nucleotide mutation rendering α_4 containing nAChRs hypersensitive to nicotine (Tapper et al. 2004) demonstrates that this subunit is sufficient to induce nicotine reward (Tapper et al. 2004). The precise role of the α_7 homomeric nAChRs in nicotine reinforcing effects remains unclear since conflicting results have been obtained in mutant mice lacking this subunit and in rodents injected with selective α_7 nAChR antagonists (Markou and Paterson 2001, Walters et al. 2006). On the other hand, repeated exposure to nicotine leads to up-regulation and desensitisation of nAChRs (Quick and Lester 2002), which are involved in the development of nicotine tolerance and the appearance of a withdrawal syndrome following smoking cessation. The brain regions underlying nicotine physical dependence have not yet been fully clarified, although an involvement of nAChRs located in the medial habenula and the interpeduncular nucleus has been recently reported (Salas et al. 2009).

Recent genome-wide association studies in humans have revealed a clear linkage between genetic variations in the nAChRs and the risk for nicotine dependence (Bierut 2009). Thus, the region on chromosome 15 that includes the family of α_5-α_3-β_4 nAChR genes has been associated with the development of nicotine dependence (Berrettini et al. 2008, Thorgeirsson et al. 2008) and lung cancer (Amos et al. 2008, Hung et al. 2008, Thorgeirsson et al. 2008). These studies differ on whether the connection between the genetic variant at chromosome 15 and lung cancer is direct (Amos et al. 2008, Hung et al. 2008) or mediated through a modification of smoking behaviour (Thorgeirsson et al. 2008).

3.6.2.2. Involvement of glutamatergic receptors in nicotine rewarding/reinforcing effects

Nicotine stimulates nAChRs on glutamatergic terminals that release glutamate in several brain regions including the VTA (Fu et al. 2000). Glutamate receptors located on postsynaptic DA neurons are critically involved in nicotine reinforcing effects (Liechti and Markou 2008). Thus, nicotine-induced DA release in the NAc is blocked by the administration of NMDA and AMPA ionotropic receptor antagonists (Kosowski et al. 2004). In addition, the blockade of NMDA receptor decreases intravenous nicotine self-administration in rats (Kenny et al. 2009). Several studies have also involved postsynaptic mGlu5 and presynaptic mGlu2/3 metabotropic receptors in nicotine reinforcing effects. Thus, mGlu5 receptor antagonists decrease nicotine self-administration (Paterson et al. 2003) and the incentive motivation for nicotine in rodents (Paterson and Markou 2005). The administration of a mGlu2/3 agonist also decreases nicotine self-administration in rats (Liechti et al. 2007). This last result is in accordance with previous studies showing that presynaptic mGlu2/3 receptors modulate glutamate release in a negative manner (Schoepp et al. 2003). The administration of mGlu5 receptor antagonists (Bespalov et al. 2005) or mGlu2/3 receptor agonists (Liechti et al. 2007) also decreases cue-induced reinstatement of nicotine-seeking in rats. Cholinergic and glutamatergic inputs from the pedunculopontine tegmental nucleus (PPTg) to the VTA seem to play a crucial role in nicotine reinforcement since complete lesion of the PPTg reduces nicotine self-administration (Lança et al. 2000, Picciotto and Corrigall 2002. On the other hand, the negative affective changes of nicotine withdrawal are related to a hyperactivity of corticotropin-releasing-factor neurons in the central nucleus of the amygdala (Bruijnzeel et al. 2007, Panagis et al. 2000) and a decrease of DA activity in the NAc (Hildebrand et al. 1999) that seems to be modulated by the glutamatergic system. Thus, mGlu2/3 receptor antagonists, which increase extracellular glutamate in the NAc, attenuate reward deficits associated with nicotine withdrawal in rodents and could also alleviate the depression-like symptoms related to nicotine abstinence in humans (Kenny et al. 2003, Liechti and Markou 2008).

3.6.2.3. Involvement of GABA receptors in nicotine rewarding/reinforcing effects

DA neurons in the VTA are under the inhibitory control of GABAergic inputs that also participate in nicotine rewarding/reinforcing effects. Hence, the administration of the GABA-B receptor agonists such as baclofen, as well as several GABA-B receptor positive allosteric modulators, decrease nicotine self-administration in rats (Paterson et al. 2004, Paterson et al. 2008). Baclofen also inhibits nicotine-induced conditioned place preference in rats (Le Foll et al. 2008). Although GABA neurons are also activated by nicotine, $\alpha_4\beta_2$ nAChRs located on GABA cells tend to desensitise rapidly during repeated nicotine exposure (Mansvelder et al. 2002). Desensitisation of these receptors following repeated nicotine exposure contributes to the final activation of mesolimbic DA neurons induced by the chronic administration of this drug of abuse. Recent studies have reported that the GABA system also participates in nicotine relapse. Thus, the administration of GABA-B receptor agonists decreases cue-induced reinstatement of nicotine-seeking behaviour in rodents (Fattore et al. 2009, Paterson and Markou 2005). In agreement, baclofen also prevents the reinstatement of nicotine conditioned place-preference triggered by nicotine priming in rats (Fattore et al. 2009).

3.6.2.4. Endogenous opioid system in nicotine rewarding/reinforcing effects

Nicotine administration has been reported to enhance the release of endogenous opioids in the CNS. Thus, an increased concentration of β-endorphin has been found in the hypothalamus after acute nicotine administration in rodents (Marty et al. 1985). In addition, chronic nicotine has been found to increase mRNA expression of prodynorphin and μ-opioid receptors (Wewers et al. 1999) in the striatum (Isola et al. 2008). An enhancement of proenkephalin expression has also been observed in the striatum of mice following acute or chronic nicotine administration (Dhatt et al. 1995).

Nicotine induces opposite responses on anxiety-like behaviour related to the development of nicotine addiction that are modulated by the endogenous opioid system. Thus, nicotine anxiolytic-like effects were blocked by a μ-opioid antagonist, and its anxiogenic-like effects were enhanced by a δ-opioid antagonist (Balerio et al. 2005). In addition, a reduction of nicotine anxiogenic-like effects was reported in knockout mice lacking β-endorphin (Trigo et al. 2009). The opioid system also plays an important role in nicotine rewarding effects. The efficacy of naltrexone on smoking cessation in humans supports the involvement of opioid receptors in nicotine reward (Rukstalis et al. 2005). In rodents, nicotine-induced elevations of extracellular DA levels in the NAc were modulated by the activation of μ-opioid receptors localized in the VTA (Tanda and Di Chiara 1998). In agreement, nicotine rewarding properties were blocked in knockout mice lacking μ-opioid receptors (Berrendero et al. 2002) or proenkephalin gene (Berrendero et al. 2005), revealing an involvement of endogenous enkephalins through the activation of μ-opioid receptors. In addition, proenkephalin knockout mice showed a reduction of nicotine-enhanced DA extracellular levels in the NAc (Berrendero et al. 2005). Mice lacking β-endorphin also showed a reduction of nicotine rewarding effects (Trigo et al. 2009). κ-Opioid receptors and their endogenous ligands modulate nicotine reward in the opposite way to enkephalins and β-endorphins. Hence, knockout mice deficient in the prodynorphin gene showed an enhanced sensitivity to nicotine self-administration, probably due to the modulation of its aversive effects (Galeote et al. 2009).

The opioid system is also involved in the development of nicotine tolerance. Thus, chronic nicotine exposure produces cross-tolerance with morphine (Biala and Weglinska 2006, Zarrindast et al. 1999), and increases the functional activity of μ-opioid receptors in the spinal cord (Galeote et al. 2006). In addition, μ-opioid receptor knockout mice developed faster nicotine tolerance than wild-type mice, suggesting that increased activation of μ-opioid receptors could be an adaptive mechanism to counteract the establishment of nicotine tolerance (Galeote et al. 2006). The involvement of the opioid system in nicotine withdrawal has also been demonstrated. In humans, the opioid antagonist, naloxone induces somatic signs of withdrawal in heavy chronic smokers (Krishnan-Sarin et al. 1999). In rodents, opioid antagonists precipitate somatic manifestations of withdrawal in nicotine-dependent animals (Balerio et al. 2004). In addition, somatic manifestations of nicotine withdrawal were reduced in mice lacking μ-opioid receptors (Berrendero et al. 2002) or the proenkephalin gene (Berrendero et al. 2005). Different studies also indicate that the opioid system participates in the negative emotional states associated with nicotine withdrawal. Thus, naloxone induced aversive effects in nicotine-dependent rodents, which reflects the motivational manifestations of nicotine withdrawal (Balerio et al. 2004, Watkins et al. 2000).

3.6.2.5. Involvement of cannabinoid receptors in nicotine rewarding/reinforcing effects

Several studies demonstrate that the endocannabinoid system plays an important role in the rewarding/reinforcing effects of nicotine (Maldonado et al. 2006). Indeed, the selective CB_1 receptor antagonist rimonabant reduces nicotine self-administration in rats (Cohen et al. 2002) and nicotine-induced conditioned place preference in rats and mice (Le Foll and Goldberg 2004, Merritt et al. 2008). In addition, rimonabant pre-treatment blocks nicotine-enhanced DA extracellular levels in the NAc (Cheer et al. 2007, Cohen et al. 2002) and in the bed nucleus of the stria terminalis (Cheer et al. 2007). Nicotine conditioned place preference was also absent in knockout mice lacking CB_1 receptors (Castañé et al. 2002, Merrit et al. 2008). The endocannabinoid system has also been involved in the relapse to nicotine-seeking behaviour (De Vries and Schoffelmeer 2005b). Thus, rimonabant attenuates the reinstatement of nicotine seeking-behaviour induced by nicotine-associated cues (Cohen et al. 2005, De Vries et al. 2005a), and reinstatement of nicotine-induced conditioned place-preference provoked by nicotine priming (Biala et al. 2009). The cannabinoid antagonist AM251 also reduced the reinstatement produced by the combination of nicotine-associated cues and a nicotine priming dose (Shoaib 2008). Based on the behavioural and biochemical results obtained in rodents, several clinical trials were developed to evaluate the efficacy of rimonabant for smoking cessation (STRATUS, studies with rimonabant and tobacco use) (Cahill and Ussher 2007). Rimonabant was effective in obtaining a significant smoking cessation in two clinical trials (STRATUS-NORTH AMERICA and STRATUS-WORLD WIDE), although this effect was not significant in the STRATUS-EUROPE trial. The different clinical trials performed with rimonabant have reported several gastrointestinal and psychiatric side effects including nausea, anxiety and depression. Due to these psychiatric side effects, the European Medicines Agency (EMA) recommended the suspension of the marketing authorisation for rimonabant on 23 October 2008. In spite of the withdrawal of rimonabant, the CB_1 receptor remains a promising target to develop new compounds to treat drug addiction.

3.6.2.6. Other neurotransmitters involved in nicotine rewarding/reinforcing effects

The serotonergic (5-HT) system, mainly through the activation of the 5-HT_{2c} receptor subtype, seems to be involved in nicotine reward/reinforcing by exerting an inhibitory influence on DA activity in the VTA (Di Matteo et al. 1999). Thus, 5-HT_{2c} agonists reduce nicotine-self-administration (Grottick et al. 2001), although responding for food was also attenuated by these antagonists. In contrast, no modification on nicotine-induced conditioned place preference was observed by a 5-HT_{2c} agonist in a recent report (Hayes et al. 2009). On the other hand, tobacco smoke contains monoamine oxidase (MAO) inhibitors which are thought to enhance the reinforcing effects of nicotine. Behavioural studies have confirmed this statement since nicotine self-administration was facilitated in rats pre-treated with MAO inhibitors (Villégier et al. 2006a, Villégier et al. 2007). Recently, the hypothalamic neuropeptides hypocretins acting in the insula have also been involved in nicotine reward (Hollander et al. 2008).

3.6.2.7. Conclusions on addictive properties of nicotine

Animal models of nicotine reward/reinforcement have enabled the neurobiological substrate involved in this behavioural response that is crucial for nicotine addictive processes. Similar animal models have been widely used to define the neurobiological substrate of the addictive properties of all drugs of abuse. Results obtained in these models suggest that the neurobiology of nicotine addiction is complex involving various transmitter systems in the CNS. Multiple neurotransmitter pathways are activated by nicotine, including dopaminergic, GABAergic and opioidergic pathways. The complexity of the mechanisms of addiction is further underlined by the involvement of the endocannabinoid system, and the serotonergic system also seems to be involved. Dose-dependency appears to have been shown in animal studies. In general, an inverted U-shaped dose-response has been revealed, which suggests that, such as for other drugs of abuse, the addictiveness of nicotine is not directly linear with the dose. The experimental animal models used for evaluating addiction are described in section 3.9.

3.6.3. Conclusions on nicotine

The action of nicotine on the CNS is multifaceted and the mechanisms of addiction are still poorly understood. There are substantial inter-individual differences in the action of nicotine and in its metabolism, which are in part genetically determined. A number of different compounds may in principle interfere with the binding of nicotine with its receptors, while others may interfere with the metabolism of nicotine via the cytochrome P450 system or other pathways. Addiction to nicotine is difficult to measure directly and is usually assessed experimentally with reference to reinforcement assessed in self-administration paradigms.

3.7. Possibilities to make tobacco more addictive or attractive

3.7.1. Introduction

Tobacco products are manipulated by tobacco companies by the addition of chemical compounds, most of which are flavours. Obviously, the flavours are added to the natural tobacco to give the product a better taste thereby increasing the attractiveness of these products. This includes the addition of humectants which keep the humidity of the tobacco product at a desired level; dry tobacco generates an unpleasant harsh smoke.

"Light" cigarettes were introduced on the market in the 1970s. Typical for light cigarettes is their high grade of ventilation. Due to the delivery of less tar, the impact and taste of the "diluted" smoke is also decreased. It is therefore probable that the light cigarettes were "enriched" by adding more substances, and in higher amounts, to compensate for reduced taste and impact. For details see sections 3.5.5 and the different sections reviewing specific tobacco additives such as section 3.8.

An important reason for using additives is to give the product a specific and standardised taste. A specific taste is important for the company to be competitive on the consumer market in view of the large variety of brands available. A unique product binds the customer/consumer to this specific product. The specific taste of a certain product must be preserved (standardised) to compensate for the yearly variation of the natural tobacco, because consumers do not like to smoke a product that changes from year to year. To circumvent this, some 40 or more substances per product are added to the majority of the brands in order to mask the variation.

3.7.2. Additives with direct or indirect addictive potency

In the following two sections, various approaches to increase the addictive and attractive potency of tobacco products have been briefly described. Details of these additives and further information about their effectiveness can be found in later sections (see section 3.8.1).

The addictive potency of tobacco products may in theory be increased by:

1. Direct enhancement of the nicotine content;
2. Addition of substances which increase the bioavailability of nicotine;
3. Addition of substances which facilitate the inhalation of tobacco smoke;
4. Addition of substances which generate compounds in the mainstream smoke which increase the addictiveness of nicotine;
5. Changing the physical properties of tobacco smoke, e.g. particle size.

The five approaches are briefly described below.

1. Direct enhancement of the nicotine content

No examples of increasing the content of nicotine in tobacco are known. Moreover, in cigarettes sold (or produced) in the EU nicotine yield has to remain below a maximal level of 1 mg per cigarette. Some Member States also have upper limits for roll your own (RYO) tobacco. Genetic techniques or classical selection of variants are available to produce tobacco with relatively high nicotine content. From public sources it cannot be deduced or concluded that such approaches are indeed used by tobacco growers or tobacco companies.

2. Addition of substances which increase the bioavailability of nicotine

a) Increase the bioavailability of nicotine by adding alkalising ingredients which increase the pH of tobacco (such as ammonium compounds). At higher pH (pH>8.0) more nicotine is in its free uncharged form, which would therefore more easily pass the (lung) membrane i.e. higher absorption leading to higher blood and brain nicotine levels. For details see section 3.8.3.2 on ammonia and other compounds affecting smoke pH.

b) Increase the bioavailability of nicotine by adding ingredients which serve as a carrier for nicotine.

c) Increase the effect of nicotine by inhibiting its metabolism.

3. Addition of substances which facilitate the inhalation of tobacco smoke

a) Certain ingredients have local anaesthetic effects. As a result coughing due to inhalation of irritating smoke is dampened and the smoker can inhale the smoke deeper (and more frequently). Examples are etheric oils, such as menthol and thymol. For details see later sections e.g. section 3.8.1.

b) Compounds which have bronchodilating properties (opening/broadening the airways) would enable the smoker to inhale deeper (a larger volume of) tobacco smoke implying an increase in the bioavailability of nicotine. It has been proposed that theobromine, generated from cocoa, caffeine and glycyrrhizine, serves such a function.

4. Addition of substances which generate compounds in the mainstream smoke which increase the addictiveness of nicotine

a) Certain natural components in tobacco have been suggested to promote the addictiveness of nicotine. Examples are components like sugars, which when pyrolysed generate acetaldehyde. The combination of acetaldehyde and nicotine appears to be more addictive than nicotine alone. The addition of sugars may thus increase the addictive nature of tobacco products. In tobacco smoke or *in vivo*, tryptophan may react with aldehydes to form beta-carbolines, like harman and norharman. Both beta-carbolines are inhibitors of monoamine oxidases (MAO). Monoamine oxidases are enzymes that degrade neurotransmitters involved in addiction such as dopamine, serotonin and noradrenaline. As such, tryptophan as an ingredient may potentiate nicotine addiction.

b) Acetaldehyde can react *in vivo* with biogenic amines to yield carbolines or isoquinolines, which have affinity for the opiate receptor. These ligands are, however, formed in very low amounts.

5. Changing the physical properties of tobacco smoke, e.g. particle size

Changing the particle size of the tobacco smoke aerosol. Considering the entry of particles to deeper lung levels, there is probably an optimum in size. Cigarette paper and/or filters can be modified in a technological way to attain an optimal particle size (see section 3.5).

The size and its distribution of smoke particles can be changed to obtain an optimum so that particles enter deeper levels of the lungs. As a result, a more efficient absorption of nicotine from the particles and higher blood nicotine levels can be attained. Examples of such applications are the use of cigarette paper with a higher porosity and filters with higher ventilation (see section 3.5).

3.7.3. Additives with attractive properties

A large number of tobacco additives are flavours, which are mostly aromatic compounds or generate aromatic compounds found in the smoke. Flavours are mainly applied for two reasons: firstly, to enhance the attractiveness of a product (appeal to consumers); and secondly, to produce a unique product, typical in "taste" and markedly different from competitor products. The aim here is to get and maintain a certain and stable market share. Note that each of the many flavours is added to tobacco in minute amounts (nano to microgram range per unit). As reported by the tobacco industry to several national competent authorities and as described on tobacco industry websites, cigarettes contain up to 40 (sometimes even more) different additives.

Sugars are natural components of tobacco, but they are also added to tobacco products during manufacturing. The heating of sugars in the tobacco product initiates a caramalisation, generating secondary products which have an attractive smell and taste.

Other additives which may increase the attractiveness of tobacco products, e.g. menthol, are mentioned later (see section 3.8.).

A number of additives have an effect on colour, smell, visibility, taste, and harshness of the smoke.

Note that some additives may fall into several of the above mentioned groups.

3.7.4. Conclusions on addictive and attractive additives

Section 3.7 has provided a preview of the additives used in tobacco which may have addictive or attractive properties. Conclusions about their efficacy are found at the end of the individual sections, which describe their effects in full detail. The addictiveness of tobacco products can theoretically be increased by additives in a number of ways including enhancing the bioavailability of nicotine, promoting smoke inhalation, and influencing particle size. Attractiveness can similarly be improved in a number of ways, such as by adding flavours. Importantly, some additives may at the same time have addictive and attractive properties, or may influence addictiveness indirectly, for example by promoting smoke inhalation.

3.8. Classification of additives

According to the EU Tobacco Products Directive (2001/37/EC) tobacco companies are obliged to provide information about the ingredients added to tobacco products, and their function, to the local authorities. In Germany, this information is published on the website of the Federal Ministry of Nutrition, Agriculture, and Consumer Protection[7]. Consumers can search for brands and ingredients. The reports from 2008 showed the amount of each ingredient listed. However, only the amounts of major ingredients such as sucrose, propylene glycol or cocoa are disclosed to the public. Furthermore, only 22 of the 50 most-used ingredients have been specified by name. In the reports for the general public the tobacco industry does not reveal the nature of all flavourings, colours, or adhesives used. Quantitatively, sugars and humectants (e.g. glycerol, propylene glycol) are the dominant additives in cigarettes. Furthermore, compounds which influence the taste of the cigarette are used in many brands; relevant substances are cocoa (incl. cocoa powder, cocoa extracts, shells of cocoa bean etc.) and liquorice (incl. liquorice extract). Other ingredients are part of the cigarette paper, the filter or are used as glue. Even if the tobacco companies are secretive about the exact amount of flavours used in each brand, some information is available on the websites of the tobacco companies (e.g. BAT[8]). Most of the tobacco companies disclose only the highest amount of ingredients used in their brands (i.e. Quantity Not Exceeded (QNE)). Therefore, it is not possible to draw conclusions about the average amount added or about the percentage of brands that contain a particular ingredient. As an example the information on the Philip Morris website[9] for German cigarettes has been evaluated. In the compilation the maximum use levels are given, i.e. Philip Morris only discloses the highest amount used in its brands. Most of the flavours are added in very small amounts. On the other hand, menthol and lactic acid are flavours used in milligram amounts per cigarette (see table 2). For the calculation it was assumed that each cigarette contains about 700 mg of tobacco.

[7] http://service.ble.de/tabakerzeugnisse/index2.php?site_key=153&site_key=153

[8] http://www.bat-ingredients.com/

[9] http://www.pmintl-technical-product-information.com/aspx/IngredientsInformation.aspx

Table 2 **Ingredients added to the tobacco based on a table presented by Philip Morris International (PMI) on cigarettes manufactured for sale in Germany[9]**

Ingredient	maximal use level (w/w%)	maximal use level (mg/cigarette (700 mg))
sucrose	4.2	29.4
propylene glycol	3.9	27.3
glycerol	2.2	15.4
invert sugar	2.1	14.7
l-menthol	1.1	7.7
d-sorbitol	1.1	7.7
liquorice extract	0.9	6.3
lactic acid	0.7	4.9
guar gum	0.6	4.2
benzoic acid	0.3	2.1
benzoic acid sodium salt	0.3	2.1
carob bean and/or extract	0.2	1.4
cocoa and cocoa products	0.2	1.4
acetic acid	0.01	0.07
lovage extract	0.01	0.07
peppermint oil	0.01	0.07
vanillin	0.01	0.07
benzoin, resinoid	0.005	0.035
phenylcarbinol	0.005	0.035
coffee extract	0.005	0.035
ethyl acetate	0.005	0.035
ethyl hexanoate	0.005	0.035
ethyl vanillin	0.005	0.035
fenugreek extract	0.005	0.035
maltol	0.005	0.035
methyl-cyclopentenolone	0.005	0.035
3-methyl-butyraldehyde	0.005	0.035
orange oil, sweet	0.005	0.035
piperonal	0.005	0.035
spearmint oil	0.005	0.035
veratraldehyde	0.005	0.035
bergamot oil	0.001	0.007
ethyl heptanoate	0.001	0.007
ethyl maltol	0.001	0.007
isoamyl acetate	0.001	0.007
isoamyl formate	0.001	0.007
orris root extract	0.001	0.007
2,3,5,6-tetramethylpyrazine	0.001	0.007
valerian root extract	0.001	0.007

3.8.1. Addictiveness

3.8.1.1. Introduction

Only few scientific articles have addressed the possibility that individual additives may cause addiction. It is probable that many additives have not been examined/analysed or the results (either positive or negative) have simply not been described in publicly available literature.

The available documentation on additives in respect to a direct addictive effect is reviewed in section 3.8.1.2. Examples of additives causing addictiveness indirectly are provided in section 3.8.1.3. Finally, an assessment of how different forms of sugar may have an indirect addictive effect due to combustion products such as acetaldehyde is presented in section 3.8.1.4.

3.8.1.2. Additives with addictive properties (direct effect)

In the peer-reviewed scientific articles assessed there is no documentation for certain individual additives to cause addiction directly.

The following compounds, used as tobacco additives, may have an effect on the central nervous system: acetophenone, isoamyl alcohol, valerian oil, theobromine, and valerenic acid (Lington and Bevan 1994, Moreno 1978a, Moreno 1978b, Moreno 1978c, Oliva et al. 2004, Ortiz et al. 1999, Reynolds 1983a, Reynolds 1983b, Reynolds 1998, Simons et al. 1985, Yuan et al. 2004). However, the fact that these additives may have an effect on the central nervous system (CNS) does not imply that they are addictive. Moreover, they are present in the products in very low amounts.

Although several articles point out that some of the above mentioned additives may create dependence, it is probably more likely that they are acting by attractiveness, as they induce a more pleasant experience of smoking and therefore reduce the barrier in relation to smoking initiation.

3.8.1.3. Additives enhancing addictiveness indirectly

Additives which increase the absorption of nicotine or potentiate in whatever way the effect of nicotine on the nervous system implicitly increase the addictiveness of tobacco products.

Examples of additives

<u>Ammonium salts</u>

It has been proposed that the free nicotine content of smoke increases with increasing pH, which would lead to a higher uptake of nicotine in the bloodstream. A higher pH also increases the nicotine/tar ratio (Wayne and Carpenter 2009) as well as the harshness of the smoke (Hurt and Robertson 1998). The increased harshness will be disguised by using different additives that remove the smoker's sensation of harshness. Ammonium salts are used as additives to increase the pH of tobacco. See Section 3.8.3.2 for full description of ammonia technology.

<u>Menthol</u>

Because of its local anaesthetic properties, menthol allows a deeper inhalation of the irritating tobacco smoke. As such, more smoke could be inhaled and deeper puffs could be attained, resulting in a higher nicotine dose. See section 3.8.3.1 for detailed description of the action of menthol.

<u>Theobromine</u>

Theobromine is found in cocoa beans; therefore this substance is present in cocoa and chocolate, both of which are used as additives in tobacco. Theobromine is a bronchodilator and has been used in the treatment of asthma (Simons et al. 1985). It has been proposed that the bronchodilating effect of the substance may contribute to the absorption of nicotine in connection with smoking (Bates et al. 1999, Fowles 2001). In a document from the New Zealand Ministry of Health (Fowles 2001) it is reported that up to 3% of the weight of cigarettes is cocoa extract and another 0.2% is chocolate. There is typically 0.2% theobromine in cocoa (Rambali et al. 2002). In most of the types of cigarettes containing cocoa and chocolate, which were reported to the Danish competent authorities[10] in 2006, the contents of cocoa and chocolate are 0.3-0.5% and 0.2%, respectively. Based on the information available on the PMI and BAT websites the percentage of cocoa used in cigarettes ranges from 0.2% to 0.66%. Taking this information into account, the content of theobromine per cigarette will be too low to have a bronchodilating effect on the lungs and thereby increase the absorption of nicotine.

Eucalyptol

Like theobromine, eucalyptol has an effect on the lungs as a bronchodilator (Hasani et al. 2003, Juergens et al. 2003). For eucalyptol it is also clear that the contents per cigarette are not large enough to exert this effect. However, even though the doses of theobromine and eucalyptol are so low in cigarettes that they probably do not have a bronchodilating effect, it cannot be excluded that there are other additives with a similar effect.

Lactones

The addictive effect of nicotine may be increased if the metabolism rate of nicotine is reduced. Reduction of the metabolic rate of nicotine, e.g. by inhibition of the metabolic enzymes involved in nicotine degradation, implicates a higher bioavailability of nicotine (nicotine is present in the body for a longer time or at a higher blood level). The additives gamma-heptalactone, gamma-valerolactone, gamma-decalactone, delta-decalactone, gamma-dodecalactone, delta-undecalactone and gamma-hexalactone are mild to weak inhibitors of CYP2A6, an enzyme within the P450 enzyme system, involved in the metabolism of nicotine (Juvonen et al. 2000). However, with IC_{50}-values in the range 560-12,000 µM it seems unlikely that these compounds will inhibit nicotine metabolism at the amounts used in cigarettes.

3.8.1.4. Additives enhancing addictiveness indirectly by combustion of sugar

Sugar is already present naturally in considerable amounts in the tobacco leaf (up to 20%) and the quantities remaining in the final product depend on the curing methods. Sugar in different forms is also one of the most common additives in tobacco (see table 2 in section 3.8). When the sugars, including complex polysaccharides like cellulose (Seeman et al. 2002) in the tobacco product are combusted, various aldehydes are generated. Acetaldehyde is claimed to increase the addictiveness of nicotine in a synergistic way (Belluzzi et al. 2005, Charles et al. 1983, Philip Morris 1992). The mechanism of action may be that acetaldehyde forms secondary condensation products which inhibit monoamine oxidase (MAO).

However, one study showed that even during heavy smoking, acetaldehyde in breath rose six-fold in smokers although only minor amounts of the acetaldehyde in the smoke is absorbed into the blood stream (McLaughlin et al. 1990), suggesting no (indirect) addictive effect of sugars when used as a tobacco additive. Alcohol consumption leads, in contrast to smoking, to a significant increase in the acetaldehyde blood level by its

[10] http://www.sst.dk/Sundhed%20og%20forebyggelse/Tobak/Indberetning/Indberetninger.aspx

metabolism. Acetaldehyde is very reactive and forms adducts with proteins and DNA. Chen et al. (2007b) found only a small contribution of chronic smoking to the formation of acetaldehyde DNA adducts, whereas alcohol consumption had a much higher effect, suggesting again that in chronic smokers lower amounts of acetaldehyde enter the circulation than in alcohol consumers.

Finally, the addition of sugars to tobacco increases the content of acids in the smoke, which results in a lower pH value of the tobacco smoke. This may be one of the reasons why ammonia compounds are added to neutralise these acids.

Examples of sugar additives

The sugars added to tobacco are mainly inverted sugar (fructose and glucose), and sucrose (Philip Morris 2002, Seeman et al. 2003), and are often added in the form of syrups (Covington & Burling 1992, Reynolds 1985). The main part of sugar substances in tobacco is non-volatile and only a small part is transferred unmodified into the mainstream smoke. The sugar substances are not hazardous to health by oral consumption, but are transformed to a number of toxic compounds under pyrolysis. These mainly include formaldehyde, acetaldehyde, acetone, acrolein and furans (Burton 1976). The pyrolysis products have a hazardous effect on health; formaldehyde is classified as a carcinogen to humans (IARC 2006, IARC 2009), whereas acetaldehyde and acrolein are highly irritating to the respiratory tract.

Mono- and disaccharides (natural sugars like glucose, fructose, sucrose)

Mono- and disaccharides are derived from a number of sources including brown sugar, honey, corn syrup, molasses, sugar cane, fig juice and prune juice. Sugars are flavourings that constitute the largest part of additives in cigarettes (Bates et al. 1999). According to table 2 in section 3.8 the levels of sugars applied to the cigarette tobacco blends constitute more than 10% of the total amount of additives. They are added to the tobacco in order to contribute to the taste and flavour (Philip Morris 2002, Reynolds 1985, Reynolds 1994) and increase the content of acids in the smoke, which results in a lower pH value of the tobacco smoke. This reduces irritation and makes the taste milder (Covington & Burling 1986, Covington & Burling 1987a, Seeman et al. 2002).

Inverted sugar is responsible for a large part of the contents of formaldehyde in smoke and also contributes to the formation of furfural, furan, levoglucosan, and acetaldehyde (Baker et al. 2004b, Philip Morris 2002).

Polysaccharides (e.g. cellulose, pectin, starch)

Apart from the sugar substances mentioned, cellulose fibres are a natural part of the tobacco, and are also added as a binding agent (Baker et al. 2004b, Baker 2006, Fox 1993). Pyrolysis of cellulose fibres results in the formation of volatile aldehydes and levoglucosan (Seeman et al. 2002). The amount of pyrolysis products varies depending on the sugar contents and the temperature within the cigarette. It is difficult to estimate the relative contribution of pyrolysis products of simple sugars in relation to polysaccharides (Covington & Burling 1986). The pyrolysis products of polysaccharides and simple sugars are similar, but their yields differ (Fox 1993, Rodgman 2002, Sanders et al. 2003, Seeman et al. 2002). It is estimated that more formaldehyde and less acetaldehyde and acetone are generated from the pyrolysis of simple sugars compared to polysaccharides (Burton 1976).

Addictive potential of acetaldehyde

Animal studies have shown that acetaldehyde can maintain self-administration behaviour equal to, or probably more effectively than, nicotine (Charles et al. 1983, Philip Morris 1992). Belluzzi et al. (2005) found that acetaldehyde has reinforcing properties (Belluzzi et al. 2005).

A number of studies have elaborated on the interaction between nicotine and acetaldehyde (Belluzzi et al. 2005, Cao et al. 2007, Charles et al. 1983, Philip Morris 1992). The combination of nicotine and acetaldehyde increases the degree of self-administration in young rats (Belluzzi et al. 2005). It is possible that norepinephrine contributes to the age-dependent difference in acetaldehyde uptake in rats (Sershen et al. 2009). A study by Cao et al. (2007) shows that acetaldehyde potentiates hyperlocomotive effects of nicotine in young as well as adult rats, but that these effects are more pronounced in adult rats. No effect of acetaldehyde on the nicotine level in the brain was observed (Cao et al. 2007). In Philip Morris publications, the interaction between nicotine and acetaldehyde is examined with the purpose of increasing the reinforcing effect of tobacco (Charles et al. 1983, Philip Morris 1992). The synergistic interaction between nicotine and acetaldehyde is substantiated by experiments where the combination of nicotine and acetaldehyde results in a rewarding effect that exceeds the additive effects of each substance in rats (Philip Morris 1992). It is likely that the combination of nicotine plus acetaldehyde is more reinforcing than nicotine alone, as a long-lasting instrumental conditioned response in young rats was observed (maintains lever pressing at a higher rate than nicotine alone) (Charles et al. 1983, Philip Morris 1992). However, the effect of acetaldehyde seems not to be mediated by opioid receptors in the CNS and the substance does not cause physiological addictiveness (Charles et al. 1983). It is discussed whether acetaldehyde may pass the blood-brain barrier and directly affect the CNS (Cao et al. 2007). It is proposed that acetaldehyde has to be present in high concentrations (>100 μM) in the blood to overcome aldehyde dehydrogenase in the blood brain barrier (Tabakoff et al. 1976). It should be noted that the experiments in animals used intravenous infusion of acetaldehyde, and as mentioned before, it is uncertain whether the acetaldehyde in smoke contributes significantly to the blood level of this substance (Chen et al. 2007b, McLaughlin et al. 1990).

Proposed mechanisms of action

The reinforcing effect of acetaldehyde may be due to the reaction between acetaldehyde and catecholamines, which results in the formation of tetraquinolines (beta-carboline and tetrahydroquinoline) (DeNoble 1994, Philip Morris 1992, Rahwan 1975). Tetraquinoline derivatives may act as false neurotransmitters and therefore promote addictiveness of the product (DeNoble 1994, Rahwan 1975).

Others argue that acetaldehyde has an addictive effect because of the formation of the condensation products harman and norharman, which inhibit the enzyme monoamine oxidase (MAO). Inhibition of MAO results in a slower metabolism of the biogenic amines, like dopamine, noradrenaline and serotonine in the brain, so that the brain levels are increased by MAO-inhibition. However, it is only proven that harman could have significance for tobacco addiction by virtue of its inhibitory effect on MAO-A (Guillem et al. 2006). Indeed, harman is formed in the smoke (0.1 to 5.8 microgram per cigarette). At this level, harman, following its absorption, may be responsible for 3 to 11% of the inhibition of MAO-A (note that drinking a cup of coffee delivers 1 to 8 microgram orally). Nevertheless, whatever the active product, one smoked cigarette decreases MAO in the monkey heart by 25% (Valette et al. 2005). Smokers have decreased MAO-A and MAO-B activities in brain (Fowler et al. 1996), which recovers following smoking cessation. The relevance of this observation in the addiction of tobacco smoking is not clear.

Open issues, acetaldehyde

The levels of isoquinolines generated in the body (by reaction of acetaldehyde with biogenic amines) are too low to be biologically significant. Formation of harman and norharman in the tobacco/cigarette smoke (reaction product of acetaldehyde with tryptophan/tryptamine) is, however, relevant considering their concentration, absorption and inhibitory potency on MAO-A (i.e. IC_{50} value).

There are, however, many conflicting data regarding the presumed pro-addictive effect of acetaldehyde.

1. Following i.v. administration acetaldehyde has an addictive effect in rodents.
2. Acetaldehyde seems not to be absorbed (or is degraded very rapidly in the circulation).
3. The mechanism of action of harman is not well established. For instance, does coffee drinking also lead to inhibition of MAO?
4. Assuming it is not harman which is the active compound, which compounds (acetaldehyde products) are formed which may be responsible for an addictive effect?

3.8.1.5. Denicotinised cigarettes

Nicotine plasma levels are associated with cigarette smoking behaviour and nicotine is considered the main factor driving cigarette addiction. In apparent contradiction to this observation, nicotine replacement therapy, as a smoking cessation treatment, does not show the expected effectiveness. Therefore, it has been assumed that non-nicotine components are important in smoking reinforcement. The exact nature of these factors (chemical composition) is largely unknown, but constituents which provide reinforcing sensory stimulation and/or minimize excessive irritation from inhaled nicotine are considered to play an important role in non-nicotine effects in cigarette smoke (Rose 2006).

In this chapter several studies with denicotinised cigarettes are briefly described to highlight the importance of the non-nicotine components in tobacco.

Denicotinised cigarettes have the appearance, draw and taste of standard cigarettes but contain (and deliver) virtually no nicotine (<0.06 mg), but deliver tar and carbon monoxide (CO) in a comparable way to traditional cigarettes (Pickworth et al. 1999).

In short term (for a few hours; maximum up to 24 hours) experiments, smoking volunteers were placed under tobacco (nicotine) abstinence and were allowed to smoke denicotinised or conventional cigarettes.

- In 1999, Pickworth et al. reported that the denicotinised cigarettes did not increase heart rate or activate the EEG, but subjects reported that both conventional and denicotinised cigarettes reduced (subjective) measures of tobacco craving and withdrawal (Pickworth et al. 1999).
- In a study by Eid et al. (2005) a stimulating effect on heart rate of denicotinised cigarettes was reported. Smoking of either denicotinised or conventional cigarettes caused a significant reduction in the craving score. The authors could not find a correlation between the nicotine yield and behavioural effects.
- Perkins et al. (2010) simulated different stressful situations (negative affects) during smoking abstinence and studied how relief was perceived after smoking. The authors did not find an association between the relief of several negative affects and smoking (also not from denicotinised cigarettes) but the relief was not dependent on nicotine intake, therefore, challenging the assumption that nicotine in smoking alleviates negative affects.
- Brody et al. (2009) found that, compared to conventional cigarettes, smoking denicotinised cigarettes (0.05 mg nicotine) resulted in a decrease in occupancy of the brain nicotine acetylcholine receptor (nAChR), as predicted on the basis of nicotine concentration. They did not observe occupancy of the nAChR with other factors, suggesting that only nicotine in cigarette smoke is capable of binding this receptor (Brody et al. 2009).

These acute studies show that denicotinised cigarettes, compared to conventional cigarettes, do not exert the same pharmacological effects, but cravings and symptoms of withdrawal can be diminished and this phenomenon is, in many cases, independent of the delivered nicotine. Some components of tobacco smoke, other than nicotine, may be biologically active; thus it has been suggested that non-nicotine components of tobacco smoke decrease brain levels of monoamine oxidase A and B which possibly change sensitivity to the actions of nicotine and/or exert independent behavioural effects (Eid et al. 2005).

Recently, Rose et al. (2010b) found that denicotinised smoke was self-administered more than any other alternative (i.v. nicotine self-administration or sham puffs) in established smokers, even after a few days of nicotine abstinence. This preference for denicotinised smoke compared to i.v. nicotine was inversely correlated with subjective ratings of "comfort" (normally) associated with nicotine; therefore non-nicotine aspects of cigarette smoking have potent reinforcing effects in established smokers. These authors, therefore suggested that in contrast to current smoking cessation pharmaco-therapies, which address only the nicotine component of nicotine (tobacco) addiction, future cessation strategies should also be designed to target non-nicotine factors such as added flavour constituents (e.g. menthol).

In conclusion, besides nicotine, a mixture of other factors in cigarette smoke probably plays an important role in craving and reinforcement. Although these unknown factors do not have pharmacological effects similar to nicotine and are probably not addictive, they definitely play a role in smoking behaviour.

3.8.1.6. Conclusions on how additives can increase the addictiveness of tobacco products

Certain tobacco additives may affect the central nervous system in smokers directly, but their concentration in tobacco products is probably too low to have a physiological effect. However, an indirect addictive effect of certain substances cannot be excluded.

Some additives increase the pH of the smoke, thereby increasing the quantity of nicotine delivered to the smoker.

Sugars generate acetaldehyde during combustion. When given intravenously to animals, acetaldehyde potentiates the addictive effect of nicotine. The mechanism of action of the reinforcing effect of acetaldehyde in animals is not clear, although an inhibition of MAO is the most likely reason. Inhibition of MAO has also been observed in human smokers. However, the acetaldehyde, generated from the sugars during combustion, is presumably not absorbed into the blood stream, and this sheds some doubt on the role of sugars in the addictiveness of tobacco products.

Natural tobacco already contains considerable amounts of sugars, especially Virginia tobacco. In addition, polysaccharides and cellulose fibres in the tobacco leaves generate acetaldehyde upon combustion. In this respect it is not clear whether the addition of sugars to tobacco leads to a significant increase in the addictiveness of the product.

The data in the literature on the presumed indirect addictive effect of sugars (exerted via the generation of acetaldehyde) are inconclusive.

3.8.2. Attractiveness

3.8.2.1. Introduction

A number of additives increase the attractiveness of tobacco products. This may be attained by creating a better experience of the product (e.g. appearance of the product, white and full smoke) or by making it easier to start smoking (e.g. by means of a cool, sweet and mild smoke, as well as causing less irritation in the lungs).

For many additives, attractiveness depends on multiple functions which may be difficult to distinguish clearly. One of the reasons to use additives is to attract the smoker to a specific product and to promote/encourage (young) people to start using the product.

Other reasons for using additives are to produce a unique product, typical in taste and markedly different from competitor products, and to maintain the stability of the taste of the product.

3.8.2.2. Better experience of the product

Preservation of humidity of the tobacco product

Humectants are added to tobacco products to retain the water, i.e. to prevent them from drying out, and consequently increase the shelf life of the products.

Examples of additives

Glycerol, propylene glycol and sorbitol.

Appearance, smell and irritation of tobacco smoke

In order to make the smoke more attractive to the smoker, but also to other people in the proximity of the smoker, it is important that the smoke is appealing and not annoying. This may be attained with additives which make the smoke whiter and more attractive to people seeing the smoke. The smell of the smoke may be also changed so that it is also more attractive and less irritating (Connolly et al. 2000, Ling and Glantz 2005).

Connolly et al. (2000) examined tobacco industry patents covering the function of environmental tobacco smoke masking. These strategies include reducing smoke odour, and reducing side-stream smoke visibility and emissions.

Methods to neutralize or reduce lingering smoke odour include addition of acetylpyrazine, anethole and limonene to modify the side-stream odour. These compounds have rather low odour thresholds, and are subsequently easily picked up, while they elicit no trigeminal nerve response. Aroma precursors, e.g. polyanethole provided a noticeable fresher, cleaner and less irritating cigarette side-stream aroma, while others (e.g. cinnamic aldehyde, pinanediol acetal) produce slightly sweet, spicy, clean, fresh, and less cigarette-like aroma. Also, more "classic" additives (e.g. vanillin, benzaldehyde, bergamot oil, cinnamon/cinnamon extract, coffee extract and nutmeg oil) modify side-stream odour.

Reduced visibility of side-stream is accomplished by the addition of magnesium oxide, magnesium carbonate, sodium acetate, sodium citrate and calcium carbonate to the wrapper (cigarette paper). This has an effect on particle size; particles become smaller and therefore do not easily scatter light and become less visible.

Reducing side-stream emissions is based on encapsulating the smoke in an impermeable cone using different types of additives such as potassium succinate, potassium citrate and magnesium carbonate.

By combining the use of additives and the look of the tobacco product, greater acceptance of the smoke may be created. Less resistance may be encountered from persons that do not smoke, and at the same time greater pleasure for the smoker may be created. The same agents may also be used to target the individual product at certain target groups (Carpenter et al. 2005a, Connolly 2004).

Taste and experience of the smoke

Cis-3-hexenol is added to increase the organoleptic characteristics of tobacco and it has a characteristic smell of new-mown grass (Alford and Johnson 1970). *Cis*-3-hexenol adds a green, foliaceous taste and a smell of chlorophyll to the tobacco smoke (Leffingwell et al. 1972). Apart from adding a taste and flavour of fresh tobacco to the tobacco smoke, the substance has another important characteristic: *cis*-3-hexenol reduces irritation (Alford and Johnson 1969).

The American tobacco company Brown & Williamson has tested the effect on the characteristics of the smoke when adding *cis*-3-hexenol to cigarettes (Alford and Johnson 1969, Alford and Johnson 1970). Cigarettes with added *cis*-3-hexenol in concentrations of 0.05, 0.10 and 0.15 mg per cigarette were tested against control cigarettes without added *cis*-3-hexenol by having an expert panel smoke the various cigarettes. All cigarettes with *cis*-3-hexenol were preferred to the control cigarettes (Alford and Johnson 1969, Alford and Johnson 1970). The effect of *cis*-3-hexenol was *"A dramatic increase in smoke freshness and acceptability. Irritation is also markedly reduced."*

Harshness

According to the tobacco industry definition, harshness is a chemically induced physical effect associated with a roughness, rawness experience generally localized in the mouth and to a lesser degree in the upper reaches of the throat and the trachea due to inhalation of tobacco smoke. Harshness can also cause a drying, rasping, coarse, astringent sensation usually associated with the smoke flavour of Virginia or air-cured type tobaccos.

Harshness is classically measured in four degrees: (i) Free – an absence of harshness; (ii) Touching – a slight awareness of a sensation; (iii) Scratchy – some discomfort, a stinging effect; and (iv) Harsh – rough, raw, raspy, coarse, astringent, painful inhalation.

Reducing the harshness of the smoke makes it possible to inhale deeper and increase the number of puffs, as more physical barriers will be reduced (Wayne and Henningfield 2008b).

The ratio between nicotine and tar is an important parameter in relation to the smoker's experience of the cigarette. If the concentration of nicotine in relation to tar is too high, the harshness of the smoke will be much higher (Hurt and Robertson 1998). Nicotine is irritating in high doses compared to other substances in the smoke (Baker 1990).

The irritating effect of nicotine on the lungs and the bad experience at too large amounts of nicotine in relation to the amount of tar may be remedied by additives that may drown or reduce the harshness of the smoke. This may also be achieved by adding nicotine salts that do not cause the same irritation, but are still delivering nicotine or keeping the nicotine effect by means of a quicker absorption by ensuring larger amounts of free nicotine (Bates et al. 1999, Keithly et al. 2005).

Smoothness

Tar provides a strong flavour and mouth sensation, masking the harsher, bitter taste of nicotine which may be unpalatable to new smokers and uncomfortable to established smokers. Certain highly flavoured additives may also have the same properties to "smoothen" or reduce the harsh irritation of nicotine in tobacco smoke.

A central feature of tobacco marketing strategy has been to promote the perception that some cigarettes are less hazardous than others, so that smokers worried about their health are encouraged to switch brands rather than quit. Products bearing the word "smooth" or using lighter coloured branding mislead people into thinking that these products are less harmful to their health. Adults and children are significantly more likely to rate packs with the terms "light", "smooth", "silver" and "gold" as lower tar, lower health risk and either easier to quit (adults) or their choice of pack if trying smoking (children). For example, more than 50% of adults and youth reported that brands labelled as "smooth" were less harmful than the "regular" variety. The colour of packs was also associated with perceptions or risk and brand appeal. For example, compared to Marlboro packs with a red logo, cigarettes in packs with a gold logo were rated as lower health risk by 53% and easier to quit by 31% of adult smokers.

Plain packs significantly reduced false beliefs about health risk and ease of quitting and were rated by the children as less attractive and appealing (Hammond et al. 2009a).

Examples:

Propylene glycol

The addition of propylene glycol (1,2-dihydroxypropane) to tobacco results in a milder smoke (Danker 1958). It was found that propylene glycol reduces the delivery of nicotine, while the formation of tar is increased (Shepperd and Bevan 1994b). In another study, also by the Brown & Williamson Tobacco Company, a reduction of nose irritation was observed and a reduced delivery of nicotine was confirmed (Shepperd 1994a). It was suggested that the sensation of reduced effect and irritation in cigarettes with added propylene glycol is caused by reduced liberation of nicotine, since the tar/nicotine ratio is of importance to the sharpness of the smoke (Danker 1958, Shepperd and Bevan 1994b).

Levulinic acid and levulinates

Based on the information submitted by the tobacco industry to the competent authorities of the EU Member States, these two substances have in many cases not been included in the reports, but have been used and mentioned several times in the internal documents of the tobacco industry.

These organic salts would also be able to reduce the harshness of nicotine, as the salts do not cause the harshness that otherwise characterise high levels of nicotine (Bates et al. 1999). In a study of the published literature up until 2004, Keithly has also shown that the primary purpose of levulinic acid as an additive in tobacco is to make the smoke sweeter and softer and at the same time increase the nicotine absorption and the effect of nicotine in the brain. Keithly also describes the use of nicotine levulinate and levulinic acid to cause less harshness (Keithly et al. 2005).

3.8.2.3. Easier to start smoking

Tobacco products may also be designed in such a way that they are easier to start smoking with. This may be attained by making it easier to inhale the smoke in the lungs and by creating a sweeter, milder or "colder" smoke. By reducing and changing the harshness of the smoke, special target groups may be reached (Carpenter et al. 2005a, Carpenter et al. 2005b, Cummings et al. 2002, Klein et al. 2008, Wayne and Connolly 2002).

In a number of countries, sweet and tasteful tobacco products are the most preferred tobacco products among children and adolescents as well as experimenting smokers (Ashare et al. 2007, Giovino et al. 2005, Klein et al. 2008).

How to make inhalation of smoke less aversive

Liquorice

Glycyrrhizin is the active substance of liquorice i.e. the root extract of Glycyrrhiza glabra and has a sweet taste (Hodge and Shelar 1979). Apart from glycyrrhizin, liquorice also contains sugar substances, cellulose fibres and essential oils (Covington & Burling 1987b).

The taste and flavour of tobacco with liquorice/liquorice root added are described as sweet, woody and round (Leffingwell et al. 1972), but adding liquorice/liquorice root also has the objective of camouflaging the unpleasant taste of tobacco (Covington & Burling 1987b).

The use of adding liquorice/liquorice root to tobacco has the following advantages (Vora 1983); it reduces the harshness of tobacco smoke, the dryness in the mouth and throat, and it provides a pleasant sweet undertone to the smoke.

Menthol

The additive menthol is relevant for how a smoker experiences the smoke in the lungs and the concentration of menthol may be an important issue for the group that the cigarette brand is targeted at. This is described further in section 3.8.3.1, which broadly outlines the potency of menthol to inhale smoke more easily and deeply.

Cooler and milder smoke

Certain substances make the smoke milder and cooler, e.g. menthol (see section 3.8.3.1), liquorice and propylene glycol. However, many more additives probably have these effects on the smoker's lungs, but they have not yet been evaluated, or have not been described in the literature.

Sweeter taste

The presence of sugars in cigarettes is associated with a more favourable taste. The experience of the smoke is less negative and the irritability is somewhat masked.

The tobacco producers have used additives that create sweetness and taste in the smoke to make it easier for new smokers to start smoking, since these tobacco products do not have the same harshness and bad experience at the first inhalations (Cummings et al. 2002, Wayne and Connolly 2002).

3.8.2.4. Conclusions on how certain additives can increase the attractiveness of tobacco products

The attractiveness of tobacco products may be increased by a number of additives. An attractive effect may be obtained in a number of ways, such as changing the appearance of the product and the smoke, decreasing the harshness of the smoke, and inducing a pleasant experience of smoking. The harshness depends partly on the tar/nicotine ratio, but may also be decreased by certain additives such as propylene glycol or levulinates. Various sugars constitute a large proportion of additives, and the sweetness of the smoke is an important characteristic.

Many different additives are used to create a specific taste/flavour in order to attract certain target groups. In order to make the smoke less aversive and permit deeper inhalation, additives such as liquorice and menthol are used. Finally, in order to make smoking more acceptable to people around, some additives have the function of reducing lingering odour or side-stream smoke visibility.

3.8.3. Most prominent additives in tobacco products

3.8.3.1. Menthol

Menthol is an important tobacco additive and it is the only additive explicitly declared to the consumer. For more than 40 years, scientific discussions have covered the health effects of the addition of menthol to tobacco. Menthol is a monocyclic terpene alcohol. It is a naturally occurring compound of plant origin which gives plants of the *Mentha* species the typical minty smell and flavour (Eccles 1994). Mentholated cigarettes have a major share of the market in the USA. However, in most European countries, the market shares for mentholated cigarettes range between 1 and 5% (Giovino et al. 2004). The menthol content has been investigated in the USA in 48 commercially available mentholated cigarette sub brands. Menthol content per g tobacco was reported to range between 2.88 and 5.75 mg menthol (Celebucki et al. 2005). In Germany, the menthol content was analyzed in non-mentholated cigarettes as well as in raw tobacco. Menthol content in raw tobacco and home grown tobacco was in the range 0.02-0.18 µg menthol/g tobacco. Menthol content per g tobacco in non-mentholated cigarettes ranged between 0.019 and 13.3 µg menthol (Merckel et al. 2006). These data clearly prove three points: firstly, menthol occurs naturally in very small amounts in tobacco; secondly, some brands contain no added menthol at all and in some brands, microgram amounts of menthol have been added; and finally, mentholated brands contain milligram amounts of menthol per g tobacco.

The tobacco industry advertises menthol as a substance which alleviates harshness and enhances taste and smoothness, but menthol may also facilitate nicotine delivery and increase the sensory impact of cigarettes.

Menthol can be applied to cigarettes in a number of ways; it can be applied directly to the tobacco or introduced into the cigarette filter, or it can be applied to the cigarette packaging (see section 3.4.).

The fate of menthol in the cigarette has only been investigated by the tobacco industry. Philip Morris showed with ^{14}C-labelled menthol that 29% of menthol went into the mainstream smoke (Jenkins et al. 1970). The transfer of menthol from tobacco into smoke was investigated by another company in 11 cigarette brands; the values ranged from 19 to 31% (Brozinski et al. 1972).

A report by Schmeltz and Schlotzhauer raised concerns about the pyrolysis of menthol. The authors pyrolysed menthol under nitrogen at 860°C and analysed the pyrolysate by paper-chromatography and thin-layer chromatography. They found approximately 400 µg benzo[a]pyrene per g menthol (Schmeltz and Schlotzhauer 1968). In the following 40 years only one study conducted by the tobacco industry addressed this question again: Baker and Bishop heated menthol at 30°C per second from 300 to 900°C under a flow of 9% oxygen in nitrogen. The products were analysed by gas chromatography and mass spectrometry. The authors found 99% of menthol was unchanged in the gas phase; additional products were menthon (0.9%) and menthen (0.1%) (Baker and Bishop 2004a). No further data have been found on this topic.

Some companies have investigated the influence of tobacco additives on the composition of smoke constituents. For example, Philip Morris studied experimental cigarettes with many additives. They prepared two sets of cigarettes containing, among other additives, 18.000 ppm menthol, yielding 13 mg menthol per cigarette (Carmines 2002). The cigarettes were machine-smoked and compared to control cigarettes without ingredients added. The benzo[a]pyrene content in the smoke of menthol cigarettes was significantly higher compared to the smoke of the control cigarettes. The smoke of the control cigarettes contained 5.1 ng benzo[a]pyrene per cigarette in comparison to 5.63 and 5.51 ng benzo[a]pyrene per cigarette in menthol cigarettes (Rustemaier et al. 2002).

The hypothesis that smoking mentholated cigarettes increases lung cancer risk compared with smoking non-mentholated cigarettes was tested in several epidemiological studies. Sidney and colleagues found a 1.45-fold increase of the relative risk for men smoking mentholated cigarettes for 20 years and more (Sidney et al. 1995), whereas three other studies (Brooks et al. 2003, Carpenter et al. 1999, Stellman et al. 2003) did not find a difference between menthol smokers and non-menthol smokers.

Menthol has a cooling effect on the skin or mucosal surfaces. The perceived temperature effect is not caused by evaporation of menthol. Furthermore it is not due to vasodilatation, but is due to a specific action on sensory nerve endings (Eccles 1994). Menthol activates a transient receptor potential channel (TRPM8). This channel is expressed in small-diameter primary sensory neurons (Clapham et al. 2005). The use of menthol causes a subjective sensation of improved airflow without any change in nasal airway resistance, breathing pattern or ventilation (Eccles 1994, Nishino et al. 1997). Furthermore, menthol has a local anaesthetic activity (Galeotti et al. 2001).

It is important to take into account that this cooling and anaesthetic effect may mask early symptoms of tobacco induced respiratory disease (Garten and Falkner 2003). In a follow-up paper, it was postulated, that there is a greater opportunity for exposure and transfer of the contents of the lungs to the pulmonary circulation. For the smoker of mentholated cigarettes this could result in a greater exposure to nicotine and the particulate matter of the smoked cigarette (Garten and Falkner 2004). Additionally, it was postulated that menthol increases the absorption with other chemicals through permeability and increased salivation. This would mean that menthol facilitates the absorption of other substances from the smoke (Ahijevych and Garrett 2004, Eccles 1994). Two recent biomarker studies addressed the question if the use of mentholated cigarettes would lead to higher exposure to toxic compounds from smoke (Heck 2009, Muscat et al. 2009). Muscat and colleagues investigated a group of 525 smokers and stratified them for sex and race. In the United States, most African Americans smoked mentholated cigarettes (90% and 82%, respectively); whereas European Americans smoked predominantly non-mentholated cigarettes (percentage of menthol cigarettes smoked was 25% and 31%, respectively). European Americans smoked significantly more cigarettes per day than African Americans. There were no significant differences in the mean concentrations of all cigarette smoke metabolites (plasma cotinine, urinary cotinine, plasma thiocyanate and urinary 4-N-nitrosomethylamino)-1-(3-pyridyl)-1-butanol (NNAL)) between menthol and non-menthol cigarette smokers in African Americans and European Americans, after adjustment for sex and other factors (Muscat et al. 2009). However, the ratio of NNAL-glucuronide to NNAL, a possible indicator of lung cancer risk, was significantly lower in menthol versus non-menthol cigarette smokers. The NNAL-Gluc/NNAL ratio was 34% lower in European Americans ($P<0.01$) and 22% lower in African Americans (Muscat et al. 2009). In subsequent human liver microsome studies, menthol inhibited the rate of NNAL-*O*-glucuronidation and NNAL-*N*-glucuronidation. These results suggest that menthol may modify the detoxification of the potent lung carcinogen NNAL (Muscat et al. 2009).

A similar study has been performed and published by the tobacco industry (Heck 2009). They investigated 112 smokers (28 African Americans and 84 European Americans; 54 menthol cigarette smokers and 58 non-menthol cigarette smokers). Smokers continued smoking *ad libitum* throughout the one week study interval. The participants were provided with a commercially available menthol cigarette brand and several non-mentholated brands of similar smoke yield. Menthol content in smoke was determined as 0.34 mg/cigarette. Content of 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) was determined as 63 ng/cigarette in the mentholated brand and with a range from 45 to 80 ng NNK/cigarette in five non-mentholated brands (Heck 2009). Neither total urinary NNAL nor urinary nicotine equivalents exhibited statistically significant differences between the menthol and non-menthol cigarette smokers (Heck 2009).

The possible influence of menthol on the metabolism of nicotine was investigated in a cross-over study in 14 healthy smokers (Benowitz et al. 2004). Subjects were randomly assigned to smoke mentholated or non-mentholated cigarettes for one week, then to cross over to the other type of cigarettes for another week. The blood levels of deuterium-labelled nicotine and cotinine were measured after intravenous infusion of these compounds. It was demonstrated that, when smoking similar numbers of mentholated and non-mentholated cigarettes of similar machine-determined yield and nicotine content, the systemic intake of nicotine and carbon monoxide during non-menthol cigarette smoking is on average not affected by mentholation. Furthermore, it was shown that mentholated cigarette smoking inhibits the metabolism of nicotine. Inhibition of nicotine metabolism by menthol most likely involves inhibition of both oxidative metabolism to cotinine, and glucuronide conjugation (Benowitz et al. 2004). *In vitro* studies using human liver microsomes showed that menthol inhibits nicotine metabolism (MacDougall et al. 2003) However, mentholated cigarette smoking did not substantially affect cotinine metabolism. Finally, the systemic intake of menthol was determined as 12.5 mg menthol from 20 cigarettes. Thus, on average 20% of menthol contained in each cigarette is absorbed systemically by the smoker (Benowitz et al. 2004).

Studies on the influence of menthol on puff numbers and puff volume gave conflicting results. Puff numbers have been investigated in seven studies, three showing a reduced number of puffs in smokers of mentholated cigarettes (Jarvik et al. 1994, McCarthy et al.

1995, Nil and Bättig 1989). Four other studies did not show any influence of mentholation on the number of puffs (Ahijevych et al. 1996, Caskey et al. 1993, Miller et al. 1994, Pickworth et al. 2002). Puff volume was investigated in six studies, three of them showing a decrease in puff volume when smoking mentholated cigarettes (Jarvik et al. 1994, McCarthy et al. 1995, Nil and Bättig 1989). Two studies did not find any effect of mentholation on puff volume (Ahijevych et al. 1996, Miller et al. 1994) and one study even showed an increase in puff volume (Ahijevych and Parsley 1999).

The results of studies on the CO exhalation in smokers of mentholated and non-mentholated cigarettes are contradictory. In a study with experimental cigarettes smokers inhaled defined volumes of cigarette smoke. The experimental cigarettes had been injected with 0 mg, 4 mg or 8 mg of menthol. The CO content in exhaled air increased from 5.6 ppm to 6.1 ppm and reached 8.1 ppm CO after use of 8 mg menthol cigarettes (Miller et al. 1994). Clark and colleagues did find a non-significant difference of 40.3 ppm CO (mentholated cigarettes) against 35.8 ppm CO (non-mentholated cigarettes) (Clark et al. 1996). In a study in women, smokers of non-mentholated cigarettes showed a higher CO exhalation (10.6 ppm) than smokers of mentholated cigarettes (6.5 ppm) (Ahijevych et al. 1996). In a cross-over study, Benowitz and colleagues did not find any significant difference in the blood carboxyhaemoglobin content in smokers of mentholated and non-mentholated cigarettes (Benowitz et al. 2004). Six other studies also did not show significant differences between CO uptake or CO exhalation in smokers of mentholated or non-mentholated cigarettes (Caskey et al. 1993, Heck 2009, Jarvik et al. 1994, McCarthy et al. 1995, Nil and Bättig 1989, Pickworth et al. 2002).

Menthol may increase the degree of dependence, or promote maintenance of smoking behaviour. Several findings suggest that menthol is involved in tobacco addiction. Some investigators have found that menthol cigarette use increases cotinine levels, and a significant correlation between cotinine and nicotine dependence has been reported, as well as a reduction in time to first cigarette of the day (Pomerleau et al. 1990).

Greater smoking urgency among menthol compared to non-menthol adolescent cessation-treatment seekers has been reported (Collins and Moolchan 2006).

Evaluating the tobacco industry documents, it was shown that cigarettes with low contents of menthol appeal to young smokers, new smokers, and smokers that do not like the harshness of the smoke. This can be due to the fact that lower contents of menthol in the smoke cover the harshness of the smoke, whereas a large dose of menthol causes harshness. On the other hand, cigarettes with a higher concentration of menthol appeal to smokers who are used to the harshness of the smoke (Kreslake et al. 2008b).

3.8.3.2. Ammonia and other additives affecting smoke pH

Armitage et al. (2004) described a study in which 10 volunteers smoked either control cigarettes, cigarettes with diammonium hydrogen phosphate (DAP) or cigarettes with urea added. The venous blood levels of nicotine were independent of the amount of DAP or urea added to the tobacco. Preliminary data of a human study performed by van Amsterdam et al. (to be published), comparing two commercial brands (one with low and one with high ammonia content) with respect to nicotine absorption, showed no difference in venous blood nicotine levels (no difference in total absorption and peak plasma of nicotine) when smoking the two brands.

The bioavailability of nicotine is dependent upon the pH as only uncharged nicotine is volatile and can be absorbed readily across cell membranes. The different ways of manipulating cigarettes so that more free nicotine is delivered have recently been reviewed (Wayne and Carpenter 2009). At lower pH the nicotine molecule will be positively charged and an equilibrium between the three forms of nicotine is created in relation to the pH (see figure 3).

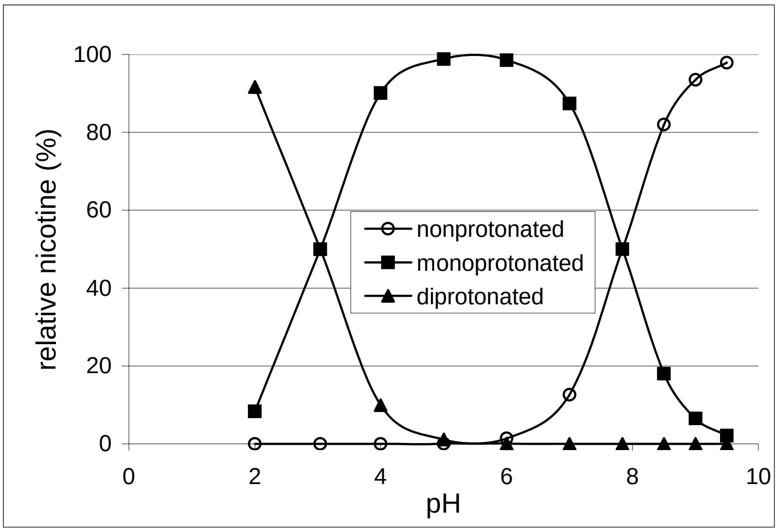

Figure 3: Chemical form of nicotine (charged or free base) and their percentages as function of pH, ranging from 2 to 9.5 (adapted from Hoffmann and Hoffmann (1997)).

Initially, cigarette smoke is lightly acidic and the nicotine is therefore poorly absorbed. However, the pH value is higher in the lungs (7.4) and some of the nicotine is found in uncharged form. Internal documents from the tobacco industry show that manufacturers started to use ammonia to increase smoke pH levels in the early 1970s (Willems et al. 2006). Particular focus has been on ammonia and related compounds, but any compound that contributes to increasing the pH value will have a potential effect in increasing the impact of nicotine and the rate at which inhaled nicotine is absorbed into the bloodstream.

While it has been shown that the absorption of nicotine in smokeless tobacco by the oral mucosa is dependent on the pH of the product (Fant et al. 1999), it is uncertain if the pH in cigarette smoke has a significant impact on the nicotine absorption in the lungs. This is due to the high local buffering capacity of the lung lining fluid which will cause free nicotine to be charged (protonated) again in the deeper airways (Willems et al. 2006). The high buffering capacity of mucus has been shown experimentally in human volunteers (Holma and Hegg 1989).

It is widely accepted that smoke from different pyrolysed tobacco delivery devices (e.g. cigarettes, cigars, waterpipes, etc.) is inhaled differently. For example, cigarette and waterpipe smoke tends to be inhaled into the lungs, while cigar smoke is typically only inhaled into the mouth (except among former cigarette smokers who have switched to cigar smoking, in which case they often smoke cigars like cigarettes). It has been argued that this may be due to the characteristics of both the delivery device (for example, waterpipes cool the tobacco smoke, thereby allowing easier, deeper inhalation) and the tobacco itself. Waterpipe smoking is associated with greater smoke exposure (a larger volume of smoke is inhaled) than cigarette smoking (Maziak et al. 2009).

This difference in inhalation may be due in part to the more acidic pH of cigarette smoke. The smoke of most cigars has an alkaline pH; as a result, nicotine contained in the smoke can be readily absorbed across the oral mucosa without inhalation into the lung. The more acidic pH cigarette smoke produces a protonated form of nicotine which is much less readily absorbed by the oral mucosa, and the larger absorptive surface of the lung is required for the smoker to receive the desired dose of nicotine. According to the

National Cancer Institute (NCI), cigarette smokers must inhale to ingest substantial quantities of nicotine (the active agent in smoke), whereas cigar smokers can ingest substantial quantities of nicotine without inhaling (NCI 1998). The difference may, however, also be explained by the fact that cigar smoke is more concentrated and contains much more nicotine than cigarette smoke.

While there has been considerable research into the effects of product characteristics on cigarette smoking behaviour (such as ventilation holes in "light" cigarettes resulting in compensatory smoking whereby smoke is inhaled more deeply to extract the required dose of nicotine), there is relatively little research into the effects of other delivery devices such as waterpipes. This is despite the rapid growth in the popularity of waterpipe smoking in European countries in recent years.

3.8.3.3. Conclusions on most prominent additives

Menthol is one of the most prominent additives in tobacco. If it is added in milligram amounts to cigarettes it dominates the taste of the smoke and the application is usually mentioned in the brand name. Menthol has a cooling effect on mucosal surfaces and a local anaesthetic activity. The use of menthol causes a subjective sensation of improved airflow without any change in nasal airway resistance, breathing pattern or ventilation. It has been proposed, that the cooling and local anaesthetic effects could lead to deeper inhalation of the smoke and higher exposure to other smoke constituents, but current data are inconclusive. However, menthol has been shown to inhibit the metabolism of nicotine. Furthermore, the taste of menthol could be an important reason for some smokers to consume mentholated cigarettes.

It has been proposed that the addition of ammonia compounds increases the absorption of nicotine in the lungs by raising the pH in smoke, but this seems unlikely because of the high buffering capacity of the lung lining fluid.

3.8.4. Additives in tobacco products other than cigarettes

3.8.4.1. Cigars

Very few additives are used in the classical manufacture of cigars; recently marketed cigarillos being an exception. In general, cigar brands contain only glue as an additive; several compounds are used as glue (e.g. ethyl-2-hydroxy ethyl cellulose, sodium carboxy methyl cellulose, gummi arabicum, methyl hydroxy ethyl cellulose). Several brands contain humectants such as propylene glycol or glycerol. Citric acid is added to influence the burning properties of the cigars. Some companies sum up their flavouring ingredients as "flavouring", whilst others mention all compounds, including the amounts used.

As written earlier, in Germany, the information about ingredients of cigars can be found on the website of the Federal Ministry of Nutrition, Agriculture and Consumer Protection[7]. Consumers can search for brands and ingredients.

Data from 2008, published on this website, showed that many of the flavourings were added in tiny amounts of 1 ppm. However, other flavourings such as 2-methylburic acid were added at a level of 60 ppm and ethyl vanillin was added at levels up to <0.5%. Some cigar manufacturers disclosed probably most, if not all of the additives, for example 211 additives are listed for the brand "7B Bonajuto" starting with 34 mg dextrose down to 8 µg clary sage oil.

3.8.4.2. Pipe tobacco

Pipe tobaccos contain humectants (e.g. glycerol and propylene glycol), preservatives (e.g. sodium benzoate, potassium sorbate), sweetening agents (e.g. dextrose, fructose, invert syrup, honey) and many flavours (e.g. cocoa, prune flavour, apple treacle concentrate, tamarind extract).

The ingredients reported in 2009 in Germany can also be found at the website of the Federal Ministry of Nutrition, Agriculture and Consumer Protection[7].

3.8.4.3. Water pipes

The use of waterpipes has increased in the eastern Mediterranean region since the 1990s with the introduction of maassel, a sweetened and flavoured tobacco (Maziak et al. 2004a). During recent years, the smoking of waterpipes has become a habit among teenagers in Germany and other European countries, and in the USA (BZgA 2008, Jackson and Aveyard 2008a, Primack et al. 2008). The mild, sweet and flavoured tobacco appeals to many waterpipe smokers, especially young smokers. No information is available about the flavours used in waterpipe tobacco. The nicotine content in flavoured waterpipe tobacco ranged from 1.8 to 6.3 mg nicotine/g tobacco; the average was 3.35 mg nicotine/g tobacco. In contrast, the traditional waterpipe tobacco without flavour contained 30 to 41 mg nicotine/g tobacco (Hadidi and Mohammed 2004).

There are major differences in the consumption of waterpipes compared to other tobacco products. In contrast to cigarettes and cigars, the tobacco in waterpipes is not burned. The waterpipe tobacco is placed in the tobacco head, which is covered by a perforated aluminium foil on which the glowing charcoal is placed. In a study in Lebanon, Shihadeh measured the temperature during a waterpipe session. Within 15 minutes the foil reached a temperature of 400 to 450°C, whereas the waterpipe tobacco reached temperatures ranging from 60°C (after 10 minutes) to 120°C (after 50 minutes) (Shihadeh 2003). To prevent the tobacco from burning, high amounts of humectants are added to waterpipe tobacco. Besides glycerol and propylene glycol, the companies use honey and molasses. The resulting smoke is very mild and it is easy to inhale, even for inexperienced smokers. Since the smoke has almost no harshness the smoker can inhale huge volumes. Some waterpipe smokers refuse to smoke cigarettes. Waterpipe smokers inhale between 0.3 and 1.0 l per puff (Eissenberg and Shihadeh 2009, Monn et al. 2007, Shihadeh 2003, Shihadeh et al. 2004) compared to approximately 0.050 l per puff in cigarette smokers (Kozlowski and O'Connor 2002).

In Germany, the addition of humectants to water pipe tobacco is restricted to an upper limit of 5%. The Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung, BfR) argued that it is possible that higher concentrations of glycerine in the waterpipe tobacco could lead to higher contents of acrolein in waterpipe smoke. Acrolein is present in waterpipe smoke as shown in a recent investigation from Lebanon. The authors not only found acrolein, but also high amounts of acetaldehyde. In one waterpipe session, 2520 µg acetaldehyde was measured in the smoke (Al Rashidi et al. 2008).

In 2008, few companies reported added ingredients in waterpipe tobacco to the German authorities. However, the values reported were interesting: in some brands the total tobacco content was small, for example the label "Al-Waha" contained per kg 200 g tobacco, 710 g fructose, 50 g glycerine and 40 g flavouring. Another label ("Sindbad") contained per kg 398 g invert syrup, 210 g water, 42 g propylene glycol, 28.6 g flavouring, 6 g ethyl alcohol and 1.92 potassium sorbate, leaving 313 g of tobacco. The tobacco content of waterpipe tobacco is thus not very high (20 to 31%).

Studies from Syria show that waterpipe use can be addictive. The frequency of waterpipe use was strongly correlated with the participants' subjective judgement of how hooked they are on waterpipes (Maziak et al. 2004b).

3.8.4.4. Smokeless tobacco

There are many different types of smokeless tobacco in use around the world, some being more toxic than others.

In Europe, smokeless tobacco is widely used in Sweden (24% of men, 3% of women), in particular in the form of moist snuff called "snus". Snus is sold in loose weight in boxes or in small "tea-bag"-like sachets. The sale of snus is banned in all other EU countries. As described in the SCENIHR opinion on smokeless tobacco products (SCENIHR 2008), the frequency of smokers in Sweden is lower than in other European countries and the morbidity due to tobacco related diseases is also lower. The use of smokeless tobacco itself is less pronounced in the neighbour countries Norway and Finland than in Sweden.

Due to immigration, many different smokeless tobacco products have found their way into EU countries, and their use is typically clustered in local communities. A similar clustering of use may be seen with now increasingly rare traditional European products such as nasal snuff.

The Swedish "snus" is, according to the manufacturers (Swedish Match[11], Fiedler & Lundgreen[12]), a standardised product using mainly air cured tobacco. Sodium carbonate is added in order to raise the pH to around 8, thus facilitating the uptake of uncharged nicotine in the mouth (Fant et al. 1999). A number of artificial or natural flavours are added according to the brand; the flavours all comply with food regulations. Two sorts of humectants are used, glycerol and propylene glycol. Snus is pasteurised and the fermentation that takes part in other tobacco products is thus inhibited, leading to a lower content of tobacco specific nitrosamines. More than 250 additives are found in snus, most of them are flavours which are used in small amounts. Table 3 shows the 50 substances that are added in greatest amount.

Gutkha is another smokeless tobacco product that is popular among Indian communities in the UK. This is a chewing tobacco that in addition to tobacco contains areca nut, catechu, lime, saffron, saccharine, mint and various flavourings. A table describing the many different smokeless products that are rarer in Europe is found in the SCENIHR report from 2008 (SCENIHR 2008).

[11] www.swedishmatch.com/

[12] www.flsnus.se

Table 3 The 50 additives present in greatest amount in snus[13]

Ingredient	Maximum percentage found in different brands
Sodium chloride	6.7
Ethanol	5.1
Propylene glycol	4.2
Coffee extract	3.7
Plant fibre	3.7
Glycerol	3.6
Sodium carbonate	2.9
Benzyl alcohol	2.1
Anethole (trans-)	1.5
Peppermint oil	1.5
Maltodextrin	1.4
Calcium carbonate	1.2
Licorice and liquorice extract	1.1
Gum Arabic	0.9
Lemon oil	0.7
Ammonium chloride	0.6
Vanillin	0.6
Lime oil	0.4
Ginger extract	0.3
Linalyl acetate	0.3
Menthol	0.3
Ethyl butyrate	0.2
Eucalyptus oil	0.2
Hydroxyphenyl-2-butanone (4-(para-))	0.2
Potassium sorbate	0.2
Sugar, invert	0.2
Acesulfame K	0.1
Acetic acid	0.1
Benzaldehyde	0.1
Buchu leaf oil	0.1
Butyric acid	0.1
Citronellol	0.1
Clary sage oil	0.1
Damascenone	0.1
Damascone (beta-)	0.1
Diacetyl	0.1

[13] Data extracted from www.swedishmatch.se

Ingredient	Maximum percentage found in different brands
Dimethyl-1,2-cyclopentadione (3,4-)	0.1
Ethyl 2-methylbutyrate	0.1
Ethyl acetate	0.1
Geraniol	0.1
Geranium rose oil	0.1
Hexen-1-ol (cis-3-)	0.1
Hexen-1-yl acetate (cis-3-)	0.1
Hexenyl butyrate	0.1
Hexenyl formate (cis-3-)	0.1
Hexyl alcohol	0.1
Hydroxy-2,5-dimethyl-3(2H)-furanone (4-)	0.1
Ionone (alpha-)	0.1
Ionone (beta-)	0.1
Jasmone	0.1
Lactic acid	0.1

3.8.4.5. Conclusions on tobacco products other than cigarettes

Compared with the widespread use of cigarettes, other tobacco products are consumed much less commonly. There is a great variety of additives which either have a specific function as humectants, glues, acidity regulators etc., or determine the specific flavour of the product or brand. Apart from sugar, most flavours are added in small amounts. There is no proof that any of the additives are by themselves contributing to addictive potential, either directly or indirectly. The additives and the design characteristics of tobacco products are likely to attract specific groups of consumers and perhaps facilitate initiation of tobacco use. The aspects of target groups will be addressed in later sections (3.10.2).

3.8.5. Overall conclusions concerning additives which can increase the addictiveness and/or attractiveness of tobacco products

For most tobacco additives, information about possible effects on addictiveness and attractiveness does not exist. A number of studies have been conducted by the tobacco industry, and there are indications that some additives have effects in relation to addictiveness and attractiveness.

The pyrolysis of sugar substances to acetaldehyde may increase nicotine addictiveness, but the data are inconclusive. Additives that reduce the acidity, and thereby the formation of free nicotine, may contribute to addictiveness, but the efficacy of these compounds has not been shown. In view of the buffer capacity of the body fluids involved (saliva, lung lining fluid), the presence of such an effect is doubtful.

A large number of additives are used to increase attractiveness. Among these, various sugars constitute an important part. Menthol is widely used in certain brands in considerable amounts while most other additives are used in small amounts, and the mixture of additives is characteristic for each brand. This is an important aspect for maintaining consistency of the tobacco products and in targeting special groups.

3.9. Experimental animal models

Several animal models are available to study particular responses that are related to nicotine addiction. Thus, predictive models are available in animals to evaluate the development of nicotine tolerance and physical dependence as well as the rewarding/reinforcing effects produced by nicotine. The animal methods currently used to evaluate nicotine addictiveness are mainly based on the evaluation of its rewarding/reinforcing properties. New complex behavioural models that resemble the main diagnosis for drug addiction in humans have been developed very recently. However, these new models can only be applied for some particular drugs and are not yet available for nicotine addiction.

3.9.1. Experimental models to evaluate the development of nicotine tolerance and physical dependence

Long-term consumption of nicotine produces adaptive changes in the central nervous system leading to the development of tolerance and physical dependence that can be easily evaluated in animal models. Thus, chronic nicotine administration produces tolerance to most of its pharmacological effects (Benowitz 2008). Tolerance to several nicotine responses such as hypolocomotion, convulsive effects, hypothermia or antinociception has been widely described in animal models, whereas an absence of tolerance to the effects on cognitive processes has been currently reported in these studies (Benowitz 2008, Collins et al. 1988, Damaj and Martin 1996, Marks et al. 1986, Miner and Collins 1988).

In humans, cessation of tobacco intake precipitates both somatic and affective symptoms of withdrawal which may include severe craving for nicotine, irritability, anxiety, loss of concentration, restlessness, decreased heart rate, depressed mood, impatience, insomnia, increased appetite and weight gain (Hughes and Hatsukami 1986, Hughes 2007). In rodents, nicotine withdrawal is also characterised by the manifestation of both somatic signs and affective changes similar to those observed in humans. The somatic signs include teeth chattering, palpebral ptosis, tremors, wet dog shakes, and changes in locomotor activity and other behavioural manifestations (Malin et al. 1992). Although the development of nicotine tolerance and physical dependence is concurrent to the development of addiction, they are not aetiologically related to nicotine addiction (Volkow and Li 2005). However, the affective manifestations of nicotine withdrawal seem to play an important role in the maintenance of the nicotine addictive process. These manifestations can be evaluated in rodents by measuring several emotional symptoms such as increased anxiety, aversive effects and reward deficits (Jackson et al. 2008b, Johnson et al. 2008). The aversive manifestations of withdrawal are mainly evaluated in rodents by using the place conditioning paradigm, whereas the associated reward deficits are currently evaluated using intracranial self-stimulation techniques. Both behavioural paradigms have also been extensively used to evaluate nicotine rewarding effects and will be described in the next section.

3.9.2. Experimental models to evaluate nicotine rewarding effects

Drug consumption is promoted and maintained by the rewarding properties of the drug. However, it is important to underline that drug consumption is a requirement for the development of addiction, although addiction is not a necessary consequence of drug intake.

3.9.2.1. Self-administration paradigms

Self-administration methods are widely used to directly evaluate the reinforcing properties of a drug. The procedures are considered by most researchers to be valid and reliable models of drug consumption in humans, and to have a high predictive value. It is assumed that the neurobiological mechanisms involved in drug self-administration in animals are similar to those underlying drug-intake in humans (Sanchis-Segura and Spanagel 2006). Self-administration methods can be classified considering the route of administration and the behavioural paradigm. From a behavioural perspective, these methods can be classified as operant and non-operant procedures. Non-operant paradigms are centred on the amount of drug consumed whereas the operant procedures require a conditioned response in order to obtain the drug, and the analysis of this response provides valuable information about different behavioural aspects of drug consumption. Non-operant paradigms in animals are mainly restricted to oral self-administration and they are very useful for alcohol research considering the similarities with the route of alcohol consumption by humans. The use of the appropriate route of self-administration for each drug of abuse provides an additional source of validity to these animal models, and these non-operant paradigms are therefore not useful in evaluating nicotine rewarding effects.

The use of operant models is based on the learning contingency defined as "positive reinforcement". In these models, the drug constitutes a positive reinforcer that is delivered contingently to the completion of the schedule requirements (Sanchis-Segura and Spanagel 2006). The operant chambers are equipped with one or more manipulandi, transmitting the operant response and devices to deliver the drug (reinforcer). Usually, there is an active manipulandum that is linked to the delivery of the drug and an inactive one, which results in the delivery of the drug vehicle or lacks any programmed consequence. The programmes of reinforcement commonly used are the fixed ratio and the progressive ratio schedule and the animal species currently used for nicotine self-administration is the rat. It is suggested that fixed ratio schedules measure the pleasurable or hedonic effects of a drug (McGregor and Roberts 1995, Mendrek et al. 1998), whereas progressive ratio schedules are more related to motivation and provide a better measure of incentive salience or craving (Arnold and Roberts 1997). Under a fixed ratio schedule, the drug is delivered every time that a pre-selected number of responses are completed. For nicotine self-administration, the number of responses required to obtain the drug is generally kept low, and the most used is the fixed ratio 1 (a nicotine delivery after each response in the active manipulandum), although fixed ratio 3 and 5 schedule of reinforcement have also been used (for instance, Shram et al. 2008). Multiple studies have demonstrated that rats easily maintain an operant behaviour to self-administer nicotine under these fixed ratio experimental conditions (Maldonado and Berrendero 2009). In contrast with other drugs of abuse, when dose-response curves have been constructed for nicotine self-administration in rats, they have been relatively flat or inverted U-shaped, which may be because of the aversive effects and toxicity associated with high doses of nicotine (Corrigall and Coen 1989, Shoaib et al. 1997). In a large number of studies the dose of 0.03 mg/kg (free base) per infusion showed very robust self-administration behaviour in rats (Corrigall and Coen 1989, Donny et al. 1999, Shoaib et al. 1997).

Under the progressive ratio schedule, the response requirement to deliver the drug escalates according to an arithmetic progression. The common index of performance evaluated in this schedule is the break point defined as the highest number of responses that the animal accomplished to obtain a single delivery of drug. In rats, several studies have also revealed that nicotine can maintain self-administration on a progressive ratio schedule of reinforcement. The break point achieved for nicotine self-administration has been compared by the authors with other drugs of abuse. They found that it was lower than the final ratio obtained for cocaine under an identical schedule of reinforcement, higher than that reported for heroin under similar progressive ratio schedule, and slightly lower than heroin when a slowly accelerating schedule was used (Donny et al. 1999). However, comparison across studies and drugs is difficult due to procedural differences in training parameters, sequence of progressive reinforcement or degree of drug dependence (Stafford et al. 1998). Increasing doses of nicotine usually resulted in a more linear increase in the performance in the progressive ratio schedule than in the fixed ratio schedule (Donny et al. 1999). The maximum break points usually reached by the adult rats when using the progressive ratio schedule are around 50 responses to obtain a single nicotine injection (Shram et al. 2008). Interestingly, higher break point values were obtained in adolescent rats (around 95) than in adult rats (Shram et al. 2008).

Operant nicotine self-administration has been difficult to establish in mice. A recent study has reported the validation of a new reliable operant model of nicotine self-administration, extinction and relapse in mice. This model was developed in C57BL/6 mice which are particularly sensitive to the behavioural effects of nicotine (Martín-García et al. 2009). Mice were successfully trained to self-administer a dose of nicotine similar to that previously used in rats (0.03 mg/kg, free base) under a fixed ratio 1 schedule of reinforcement. An inverted U-shaped dose-response function was also obtained using mice to self-administer different doses of nicotine (Galeote et al. 2009). Similar to other drugs of abuse, the break point achieved for nicotine self-administration in mice was lower than in rats. Indeed, the maximum break point (27 responses to obtain a single nicotine injection) was reached by the mice when using the dose of nicotine of 0.042 mg/kg (free base) (Galeote et al. 2009).

3.9.2.2. Conditioned preference paradigms

In the conditioned preference paradigms, the subjective effects of the drug are repeatedly paired to a previously neutral stimulus. Through this repeated conditioning process, this stimulus acquires the ability to act as a conditioned stimulus, and the animal will prefer or avoid this conditioned stimulus depending on the rewarding or aversive effects produced by the drug. The most commonly used paradigms apply a spatial environmental stimulus as conditioned stimulus and the animal will show a conditioned place preference or a conditioned place aversion for the environment associated to the effects of the drug or its withdrawal. Although a conditioned approach/avoidance towards specific stimuli can also occur in humans as a result of drug consumption (Bardo and Bevins 2000), the place conditioning paradigms are not primarily intended to model any particular feature of human behaviour. These paradigms mainly represent an indirect assessment of the rewarding or aversive effects of a drug or its withdrawal, by measuring the response of the animal towards the conditioned stimulus. Drugs of abuse display a differential ability to produce conditioned place preference. Opioids and psychostimulants easily produce robust place preference over a wide rang of experimental conditions, whereas other drugs such as ethanol, cannabinoids or nicotine produce more inconsistent results (Sanchis-Segura and Spanagel 2006). Thus, nicotine has been shown to induce in rodents conditioned place preference across a wide range of doses in some experiments, although inverted U-shaped dose-response curves have been often reported, and the magnitude of the effect is generally small and affected by environmental stimuli or previous handling history (Castañé et al. 2006, Forget et al. 2005, Grabus et al. 2006, Le Foll and Goldberg 2004). Nicotine also produced aversive effects when used at high doses in some, but not all, studies (Grabus et al. 2006, Le Foll and Goldberg 2004). These results suggest that the rewarding effects of nicotine may be weaker than other drugs of abuse in this particular experimental paradigm (LeFoll and Goldberg 2004). Interestingly, sex differences were clearly revealed in mice exposed to nicotine in the conditioned place preference paradigm. Thus, female mice responded more to the conditioned rewarding effects of nicotine compared with males (Isiegas et al. 2009).

3.9.2.3. Intracranial self-stimulation paradigms

Intracranial electric self-stimulation procedures were essential in the discovery of the brain reward circuits (Olds and Milner 1954) and are now widely used to study the effects of drugs of abuse in the activity of the reward circuits (Sanchis-Segura and Spanagel 2006). In this paradigm, animals are trained to maintain an operant behaviour in order to obtain an electric pulse through an electrode that has been previously implanted in a reward-related brain site, most frequently the lateral hypothalamic area. The threshold of the minimal current needed to promote intracranial electric self-stimulation is estimated. A drug that stimulates the reward circuit will decrease this threshold, which would be related to its rewarding properties, whereas a drug having aversive effects will enhance the minimal current required to maintain the self-stimulation (Markou and Koob 1993). Nicotine as well as other drugs of abuse such as psychostimulants, opioids or ethanol, reduces the threshold to promote intracranial electric self-stimulation in some reward brain areas (Huston-Lyons and Kornetsy 1992, Kornetsky and Bain 1992, Wise 1996). Therefore, this behavioural paradigm clearly demonstrates the capability of nicotine to activate the brain reward circuits.

3.9.3. Experimental models to evaluate nicotine addiction

The behavioural models available to evaluate drug rewarding effects have been very useful in clarifying the neurobiological basis of drug taking. However, addiction is not just the taking of drugs, but represents a relapsing disorder characterised by compulsive drug use maintained despite adverse consequences for the user (APA 1994). Behavioural models that resemble the main diagnosis criteria for addiction are difficult to validate in animals. Recently, two independent research groups have validated behavioural models of compulsive drug seeking in rodents that resemble addictive behaviour in humans (Belin et al. 2008, Deroche-Gamonet et al. 2004, Vanderschuren and Everitt 2004). In these models the authors have evaluated the difficulties in stopping drug use by measuring the persistence of drug seeking during a period of signalled non-availability. The extremely high motivation of the addicts to take the drug has been evaluated by using a progressive ratio schedule where the number of operant responses to obtain a single drug injection was increased progressively within the same session. The maximal amount of work that the animal performs before cessation of responding (referred to as the break point) is considered a reliable index of the motivation for the drug. These new animal models of addiction report a break point over 500 to obtain a single cocaine injection in "addict rats" (Deroche-Gamonet et al. 2004). In these new animal models of addiction, the continued use of the drug despite its harmful consequences has been resembled by the persistence of the animal's responding for the drug when drug delivery was associated with a punishment.

However, these models validated for cocaine consumption are still not available for other drugs, such as nicotine. Indeed, nicotine self-administration has not been reported to be maintained when drug delivery was associated with a punishment. In addition, only moderate break point values were obtained when a progressive schedule of reinforcement was used for nicotine self-administration. Thus, the maximum break points usually reached to obtain nicotine, i.e. around 50 responses in adult rats (see for instance, Shram et al. 2008) and around 95 in adolescent rats (Shram et al. 2008), are far away from the break point values reached to obtain cocaine by the "addicted rats" (over 500, Deroche-Gamonet et al. 2004).

In contrast, recent advances using animal models of relapse have shown that nicotine seeking after extinction of the operant behaviour can be triggered in rats and mice by nicotine-associated (conditioned) cues (Caggiula et al. 2002, Liu et al. 2007, Martín-García et al. 2009), stressors (Bilkei-Gorzo et al. 2008, Buczek et al. 1999) (e.g. mild footshocks) and re-exposure to the previously experienced drug (Chiamulera et al. 1996, Dravolina et al. 2007, Shaham et al. 1997), which are the same events that trigger nicotine craving and relapse in humans. Nicotine-paired cues have a critical role in sustaining nicotine self-administration after prolonged periods of abstinence and in maintaining smoking behaviour in humans. Indeed, a critical role of the environmental stimuli previously associated with drug consumption has been attributed when explaining the high rate of nicotine relapse (Caggiula et al. 2001, Caggiula et al. 2002, Liu et al. 2007). In agreement, the exposure to the associated cues was the most effective stimulus reinstating nicotine-seeking in mice, whereas stress exposure reinstated nicotine-seeking behaviour in half of the mice, and a priming injection of nicotine only reinstates seeking behaviour in a low percentage of mice (Martín-García et al. 2009). The neurobiological mechanisms involved in the processes underlying relapse to nicotine seeking are poorly understood. Further studies will be required to clarify the mechanisms involved in nicotine relapse using these animal models now available.

3.9.4. Conclusions on experimental animals

Animal models to evaluate the rewarding and the reinforcing properties of nicotine, and the development of nicotine tolerance and dependence, are available. The models most currently used to evaluate nicotine addictiveness are based on its rewarding/reinforcing properties, are well established and have been widely used for other drugs of abuse to determine their addictive potential. Among these models, the operant self-administration paradigm is particularly useful considering its high predictive value for the abuse liability of a drug and therefore also possibly for its addictive potential in humans. A response easy to evaluate in the self-administration paradigm that has been related to the addictive potential is the break point (highest number of responses that the animal accomplishes to obtain a single delivery of a drug). A higher break point represents a direct measure of the motivation of the animal to obtain the drug and is often taken to imply an increase in the addictive potency of the drug. New complex behavioural models that resemble the main diagnosis for drug addiction in humans have been developed very recently, although these new models can only be applied for some particular drugs and experimental conditions at the present moment.

3.10. Human studies of role of additives in addictiveness and attractiveness of tobacco products

Tobacco addiction is maintained by nicotine, and tobacco products that do not deliver nicotine do not sustain addiction. However, it is important to distinguish between the stages of tobacco use, from early experimentation and initiation (prior to the development of dependence), through to regular use (and possible dependence) and possibly eventual cessation. Therefore, nicotine and additives may play different roles, or may differ in their relative importance during experimentation and initiation compared with the progression to regular use. In addition, the role of additives will differ according to whether the tobacco is delivered as a smoked or smokeless product.

Smoking and inhalation into the lungs, in particular, is a highly efficient form of nicotine administration, as the drug enters the circulation rapidly through the lungs and moves into the brain within seconds. This also allows precise dose titration, so a smoker may obtain the desired effects (Benowitz 2008). Therefore, additives and design characteristics which require the inhalation of tobacco smoke will be associated with increased dependence potential, and this will be particularly true when inhalation into the lungs (as opposed to the oral cavity only) is encouraged. In addition, various tobacco additives and flavourings can modulate the impact of nicotine, including via administration and inhalation behaviour. The impact of these additives on the attractiveness and palatability of tobacco products, in particular in naive users, may influence initiation of use and progression to regular use, before dependence is established.

Tobacco dependence is operationalised in multiple ways, but all definitions share core features of tolerance and withdrawal symptoms in relation to tobacco use. Most studies use either the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for tobacco dependence, or a proxy measure such as the Fagerström Test for Nicotine Dependence (FTND), or the number of cigarettes smoked per day. However, the number of cigarettes smoked per day is often a poor measure of dependence, given the substantial inter-individual variability in the amount of nicotine extracted from a cigarette. The majority of the variance in scores on the FTND is accounted for by the first item ("How soon after you wake do you have your first cigarette?"), and it is likely that many dependent cigarette users can be identified by how soon after waking they smoke their first cigarette. This is most likely due to the short half-life of nicotine, which means that after a period of sleep most tobacco users have very low levels of circulating nicotine, resulting in withdrawal symptoms which are rectified by tobacco use.

Human behavioural studies require either subjective or objective measures of the effects of tobacco, and this allows a comparison of these effects between tobacco products which do and do not contain specific additives. Subjective measures include self-report measures of mood and craving, which may be as simple as single visual analogue scale measures of liking (e.g. "How much do you like the taste of this cigarette"), or include validated questionnaire measures (e.g. the Positive and Negative Affect Schedule). The latter refers to a range of laboratory assessments, including actual smoking behaviour through smoking topography measurement, which allows the detailed measurement of number of puffs taken per cigarette, depth of inhalation, inter-puff interval, and so on. This may also include self-administration or cigarette choice paradigms (e.g. presenting participants with two cigarettes, only one of which contains an additive of interest, to determine which is preferentially smoked), which are more closely comparable with paradigms used in animal studies.

These measures are generally impossible or impractical to collect in survey studies, although the rates of use of different tobacco products, containing different additives, may allow their attractiveness to certain sub-groups (e.g. defined by age or ethnicity) to be inferred. A further complication is the possibility that what constitutes an attractive or palatable product may be culturally or ethnically specific.

3.10.1. Experimental and observational studies

Cigarette smoking topography describes the pattern of smoking behaviour for an individual cigarette smoker, and includes measures of puff volume, puff duration, puff flow, interpuff interval, and number of puffs per cigarette. This technology can be used to assess the effects of product design characteristics and additives on smoking behaviour. There is good evidence, for example, that cigarette smokers partially compensate for the low nicotine delivery by low tar cigarettes, possibly by inhaling more deeply, taking more puffs per cigarette, and so on (Frost et al. 1995), so that the addictive potential of low tar cigarettes may not be substantially different than high tar cigarettes (see also section 3.5.5). Similar effects have been observed when comparing nicotinised and denicotinised cigarettes (Strasser et al. 2007).

Neurological techniques have also been used (e.g. by Philip Morris) to assess the effects of additives on smokers' central nervous system functioning. Electroencephalography (EEG), pattern reversal evoked potential (PREP), and chemosensory event-related potential (CSERP) were used to measure physiological, sensory, and cognitive changes related to nicotine, flavourings and other additives (Gullotta 1994).

Other chemicals (e.g. pyrazine, vanillin, and propylene glycol) appear to increase P1-N2 amplitudes (the first positive and second negative peaks in the EEG waveform elicited by a novel stimulus, corresponding to early sensory processing) (Philip Morris 1995), and different tobacco flavourings affect CSERPs (EEG waveforms elicited by olfactory or gustatory stimuli) differently, even when smokers were unable to discriminate these subjectively (Gullotta 1994). This indicates that objective measures may be more sensitive to the modulation of smoking behaviour by specific additives than subjective measures (see also section 3.8.1 and 3.8.2).

The sensory effects of tobacco smoke may themselves acquire reinforcing properties through their repeated association with the rewarding properties of tobacco. This has been shown in various studies where, for example, denicotinised cigarettes continue to be smoked in the absence of nicotine reward but are not smoked as frequently when the upper airway is anaesthetised to block the sensory effects of the tobacco smoke (Rose et al. 1985). This may explain in part the loyalty to specific brands shown by tobacco users, since the exact sensory properties of individual brands will differ. It is also possible that extended product characteristics (e.g. pack designs) may acquire reinforcing properties through similar processes, although this has not yet been investigated systematically.

A variety of product design strategies (e.g. ventilation holes – see section 3.5) and application of additives (e.g. ammonia or ammonia-derivatives, see section 3.8.1 and 3.8.3.2) may play important roles either via smoking behaviour such as puffing characteristics (see section 3.5.5), or via more direct biological effects such as nicotine bioavailability. These may in turn influence addictive effects and appeal to the user (Baker et al. 2004c, Djordjevic and Doran 2009). As reported by the tobacco industry, approximately 600 substances are used as cigarette additives, but among the most commonly used products only one additive (menthol) is widely advertised by the industry (Ahijevych and Garrett 2004).

3.10.2. Target groups (age, ethnicity, gender, socioeconomic position)

Internal tobacco industry documents illustrate that additives and technical characteristics have been extensively evaluated in relation to their appeal to specific target groups and markets (Carpenter et al. 2007). Some of this evidence relates to the US experience, in particular with respect to ethnicity, and it is not clear whether these results will generalise, in full or in part, to the European situation. However, some findings, such as those relating to younger age groups and gender, are more likely to generalise.

3.10.2.1. Age groups

There is evidence from tobacco industry documents that flavourings have been used to target younger smokers: "[U]se the FLITE technology to inject various flavours into the blend. These flavours would be new and unconventional. Two flavours which were discussed as options were Root Beer and Brazilian Fruit Juice, both of which *tend to appeal to the younger generation* while being rejected by their parents" (emphasis added) (BAT 1997). This may act as a gateway to subsequent tobacco use in adulthood.

A survey in the US showed that 17 year old smokers are three times as likely to use flavoured cigarettes as are smokers over the age of 25 (Klein et al. 2008). Therefore, the addition of exotic flavours may be used to increase the appeal of tobacco products (including smokeless products), and in particular their appeal to naive users and younger age groups. Dutch survey data indicate that taste and smell are important determinants of brand preference among young smokers aged 10-18 years, with brands with light or mild taste regarded as less unhealthy (Talhout et al. 2009).

3.10.2.2. Ethnicity

In the USA, there is a striking difference in the use of mentholated cigarettes among African Americans and European Americans, with the prevalence of mentholated cigarette smoking much higher in the former group. Menthol is the most widely-studied additive, and therefore provides a case-study for some of the behavioural consequences of tobacco additives. This suggests that specific additives may be used to improve the attractiveness of tobacco products to specific populations or target groups.

Internal tobacco industry documents, available under the Freedom of Information Act in the USA, describe the relationships between sensory perception and the attitudes, preferences, and patterns of cigarette use among menthol smokers. Two unique types of menthol smoker are described: those who cannot tolerate the harshness and irritation associated with smoking non-menthol cigarettes, and those who seek out the specific menthol flavour and associated physical sensation (Kreslake et al. 2008b).

Additives also contribute to the effects of other tobacco products with either marginal or region-specific use. For example, clove cigarettes, used predominantly by East Asian populations, are composed of a mixture of tobacco (60–80%) and ground clove buds (20–40%), available with or without filters. Eugenol, an analgesic, is naturally occurring in cloves, and is present in milligram quantities in the clove cigarette filler. Like menthol, eugenol diminishes the harshness of the tobacco smoke (Djordjevic and Doran 2009).

3.10.2.3. Gender

While the targeting of specific groups and populations (e.g. young people, women, ethnic groups) is primarily through advertising campaigns for tobacco products, this targeting can also include the development of specific tobacco products, and the use of specific additives in these products. For example, cigarettes with perfumed scents and labelled as "slim" or "light" brands have been marketed to women. This is reflected in evidence that more women than men smoke light and ultra-light cigarettes (ONS 2007).

3.10.2.4. Socioeconomic position

Tobacco use is heavily socially patterned in developed countries, with prevalence of use being higher in lower income groups compared to higher income groups (Eek et al. 2010, Main et al. 2008). While tobacco use in general, and cigarette use in particular, has declined dramatically in wealthier socioeconomic groups over the last few decades, the decline in less wealthy socioeconomic groups has been much less pronounced. In particular, in the most economically disadvantaged groups, tobacco use prevalence has remained almost unchanged over this period. As a result, tobacco use is one of the largest causes of health disparities between socioeconomic groups in European countries. However, there is no evidence suggesting that changing patterns of use in Europe are a result of tobacco industry's targeting certain socioeconomic groups.

3.10.3. Emotional/subjective effects

Flavours impart a specific taste or aroma to a product, while other additives may be used for a specific technological purpose in the manufacture of tobacco products (Baker et al. 2004b). Both flavours and other additives can confer emotional and subjective effects. The term "impact" is widely used in tobacco industry research and documents, and is a tobacco industry term for smokers' subjective awareness of the drug effects of nicotine.

Organic acids have been used since the 1950s to improve "smoothness" of cigarettes. For example, Philip Morris found that lactic acid decreased subjective ratings of harshness and bitterness, and produced a sweeter flavour. Citric additives have been used not only for reduced harshness and flavour modification, but also to modify smoke pH, to neutralize nicotine "impact" (an industry term denoting the organoleptic sensation caused by nicotine; smokers often describe this as "throat catch" or "throat hit"). Tartaric and lactic acids likewise modify the pH of smoke. All of these organic acids increased smoothness and are associated with a decrease in nicotine "impact" (Philip Morris 1989, see also section 3.8.2.2.) However, it is unclear whether these effects are due directly to pH modification.

Unregulated botanical and chemical additives might have "multiple-use" purposes, such as enhancing flavour and producing "smoother" cigarette smoke, as well as potentially preventing or masking symptoms associated with smoking-related illnesses (Rabinoff et al. 2007).

3.10.4. Conclusions

A wide range of subjective and behavioural effects of tobacco additives have been reported in humans, but there are relatively few studies published in the scientific literature, with much information having been obtained from tobacco industry documents under freedom of information legislation. In principle, similar methods to many of those used in experimental animal models may also be used in humans. However, there is greater variability in the specific methods employed, which include subjective reports of liking, behavioural measures of drug choice, neurobiological measures of drug effects (such as neuroimaging techniques), and direct measures of drug administration (such as cigarette smoking topography). The majority of additives used appear to be flavourings, and these may be used to target specific markets, such as young people, women, or ethnic groups. There is some evidence that these additives modify objective measures of cigarette smoking behaviour (i.e. smoking topography), but this is somewhat inconsistent.

3.11. Effects of additives on nicotine-addictive properties

3.11.1. Modification of the pharmacology and reinforcement properties of nicotine

3.11.1.1. Comparison of addictive properties of nicotine vs. whole tobacco and modification of reinforcing properties of nicotine

Acetaldehyde is formed in high concentrations when cigarette constituents, including sugars, are burned. Animal research conducted by Philip Morris demonstrated a synergistic interaction between nicotine and acetaldehyde, using a lever-pressing model of self-administration in rodents (Charles et al. 1983, DeNoble et al. 1997). Rats pressed a bar more for the combination of nicotine and acetaldehyde than for either substance alone. If these results apply to humans, smokers would puff more with the combination of nicotine and acetaldehyde. As described below, an inhibitory effect of acetaldehyde on MAO-A and B is one of the possible mechanisms that reinforce the properties of nicotine. It should be noted that the contribution of acetaldehyde in smoke to the level in blood is minimal compared to, for example, the effect of ethanol consumption (Chen et al. 2007b, McLaughlin et al. 1990). There are indications that users of smokeless tobacco do not have a reduced MAO activity, suggesting that constituents of the smoke (acetaldehyde?) are needed to inhibit MAO activity (Berggren et al. 2007). In section 3.8.1.4 the action of acetaldehyde is described in more detail.

Tobacco is a potent reinforcing agent in humans, and nicotine is generally considered to be the major compound responsible for its addictive properties (Balfour et al. 2000, Dani et al. 1996, Di Chiara 2000). However, animal experiments indicate some discrepancies between the effects of nicotine and those of other drugs of abuse. For example, the capacity of repeated nicotine to elevate dopamine levels in the nucleus accumbens is controversial (Balfour et al. 1998, Di Chiara 2000, Vezina et al. 1992) and repeated nicotine treatments in rats induce a behavioral sensitisation which vanishes more quickly than that for other drugs of abuse (Ksir et al. 1985, Villégier et al. 2003). Furthermore, with the exception of ethanol which possesses potent sedative effects, most drugs of abuse, such as psychostimulants and opiates, induce a substantial locomotor hyperactivity both in rats and mice. Nicotine, however, is a weak locomotor stimulant in rats and generally fails to induce locomotor hyperactivity in mice at any dose (Marks et al. 1983, Sparks and Pauly 1999). Nevertheless, when animals are pretreated with an inhibitor of monoamine oxidases, nicotine is able to induce a potent locomotor hyperactivity, even in mice (Villégier et al. 2006a). These differences could suggest that the addictive effects of tobacco are not only due to nicotine and that monoamine oxidase inhibitors have a critical effect.

In fact, tobacco and tobacco smoke are known to contain a number of compounds, among which monoamine oxidase (MAO) inhibitors, such as harman, norharman or acetaldehyde, have been the focus of special interest (Breyer-Pfaff et al. 1996, Gaddnas et al. 2000, Rommelspacher et al. 2002). Monoamine oxidases exist under two forms; MAO-A and MAO-B. They are enzymes that degrade dopamine, serotonin and noradrenaline - three neurotransmitters involved in addiction. The inhibition of MAO increases brain monoamines levels which decrease the sensitivity of their respective receptors. Human MAO-A and MAO-B genes isolated from X chromosome-specific libraries span at least 60 kilobases, consist of 15 exons, and exhibit identical exon-intron organisation (Grimsby et al. 1991). Inhibition of monoamine oxidases by tobacco smoke does not result from the actions of nicotine (Carr and Basham 1991), but from that of other compounds also present in other psychotropic plants (Uelbelack et al. 1998). It was shown that MAO inhibitor pre-treatment allows the maintenance of behavioural sensitisation to nicotine in rats (Villégier et al. 2003), thus suggesting a role of MAO inhibitors in the addictive properties of tobacco. More recently, tranylcypromine, a cyclized amphetamine five thousand times as potent an MAO inhibitor as amphetamine (Zirkle and Kaiser 1964), was found to be able to trigger a locomotor response to nicotine in mice (Villégier et al. 2006a) and nicotine self-administration in rats (Guillem et al. 2005, Villégier et al. 2006a). Moreover, increases in extracellular 5-HT levels induced by monoamine oxidase inhibitors appeared to be crucial for these effects (Villégier et al. 2006b).

Nicotine is commonly considered as a monoamine releaser (Summers and Giacobini 1995, Summers et al. 1996) that increases serotonergic neurons firing (Li et al. 1998; Marubio et al. 1999, Olausson et al. 2001a, Olausson et al. 2001b, Olausson et al. 2002). This increased release of 5-HT, in absence of MAO inhibitors, is however transient. Indeed, an immediate inhibitory retro-control blocking the firing of serotonergic raphe neurons through the stimulation of somato-dendritic 5-HT1A receptors has been described (Engberg et al. 2000, Li et al. 1998, Mihailescu et al. 1998). It has therefore been proposed that MAO inhibitors, because of their enhancing effects on extracellular 5-HT levels, compensate the consequences of the indirect inhibition of serotonergic cells by nicotine, thus suggesting a mechanism by which MAO inhibitors contained in tobacco smoke could act in synergy with nicotine to induce addiction (Tassin 2008). Very recent experiments using 5-HT1A agonists and antagonists have indicated that MAO inhibitors contained in tobacco desensitize 5-HT1A autoreceptors to trigger the strong addictive properties of tobacco (Lanteri et al. 2009).

In humans, nicotine replacement therapies are the most widely used form of pharmacological intervention, but have proven to be remarkably unsuccessful (Medioni et al. 2005, Silagy et al. 2004). Interestingly, most tobacco smokers (> 80%) relapse after a few weeks withdrawal, i.e. when inhibition of MAO activity by tobacco and tobacco smoke is likely to have disappeared. It has also been argued that the lack of efficacy of nicotine replacement therapies was due to the continuous delivery of nicotine by gums or patches. It was indeed believed that peaks of nicotine occur in the brain after each puff of tobacco smoke. Very recent experiments, performed with PET and ^{11}C-nicotine, indicate that these peaks exist only in the arterial blood of smokers and do not appear in the brain (Rose et al. 2010a). The half-life of nicotine in the human brain is 13 minutes, which is much longer than the ~45 seconds which separates two successive puffs. Indeed, brain nicotine levels increase regularly along with the cigarette consumption (Rose et al. 2010a).

The role of tobacco smoke on MAO is even more important than originally thought. A substantial inhibition of MAO-A has been found by neuroimaging in chronic smokers (Leroy et al. 2009). Another study has shown that smokers have the methylation frequency of their MAO-B gene promoter markedly lower ($P<0.0001$) than non-smokers, thus inducing a higher quantity of MAO-B in smokers (Launay et al. 2009). Interestingly, this is also true for smokers who have quit for about 10 years. This was explained by showing that cigarette smoke induces an increase of nucleic acid demethylase activity and an epigenetic regulation of MAO-B. Altogether, these authors have shown that metabolism of 5-HT is modified in smokers but that it is also true for those who have stopped smoking for a long time (over 10 years) (Launay et al. 2009).

It seems therefore that MAO inhibitors, or any compound able to modify 5-HT metabolism and desensitize 5-HT1A autoreceptors, may provide a more complete scheme of the addictive properties of tobacco in experimental models of reward.

3.11.2. Conclusions on effects of additives on nicotine addictive properties

There is evidence that nicotine cannot, by itself, explain the high addictive potential of tobacco and tobacco smoke. The increase of nicotine in the brain resulting from smoking a single cigarette is extremely rapid due to the absorption of smoke inhaled into the lungs but the peak observed in arterial blood after a puff is not reflected in the brain where the half-life of nicotine is much higher than in blood. Converging data indicate that MAO (monoamine oxidase) inhibitors contained in tobacco and tobacco smoke act synergistically with nicotine to enhance addiction potential. Smokers have reduced levels of MAO in the brain. Among MAO inhibitors, compounds resulting from sugar combustion, such as acetaldehyde, may play a crucial role in tobacco addiction. MAO inhibitors increase serotonin extracellular levels and desensitize 5-HT1A autoreceptors, thereby allowing nicotine to activate serotonergic neurons and become addictive. As yet, data about the role of acetaldehyde are inconclusive and need further investigation before a role for sugars as indirectly addictive compounds can be established/confirmed.

3.12. Methods to assess attractiveness

3.12.1. Introduction

According to the World Health Organisation (WHO), the terms “attractiveness” or “consumer appeal” refer to factors such as taste, smell and other sensory attributes, ease of use, flexibility of the dosing system, cost, reputation or image, assumed risks and benefits, and other characteristics of a product designed to stimulate use (WHO 2007b).

Overall, attractiveness is likely to be influenced by a subtle array and interaction of any number of these factors, although at certain times individual factors may take precedence (e.g. price, particularly during a recession). In addition, certain factors might be essential for enduring attractiveness (e.g. the presence and ease of delivery of nicotine).

The factors influencing attractiveness can be broadly divided into: extrinsic factors (e.g. marketing, packaging, pricing); and intrinsic factors (e.g. taste, smell, sensory attributes, and pharmacological factors). Additives play a role mainly in the intrinsic factor category, but marketing and packaging can also reflect the presence of additives in a way to attract and maintain customers (e.g. by signalling that the tobacco product contains menthol). Given the subtle interactions between different factors however, identifying and measuring the influence of individual addictives on attractiveness of products is difficult. Separating the role of additives in enhancing addictiveness, from their role in enhancing other attractive attributes of a tobacco product is also complex.

3.12.2. Measuring attractiveness

There are two main ways of examining the influence of additives on the attractiveness of a product. Firstly, one can assess individual tobacco products, and compare their attractiveness on a number of scales/dimensions, against other tobacco products. By then examining what is known about the additive content of these products, indirect inferences can be made as to the role of individual additives in the overall attractiveness of the product, although there are important limitations to studies of this kind. Secondly, one can examine the influence of individual additives on attractiveness of a tobacco product, along a number of scales, by experimentally adjusting tobacco products to include or exclude individual additives and testing responses to them. In addition, the quantity of the additive can be varied to assess dose response and whether there is a threshold below which any impact is not observed. However, in practice this may be difficult to achieve by research groups outside of the tobacco industry, who are likely to lack the resources to manipulate additive content in this way.

Tobacco industry documents show that the tobacco companies frequently tested human smokers on their reaction to different cigarettes using focus groups, market testing, human smoking behaviour studies or consumer panels. For example, one study carried out by British American Tobacco in 1980 exposed a panel of smokers, trained to be objective in their evaluation of cigarettes, to different conditions wherein brand identification was either masked or visible, in order to understand how brand identification and imagery affected subjective evaluation of cigarettes (Ferris 1980).

The difficulties with this type of research are that ethical restrictions will usually preclude human testing of different tobacco products, particularly among non-users or children. In addition, there are technical constraints on the ability to manipulate the presence or absence of specific additives in tobacco products. While the tobacco industry may be able to achieve this, such manipulations may be beyond the resources of independent academic research groups.

Both the main methods have advantages and disadvantages and should be seen as complementary. Ideally, a variety of methods and tests would be utilised and assessors would be looking for overall consistency in the findings, in order to conclude that an additive affected attractiveness.

3.12.2.1. Measuring attractiveness of different brands

Actual brand choices

Assessing actual brand use gives an overall indicator of attractiveness of products which reflects all the factors listed at the outset of this section covering both extrinsic and intrinsic variables, of which additive content is only one factor. A major difficulty of this approach will therefore be separating the influence of these factors. The largest influence is likely to be the marketing budget. For example, the popularity of Marlboro worldwide is likely due to the substantial funding spent on its advertising and promotion. A further complication with interpretation of brand preference data over time is that the tobacco industry has been expanding the number of variants of existing brands; since 1998 brand families have increased by more than 50%. For example, Benson & Hedges increased the number of brands from four in 1998 to 12 by 2008 (ASH 2010).

Brand choices can be examined cross-sectionally in populations (nationally and globally) but longitudinal data enable trends in brand preferences to be examined over time and in relation to changing product make up (content and design) as well as tobacco control policies and other factors. Brand preferences should be examined in subpopulations such as by gender, age, and sociodemographic factors, which might reflect targeting by tobacco companies. Brand preferences in younger age groups (e.g. 11-16 year olds) are especially important to identify as these can enable an assessment of attractiveness and appeal to children. In particular, it is important to assess which brands are used initially by children, followed by those that they progress onto over time. Products that attract children to smoking have been referred to in the literature as “starter products”. This refers to two main types of products: confectionary products which are made and packaged to look like cigarettes, thereby enabling children to imitate smoking (e.g. candy cigarettes, not discussed further here), and tobacco products which are made to look like confectionary (e.g. candy-flavoured cigarettes), thought particularly to appeal to children and ethnic minorities (Connolly 2004).

Comprehensive sources of data on brand preferences at country level broken down by socio-demographics are not readily accessible. As an example, we have selected data from the UK which suggest that brand preferences of children and adults can be quite similar. The top five brands in 2009 were identified as: Lambert & Butler King Size, Mayfair King Size, Marlboro King Size Gold, Benson & Hedges King Size Gold and Richmond King Size (Hegarty 2010). Comparable data are not available for youth from 2009 but in 2006, the most popular brands with 11-16 year olds were: Mayfair (58%), Lambert & Butler (56%), Richmond (45%), Benson & Hedges (28%) and Sovereign (23%) (Amos and Hastings 2009). Four of the brands were common to both adults and youth, and for each age group there was a dominance of economy brands. Trends over time indicate increasing popularity of economy over premium brands suggesting price may be playing a key role in current brand choices. As indicated in section 3.13.2., there may be a trend in the UK for preferring brands marketed as containing no additives, but this observation needs confirmation.

Careful monitoring of brand preferences over time will be important for future research, as will disclosure by the tobacco industry of detailed product content information for all brands on the market.

Perceived brand preferences

By showing different brands to consumers, assessments can be made about how attractive the products are perceived to be. For non-tobacco users, responses will largely reflect extrinsic factors such as the packaging, but will also reflect their knowledge of experiences of others with the products. For users, such assessments also reflect knowledge and experience of using the products in addition. The role of additives therefore will need to be assessed and inferred alongside these other factors, assuming that differences in additives between the different brands are known. As stated above, this research involves examining the look of a pack, and its design and packaging.

Packages can be digitally altered experimentally to test the responses of the presence or absence of attributes (e.g. whether listing an additive such as menthol alters how people respond to the product). However, studies have shown that colours of packs quickly become associated with certain attributes; for example, one study in New Zealand found that green colouring indicated the presence of menthol (Peace et al. 2007). In these types of studies, different population groups should be compared to test if some products are more appealing than others. For example, one experimental study indicated that some adolescents had more favourable impressions of tobacco brands that featured cherry flavouring in the packaging (Manning et al. 2009).

This type of research has now been carried out in a variety of settings (e.g. internet, supermarket, and mall intercept studies) and using a variety of qualitative and quantitative research techniques (Hammond et al. 2009a, Hammond and Parkinson 2009b, Manning et al. 2009). The products have been assessed along several attributes including their perceived attractiveness, harmfulness, ease of initiation or cessation. Standardised designs, methodologies and questions therefore exist which can be utilised to facilitate comparative analysis.

Sensory attributes to users and others

Consumer perceptions of sensory attributes such as taste or palatability, smoke irritation and odour, can also be useful for indicating differences in brands. Although there is likely to be some impact of packaging and design on expectations of sensory effects, this area of testing will be more focused on attributes of the content and emissions of the product itself. This research can be done in two main ways:

a) Through surveys of smokers in which questions cover reasons for selecting the brands they smoke and the role of sensory attributes.

b) Experimentally, using panels of test subjects trying products and expressing preferences using, for example, visual analogue scales (see section 3.10). However, whilst perceived responses to these attributes are important, it is also useful to see how sensory differences translate into topography measurements and the presence of biomarkers, such as cotinine (see below).

These factors could be attractive to a smoker as they make it less troublesome for others in their presence, who are then less likely to complain about their smoking. The sensory attributes to be measured here would include smoke irritation, smoke odour, and visibility of sidestream and mainstream smoke. These assessments can be made as described above, but of non-smokers who live, work or are in the presence of smokers.

3.12.3. Conclusions on methods to assess attractiveness

Attractiveness depends on multiple factors that combine to stimulate use. These include extrinsic factors such as marketing, packaging and price, and intrinsic factors such as taste and smell. It is very difficult to identify the role of individual additives in enhancing addictiveness or enhancing other attractive attributes of tobacco products. The attractiveness of a product may be assessed by the direct comparison of different products by surveys, experimental measures or human testing.

Another way to examine the attractiveness of individual additives is to test a certain tobacco product by introducing the additive in different doses. When additives are thought to act in synergy, they may be tested together. In practice, however, overall attractiveness is assessed by comparison of brand choice in subpopulations according to gender, age and sociodemographic factors. By showing different brands to consumers, assessments can be made about their perceived attractiveness.

Sensory attributes such as taste, irritation etc. may be tested by surveys of users or experimentally on panels of test subjects. In general, methods similar to those described in section 3.10 may be used.

The main disadvantage of using any of the data described above is the lack of detailed information available on additive content of different brands and the extent to which additives contribute to any differences observed, over and above other factors intrinsic to the brand, and the price and marketing of the brands.

3.13. Tobacco use in the European Union

Manufactured cigarettes are by far the most preferred tobacco products in the 27 Member States of the European Union. Cigarettes constitute well over 90% of the tobacco sold whereas tobacco used in pipes and for RYO cigarettes (roll-your-own) amounts to about 5%. In most Western EU countries, smoking prevalence among men and women has in general stabilized or is decreasing. The number of smokers has also started to decrease in some countries in the eastern part of EU, although generally it is only stabilizing among men, with no clear overall trends, and in some cases a slight rise in prevalence among women is being recorded. In the EU as a whole the situation has been stable in the last decade (WHO 2007a).

The use of smokeless tobacco (snus) is common among males in Sweden. The sale of snus is banned in all other countries in the EU but other oral tobacco products may be sold. In the United Kingdom, both male and female migrants from the Indian subcontinent use a wide variety of smokeless tobacco products. Elsewhere, smokeless tobacco use is rare but a wide variety of tobacco products do find their way to Europe through migration (SCENIHR 2008). Similarly, waterpipe smoking is spreading through cultural influence, mainly by migrants from the Middle East. However, during recent years, waterpipe use has become increasingly popular among teenagers in the general population.

The latest comprehensive data from the 27 Member States were collected for 2002 and 2005, respectively (WHO 2007a). Where data were missing other sources have been used in an effort to get the full picture. The information for some countries may not have been collected during the 2005 survey and the figures may therefore not be entirely correct.

3.13.1. EU adult smoking rates 2005

The overall adult daily estimated smoking prevalence (population-weighted) has stabilized at around 28.5% in the EU. The estimated average smoking prevalence among males is 34.2% in 10 (mostly Eastern European) countries and there is a higher prevalence rate of male smoking, while in eight (mostly Western European) countries the male smoking prevalence is below 30% (see figure 4). The estimated average female smoking prevalence in the EU is 22.6% in 14 (mostly Western European) countries and the prevalence rate is higher, while in only three countries is it below 15% (see figure 5).

3.13.1.1. Gender differences

In all but one country (Sweden), smoking prevalence is higher among men than among women. Data from Latvia show the widest gender gap of 29%. A small difference between male and female smoking prevalence of less than 10% can be found in 11 (mostly Western European) countries.

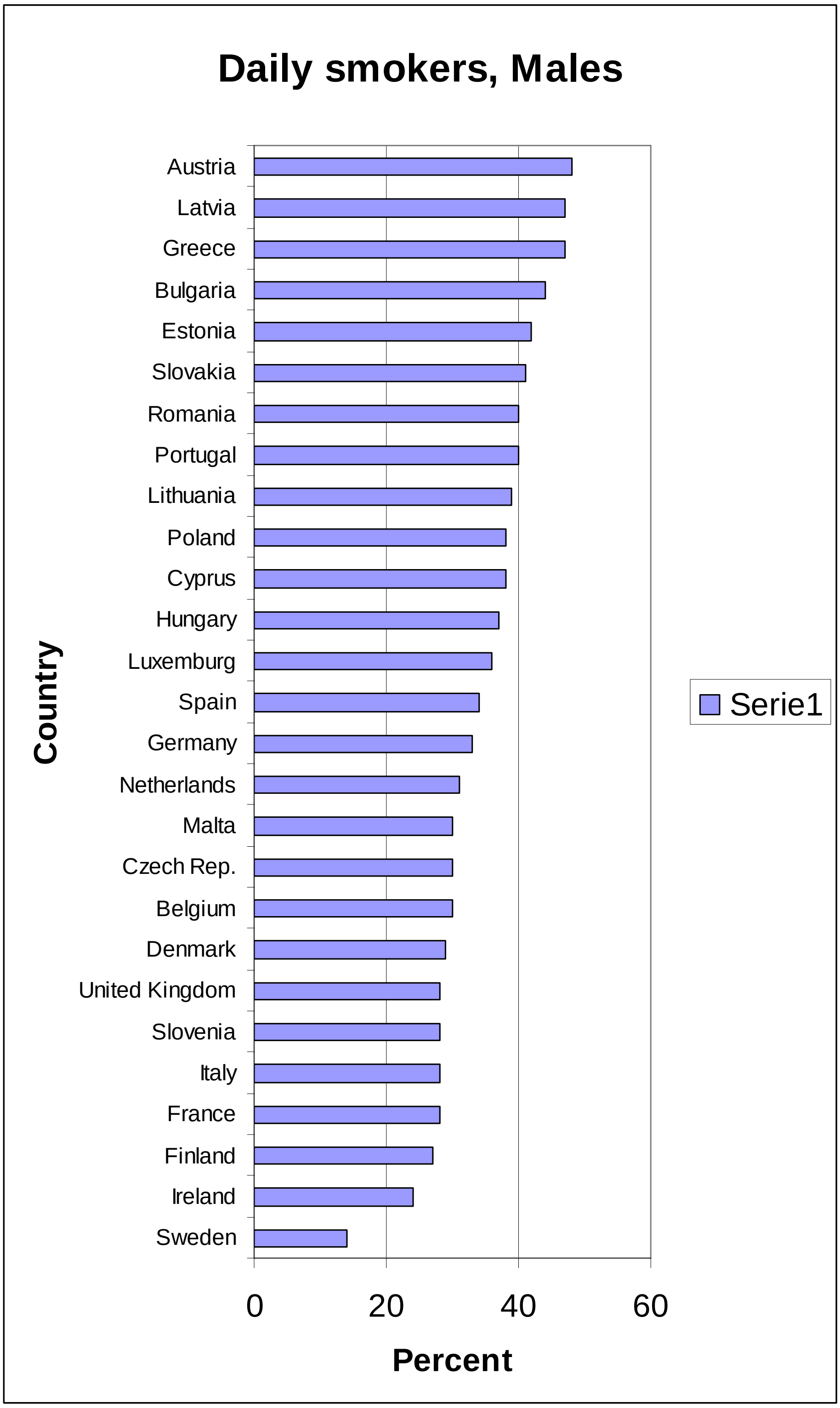

Figure 4: Rates of daily smokers among males in EU countries (WHO 2007a)

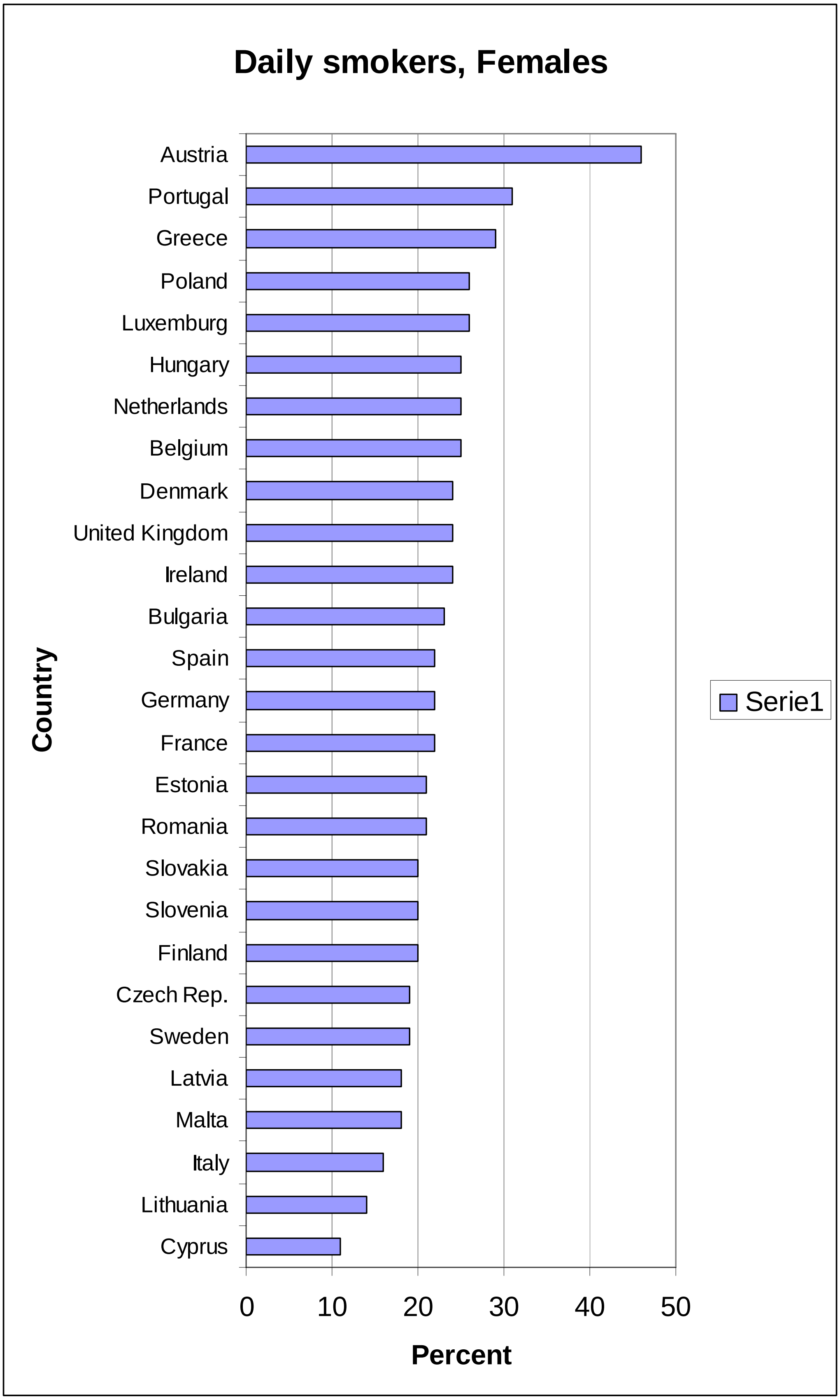

Figure 5: Rates of daily smokers among females in EU countries (WHO 2007a)

3.13.1.2. Changes in smoking prevalence

Estimates for male and female smoking prevalence for 2002 and 2005 are available for 24 of the 27 EU countries. Only relative differences of more than +/-10% have been taken into account as noteworthy changes when comparing data for these two years.

Since the 2002 European report on tobacco control policy, smoking prevalence among the male population has in general stabilized across the EU. A notable decrease has been reported for Sweden (16.3% to 14.4%), but in most countries in the EU male smoking prevalence did not show a significant change between 2002 and 2005. There was no significant change in female smoking prevalence although slight increases were observed in many countries.

In May 2010, near completion of the present report, the Special Eurobarometer 332/72.3 was published (EC 2010). This Eurobarometer, performed upon request of Directorate General Health and Consumers (SANCO) of the European Commission, reports on the results of an EU-wide telephone survey on tobacco conducted in late 2009. The survey method is standardised but the results are not directly comparable to the WHO reports quoted above. Furthermore, they are not comparable to an earlier Eurobarometer published in 2006 (EC 2006) due to changes of design (EC 2006, EC 2007a). Still, some additional information can be extracted. The Eurobarometer (EC 2010) reports the proportion of smokers as 29% (males 35%, females 25%) but does not distinguish between daily and non-daily smokers. It is not possible to ascertain whether this represents a further drop in adult daily smoking rates compared to the WHO report from 2007 mentioned above.

However, Eurobarometer (EC 2010) provides other data of interest. The average number of cigarettes consumed is 14.4/day, ranging from 22 in Cyprus to 10 in Sweden. Men smoke, on average, three cigarettes/day more than women. When asked to single out the most important reason for choice of brand, taste is most important for 22% of smokers in the EU 27 while price is most important for 6%. The package scored 0%. One out of 10 smokers in the EU believes that a less harmful cigarette can be identified by taste (ranging from 27% in Hungary to 3% in Denmark). Unique for the Eurobarometer (EC 2010) is the data on waterpipe smoking. On average, 11% of EU adults have tested or use a waterpipe occasionally, whereas 1% smoke it daily. Differences of use vary between countries but being a young adult appears to increase the probability of use.

3.13.1.3. Conclusions on tobacco use in different EU countries

Manufactured cigarettes are by far the most preferred tobacco products in the 27 countries of the European Union and constitute well over 90% of smoked tobacco. The overall adult daily estimated smoking prevalence (population-weighted) has stabilized at around 28.5% in 2005 (males 34.2%, females 22.6%) but higher rates are found mainly in Eastern European countries. Smoking rates have not changed significantly between 2002 and 2005. Smokeless tobacco is used by over 10% of the population in Sweden but its use is rare in other EU countries.

3.13.2. Brand preference in selected countries

United Kingdom

The cigarette market in the UK (and Ireland and Malta) is quite divergent from the continental European countries. This is mainly because in the UK some typical “English” brands are popular and have a large market share. Some quite surprising observations can be made when looking at the top-10 brands marketed in the UK (Hegarty 2010):

1. Of the top-10 brands (according to market share), three brands (Lambert & Butler King Size, Richmond King Size and Richmond Superkings) contain no additives (water is not considered as an additive).
2. Five brands contain up to 10 additives.

3. Two brands (Marlboro King Size Gold of PMI and Royals King Size Red of JTI) both contain over a dozen additives.

4. Lambert & Butler King Size is by far the most sold cigarette brand. Brands without additives have a market share of 42%, whereas those with 1 to 10 additives have a market share of 48%. Brands containing over a dozen additives have a market share of only 10%.

The "taste" of a tobacco product is not only defined by additives but also by blend-selection. English brands i.e. the typical UK brands are made predominantly from flue-cured Virginia tobacco, which contains relatively high amounts of sugars. Marlboro for instance uses the "American blend" (a mixture of Virginia, Burley and Oriental tobaccos) as a base to which many compounds are added during the manufacture.

By blending, it is possible to manufacture cigarettes with a characteristic taste, without using additives. Imperial Tobacco has thus succeeded in producing a typical brand (Lambert & Butler King Size) via the blending approach. In addition, cigarettes marketed as "additive free", may appeal to smokers that prefer "natural products".

Tobacco products in Central and Eastern European countries before and after 1990

Before 1990, the tobacco used for making cigarettes was usually domestic black shag, and most cigarettes were made with low amounts of additives. Cigarettes were sold without filters and tar levels of 20 to 30 mg per cigarette have been reported. The average nicotine content in Poland in the 1980s was 2 mg per cigarette implying that levels were 1.5 to 2 times the level in Western Europe. After 1990, the large international tobacco companies quickly took over and cigarettes were manufactured in Central and Eastern Europe according to international standards. Most cigarettes are manufactured from light tobacco and the proportion of filter cigarettes rose to 90%. The properties of cigarettes, additives and taste enhancers are now similar to those used in Western Europe and follow the European Union requirements (Zatonski 2008). Availability, marketing, trends, taste, and attractiveness are all factors that may have contributed to the rapid market change.

3.13.3. Smoking prevalence among young people / Target Groups

The analysis of smoking prevalence among young people is from the European Tobacco Control Report 2007 based on the WHO Health Behaviour in School-aged Children (HBSC) study, a cross-national research study conducted every four years: 1993/1994, 1997/1998 and 2001/2002 (WHO 2007a). The 2005/2006 survey was launched in 41 countries and regions and no comparable data are yet available. Information based on a second survey instrument, the Global Youth Tobacco Survey (GYTS) was also used (GYTS Collaborative Group 2002). The GYTS was developed by the United States Centers for Disease Prevention and Control (CDC) and WHO and has been carried out in a large number of countries in the European Region (see table 4). With more and more countries carrying out and repeating the GYTS, comparisons should be possible in the coming years.

3.13.3.1. Current status

According to the HBSC study, weekly smoking prevalence rates were on average 2% among 11-year-olds, 8% among 13-year-olds, and 24% among 15-year-olds. In general, smoking prevalence rates increased more steeply between the ages of 11 and 13 years than between 13 and 15 years. The results of the HBSC and GYTS studies show that weekly smoking prevalence rates in 15-year-old boys were especially high (>30%) in some Eastern European countries (Estonia, Latvia and Slovakia). The highest smoking prevalence rates (>30%) among 15-year-old girls were found mostly in Western European countries such as Austria, the Czech Republic, Finland and Spain. The lowest smoking prevalence rates among 15-year-old boys (<15%) were in Greece and Sweden. Smoking prevalence rates among girls were below 10% only in Greece. An overview of smoking prevalence rates among young people in the EU obtained by the HBSC and GYTS is provided in table 4.

Table 4 Weekly smoking rates among boys and girls in EU countries (WHO 2007a)

	HBSC				GYTS		
Country	**1997-1998**		**2001-2002**		**2001/2004**		
	Boys	**Girls**	**Boys**	**Girls**	**Year**	**Boys**	**Girls**
Austria	30	36	26.1	37.1			
Belgium	28	28	21.3	23.5			
Bulgaria					2002	28.7	26.4
Czech Rep.	22	18	28.7	30.6	2002	29.9	32.8
Denmark	20	28	16.7	21			
Estonia	24	12	30.4	18.2	2002-3	31.8	23.0
Finland	25	29	28.3	32.2			
France	28	31	26.0	26.7			
Greece	18	19	13.5	14.1	2003	16.3	9.5
Hungary	36	28	28.2	25.8	2003	24.1	27.4
Ireland	25	25	19.5	20.5			
Italy			21.8	24.9			
Latvia	37	19	28.9	21.1	2002	30.2	22.1
Lithuania	24	10	34.9	17.9	2001	29.0	20.5
Malta			16.9	17.4			
Netherlands			22.5	24.3			
Poland	27	20	26.3	17.0	2003	20.8	14.3
Portugal	19	14	17.6	26.2			
Romania					2004	16.8	12.8
Slovakia	28	18			2003	31.3	28.8
Slovenia			29.5	29.7	2003	24.2	28.8
Spain			23.6	32.3			
Sweden	18	24	11.1	19.0			
United Kingdom	25	33	21.1	27.9			

3.13.3.2. Gender differences

The prevalence of weekly smoking among 15-year-old girls was higher than that of 15-year-old boys in 16 mainly Western European countries of those that implemented the HBSC study in 2001/2002 (Austria, Belgium, the Czech Republic, Denmark, Finland, France, Greece, Ireland, Italy, Malta, the Netherlands, Portugal, Slovenia, Spain, Sweden and the United Kingdom). In Austria, Belgium, Sweden and the United Kingdom, this difference was even greater than in the late 1990s. In the remaining (mainly Eastern European) countries (Estonia, Hungary, Latvia, Lithuania, Poland), smoking prevalence in girls was lower, but in many of these 10 countries, it was catching up and, in two countries (Czech Republic and Hungary), even overtaking smoking prevalence in boys. The GYTS data in general confirmed the pattern of higher rates of smoking prevalence among boys than girls in Eastern Europe.

3.13.3.3. Changes in smoking prevalence

Sixteen countries implemented the HBSC survey both in 1997/1998 and 2001/2002.

A comparison of the results from these two surveys shows that weekly smoking prevalence rates in 15-year-old boys decreased in 11 (mostly western European) countries of the 16 countries, increased in four countries and remained stable in one. The picture among 15-year-old girls is quite similar: weekly smoking prevalence rates decreased in nine out of the 16 countries, and increased in seven.

A calculation of the averages from these two HBSC surveys shows that the average weekly smoking prevalence among 15-year-old boys and girls did not change significantly between the two periods, although a slight downward trend in boys and a slight upward trend in girls can be observed.

3.13.3.4. Conclusions on smoking according to different groups of young people

Weekly smoking rates among children and adolescents living in the European Union increase four-fold from about 2% at age 11 to 8% at age 13, and another 3-fold increase to 24% at age 15. The highest rates among boys are found in some Eastern EU countries whereas the highest rates among girls are seen in some Western EU countries. From the year 2000, non-significant trends towards decreased smoking among boys and increased smoking among girls have been observed. Smokeless tobacco use is common among adolescent boys in the Nordic countries but rare elsewhere.

Referring to section 3.12 it is clear that the tobacco industry not only has aimed to target different groups of users through advertising and promotion. They have also manipulated the cigarettes themselves. We have very limited data on market share by brand. Top ten lists have only been found from the UK (2009) and Germany (2007). Detailed information on annual cigarette sales in individual EU countries can be purchased dearly from commercial sources[14].

However, even in those publications no data on brand preferences according to gender, age, ethnicity or culture/region are presented. Again, referring to section 3.12 it is conceivable that such information is collected by the manufacturers but treated as secrets of trade.

Information about top selling individual brands in EU countries is available from commercial sources. In the public domain, only limited data are available. Data on brand preferences according to gender, age, ethnicity or culture/region are almost non-existent

[14] http://www.euromonitor.com

with a couple of limited reports from the UK being the exception. Referring to section 3.12 it is conceivable that such information is collected by the tobacco companies but treated as trade secrets.

3.13.4. Conclusions on EU

European Union tobacco smokers prefer manufactured cigarettes. The overall adult daily estimated smoking prevalence (population-weighted) had stabilized at around 28.5% in 2005 (males 34.2%, females 22.6%) but higher rates were found mainly in Eastern European countries. Smoking rates had not changed significantly between 2002 and 2005. The prevalence of weekly smoking among 15-year-old girls was higher than that of 15-year-old boys in 16 mainly Western European countries whereas the opposite was found in most Eastern European countries. In some countries (e.g. the UK) a large proportion of smokers preferred cigarettes marketed as “additive free”. Significant use of oral tobacco was seen only in Sweden and the UK.

3.14. Gaps of knowledge

In a number of areas, it was felt that insufficient information was available concerning tobacco additives:

- Smoke composition of tobacco products other than cigarettes (cigars, cigarillos, waterpipes).
- Importance of different sugars for the addictive potency of nicotine and tobacco products.
- Objective measures for attractiveness of tobacco products and additives.
- Information about which brands are preferred by new smokers and the reasons for brand choice.

3.15. Research Recommendations

It is evident that advanced studies on the action of nicotine and tobacco additives need considerable financial resources that are generally not available in public laboratories. Technological advances have been made in recent years that permit new information to be obtained, for instance on smoke composition and neural networks (functional neuroimaging). We propose either calls for European collaborative projects addressing questions about nicotine and additives or the creation of a European Institute for testing and research on drugs of abuse. A better knowledge in these areas would allow evidence based regulation of the manufacture and marketing of tobacco products to be established. Among the potential research areas we would like to mention:

- Investigate the effect of different sugars with respect to their presumed pro-addictive potency: (1) mode of action, (2) relative efficacy of various sugars, (3) generation of relevant biologically active compounds in the smoke or following entry of acetaldehyde into the blood stream, (4) the capacity of different tobaccos (i.e. Burley vs. Virginia) to form acetaldehyde and inhibit MAO in situ.
- Investigate the reasons why certain brands (e.g. certain typical UK brands) are popular in some countries although no additives have been used in their manufacture and study whether cigarettes without additives are less addictive than those with additives.

- Perform innovative neuroimaging techniques to assess the attractivity of tobacco additives objectively. The methods should be sufficiently sensitive to detect the contribution of a single additive added to a tobacco product.
- Determine what makes a specific brand attractive for new smokers. Is it the image, popularity, peer influence or taste?
- Determine, by neuroimaging studies, whether nicotine alone (given as pills) induces signals in the brain of dependent smokers that are different from non-smokers.
- Determine in animal studies (e.g. by neuroimaging, neurochemical, and behavioural approaches) the influence of different tobacco additives on the addictive potential of nicotine. These studies are crucial to define the exact role of the multiple tobacco additives in the final high addictive potential of tobacco (in humans).

3.16. Conclusions

In the present report we have evaluated the available scientific evidence for the role of additives in the addictiveness and attractiveness of tobacco products. The main addictive substance in tobacco leaves is nicotine, but pure nicotine is only weakly addictive in animal studies, and great variations are found between individual animals. In humans, pure nicotine products are not very efficient for cessation of tobacco use and other substances in tobacco products are likely to play a role in addiction. The vast majority of tobacco products are consumed as cigarettes, and they typically contain around 10% additives by weight; mainly sugars, humectants and various flavours. Most of the additives are used in small amounts. We have indicated various gaps of knowledge and made some recommendations for research in order to permit filling the gaps. In the following opinion chapter (section 4), we summarise the scientific evidence detailed in the previous sections in order to answer the questions concerning the contribution of additives to addictiveness and attractiveness of tobacco products.

4. OPINION

In the light of the most recent scientific information, the Scientific Committee is requested to answer the following questions:

1. Which are the criteria which will define whether an additive or a combination of additives increases the addictive potency of the final tobacco product?

In human studies there are clinical criteria for dependence (e.g. DSM, difficulty in quitting), laboratory measures of self-administration (e.g. neurobiological measures) and smoking frequency and depth of inhalation, as well as preference studies. These criteria indicate that tobacco in humans has a high addictive potential, but they have limitations when assessing the addictiveness of individual additives in the final tobacco product. There is no widely-agreed universal standard for human studies and as a result various possible endpoints exist. An addicted individual can be considered as someone who is suffering from a specific set of chronic conditions related to a modification of the regulation of their neural networks. It is the potential to induce these modifications which should be the criteria used to define the addictive potency of a product.

In animal studies the reinforcing potency of a drug is used as a criterion for addictive potential. However, some self-administration studies indicate that nicotine could have a weak addictive potential. At present it is not possible to evaluate whether additives increase the addictive potency of the final tobacco product. Drugs of abuse such as nicotine induce different types of behavioural and neurochemical dysregulations in animal studies but no consensus about which of those are directly related to the addiction process in humans has yet been attained among scientists.

In conclusion, the criteria for defining dependence indicate that tobacco is highly addictive in humans. Animal studies that use intravenous administration show that nicotine could have a weak addictive potential. An evaluation of the role of additives has not yet been done in animals.

2. What are the methods currently used for assessing the addictive potency of a substance and are they considered adequate?

Many different methods are used in humans, but there is a lack of consistency between these methods. Human studies have many limitations in design (e.g. the use of conditioned cues and the need to work with smokers). Furthermore, ethical issues may arise when testing substances in humans.

There is currently no animal model to assess the addictive potency of the final tobacco product; however, pure nicotine has been studied extensively.

The methods currently used in animals to evaluate the addictiveness of any drug of abuse, including nicotine, are mainly based on the evaluation of the re-inforcing properties of the drug. These experimental animal models are mainly based on self-administration protocols in rodents, usually rats. The model with the highest predictive validity is the operant self-administration paradigm. A response which is easy to evaluate is the break point. This is defined as the highest number of responses that the animal completes in order to obtain a single delivery of a drug. A higher break point represents a direct measure of the motivation of the animal to obtain the drug and is often taken to imply an increase in the addictive potency of the drug.

Other models have also been used, such as the intracranial self-stimulation and the conditioned place preference paradigms. New complex behavioural models that resemble the main diagnosis for drug addiction in humans have been developed very recently, although these new models can only be applied for some particular drugs and experimental conditions at the present moment. The methods have additional limitations as in animal studies pure nicotine is injected intravenously and shows only a weak addictive potential whereas in humans tobacco is used differently (e.g. inhalation, oral consumption). The operant self-administration paradigm has been widely accepted as a reliable animal model with high predictive value for the abuse liability of a drug and therefore, possibly also for its addictive potential in humans. However, a consensus between scientists has not yet been attained on whether this method, which is appropriate to define the abuse liability, would also be the most suitable method to define the addictive potential of a drug.

In conclusion, there are many methods for assessing the addictive potency of a substance in humans, but they have limitations in design and ethical issues may arise. Animal studies using self-administration protocols evaluate the reinforcing properties after intravenous injection of the drugs but there is no consensus concerning the most suitable method for defining the addictive potential. The current methods can thus not be considered adequate.

3. Is the development of nicotine addictiveness dose-dependent?

In humans, there are little data available on pure nicotine use. However, when consumed in tobacco, frequency of use (number of cigarettes smoked per day) is positively correlated with dependence. This suggests that individuals who maintain higher nicotine levels in blood are more dependent than individuals who maintain low levels.

Based on the criteria described in Question 1, dose-dependency appears to have been shown in animal studies. In general, an inverted U-shaped dose-response has been revealed in animals, suggesting that the addictiveness of nicotine is not directly linear with the dose. In addition, pure nicotine is only weakly addictive in some animal studies.

There is substantial variation in response to nicotine and addictive potential in both animals and humans, and genetic factors probably play an important role.

4. Which additives are addictive by themselves in tobacco products?

No tobacco additives, which are addictive by themselves, have so far been identified. However, sugars, added in high quantities to most tobacco products, give rise by pyrolysis to acetaldehyde which is self-administered by animals.

However, experiments using denicotinised cigarettes show that besides nicotine, a mixture of factors in cigarette smoke probably plays an important role in craving and reinforcement. Although these unknown factors do not have pharmacological effects similar to nicotine and are probably not addictive, they definitely play a role in smoking behaviour.

5. Which additives enhance the addictiveness of nicotine and how?

A large percentage of the additives found in tobacco are sugars, or their derivatives, that by pyrolysis produce numerous toxic substances, including different combinations of aldehydes, one of which is acetaldehyde. Acetaldehyde injected into experimental animals enhances the addictiveness of nicotine, probably by inhibiting monoamine oxidase (MAO) in the brain. Smokers have decreased levels of MAO in the brain. However, there is no proof that acetaldehyde in the smoke contributes significantly to blood levels of acetaldehyde. This does not exclude that there is a biological effect of acetaldehyde, possibly by generation of harman and norharman that also may inhibit MAO.

Additives that facilitate deeper inhalation (e.g. menthol) may enhance the addictiveness of nicotine indirectly. Other substances may enhance the addictiveness of nicotine by inhibiting its metabolism. Substances such as ammonia that increase the pH of the tobacco (and the smoke) result in higher amounts of uncharged nicotine, that is more easily absorbed by the cells. However, due to the high buffer capacity of the lining fluid in the lungs it is uncertain if more nicotine is absorbed with higher smoke pH. It is unlikely that additives in smoked tobacco would increase nicotine blood levels sufficiently to enhance the addictive potential of the tobacco product. For smokeless tobacco it has been shown that more nicotine is absorbed in the mouth when the pH of the product is increased.

In conclusion, apart from the possible action of combustion products of sugars (acetaldehyde and similar compounds that enhance the action of nicotine by inhibition of MAO), there is no evidence that additives enhance the addictiveness of nicotine and therefore of tobacco.

6. Which are the methods used to quantify the potency of additives in enhancing the addictiveness of nicotine and are they considered adequate?

The methods used to quantify the potency of additives to enhance the addictiveness of nicotine or tobacco, are described in the answer to question 2. The limitations of these methods arise from technical challenges in experimentally manipulating the presence or absence of an additive in the tobacco products used in these experiments. Such experiments have probably been carried out by the tobacco industry for some additives, especially sugars and their derivatives, but they require technical and financial resources that are not generally available except to the tobacco industry. In addition, there are ethical issues if testing in humans is considered.

In conclusion, the methods used to quantify the potency of additives in humans or animals have limitations, and the available methodologies are thus not considered adequate for a reliable quantification.

7. Which technical characteristics enhance the addictive potential of tobacco products?

A number of technical characteristics of cigarettes influence the content of different substances in the smoke and the size of smoke particles. The so-called TNCO values (tar, nicotine and carbon monoxide) are determined by, amongst other things, ventilation (paper, filter), the packing of the tobacco and the geometry of the cigarettes. Smokers usually compensate for a lower dose of nicotine by increasing puff volume and frequency, and by deeper inhalation. In order to achieve the desired level of nicotine impact many smokers apparently take more puffs and inhale deeper when smoking low nicotine cigarettes.

A change of the technical characteristics of cigarettes may affect the mean particle size and, therefore, the distribution of the smoke aerosol. However, based on the limited publicly available information, it seems that exposure to nicotine cannot be substantially increased by altering the particle size of the smoke aerosol.

In conclusion, it does not seem that technical characteristics can enhance the addictive potential of tobacco products.

8. Which are the criteria based on which an additive or a combination of additives can be considered (classified) attractive?

The criterion of attractiveness is the stimulation to use the product.

Attractiveness of additives refers to factors such as taste, smell and other sensory attributes. In addition, a number of external factors (e.g. ease of use, flexibility of the dosing system, cost etc.) contribute to the attractiveness of the product.

The attractiveness of tobacco products may be increased by a number of additives. Many different additives are used to create a specific taste/flavour in order to attract certain target groups. An attractive effect may be obtained by changing the appearance of the product and the smoke, decreasing the harshness of the smoke, and inducing a pleasant experience of smoking. The sweetness of the smoke is an important characteristic for certain users. Finally, in order to make smoking more acceptable to other people nearby, some additives have the function of reducing lingering odour or side-stream smoke visibility.

In conclusion, many different factors influence the attractiveness of tobacco products, not only the additives used but also a number of external factors.

9. What are the methods currently used for assessing attractiveness and are they considered adequate?

Animal models do not currently exist to allow the assessment of attractiveness.

There are two main ways of examining the influence of additives on the attractiveness of a product which have largely been conducted by tobacco industry.

The first is to assess individual tobacco products and compare their attractiveness against other tobacco products on a number of scales/dimensions. By then examining what is known about the additive content of these products, judgements can be made as to the role of individual additives in the overall attractiveness of the product. This can be done using a variety of research methods, such as panel studies and surveys, experimental measures and human testing.

The second is to examine the influence of individual additives or combination of additives on attractiveness of a tobacco product, along a number of scales, by experimentally adjusting tobacco products to include or exclude individual additives and testing responses to them. In addition, the quantity of the additive can be varied to assess dose response and whether there is a threshold below which any impact is not observed.

The difficulties with this type of research include ethical considerations that will usually preclude human testing of different tobacco products, particularly among non-users or children.

In conclusion, it is only possible to assess attractiveness in humans, and this may be done by comparison of different products used or by adjusting tobacco products experimentally. However, such studies in human subjects are difficult to carry out due to ethical considerations and the current methods are thus not considered adequate for a reliable quantification of attractiveness in humans.

10. Which additives increase attractiveness of tobacco products?

Numerous additives are used in order to increase the attractiveness of tobacco products.

Various sugars constitute a large proportion of additives, and the sweetness of the smoke is an important characteristic of the product.

Some additives are used to attract certain target groups, because they give the product a specific taste/flavour particularly appreciated by the target group. The best known example is menthol (African Americans) and the use of fruit and candy flavours in high amounts to favour smoking initiation by young people.

A number of additives decrease the harshness and increase the smoothness of the smoke. As a result the smoke inhaled is less aversive, cooler and milder, which improves the experience of smoking and promotes smoking initiation. The harshness depends partly on the tar/nicotine ratio, but may also be decreased by additives such as propylene glycol and glycyrrhizin, a substance in liquorice. Menthol, due to its local anaesthetic effect may enable a deeper inhalation of the smoke. It also acts on sensory nerve endings, resulting in a cooling effect appreciated by smokers.

For cigarettes, certain additives yield a full and white smoke (for example, magnesium oxide, magnesium carbonate, sodium acetate, sodium citrate, calcium carbonate). Other additives reduce the lingering odour of the smoke in order to favour the acceptability of smoking to people around (for example, acetylpyrazine, anethole, limonene, vanillin, benzaldehyde).

In conclusion, many different additives have been used to increase the attractiveness of tobacco products but it is very difficult to identify the role of individual additives in enhancing attractiveness. In several countries there is a growing trend of using "natural" tobacco products advertised as containing no additives.

11. What is the association between additives and tobacco consumption (independent of any addictive potential they might have)? Which additives are used to target specific groups?

Additives considered attractive may in principle lead to brand preference or a higher consumption of tobacco products, although it is difficult to disentangle the direct effects of additives from indirect effects such as the marketing of specific products at specific groups. For example, the consumption of menthol cigarettes is much higher among African Americans in the USA than among other populations, while flavourings (e.g. fruit and candy) appear to be targeted at young people.

It is notable that waterpipe smoking is becoming increasingly popular in some EU countries (and elsewhere), potentially due to the flavoured tobaccos used and the mild smoke, which facilitate the inhalation of large volumes into the lungs. Smokeless tobacco products have gained increased interest from the tobacco industry because they may be used in places where smoking is prohibited.

Additives and design characteristics may modify consumption patterns, theoretically in a way which may impact on uptake of tobacco use and/or the development of dependence. However, in spite of the many additives commonly used, tobacco products openly marketed as containing specific additives (e.g. menthol cigarettes) command a relatively small market share in EU countries and in some markets so-called natural tobacco products are becoming popular.

In conclusion, additives have been used largely by the tobacco industry to target specific groups. However, the effect of marketing is probably very important and there is currently a trend in several countries to use products labelled "without additives".

Gaps in knowledge:

- Smoke composition of tobacco products other than cigarettes (cigars, cigarillos, waterpipes).
- Importance of different sugars for the addictive potency of nicotine and tobacco products.
- Objective, quantitative measures for attractiveness of tobacco products and additives.

- Information about which brands are preferred by new smokers and the reasons for brand choice.
- Why are certain brands, apparently without additives, popular in certain countries (UK)?
- Effect of pure nicotine in smokers and non-smokers (neuroimaging).
- Effect of nicotine and additives in experimental animals (neuroimaging).
- Role of substances other than nicotine and in the absence of nicotine, on the use of tobacco.

Recommendations:

- European funding of research on nicotine/European research institute.
- Investigate effect of sugars when pyrolysed.
- Perform epidemiogical/sociological studies on trends.
- Investigate effect of nicotine and other substances, in particular by functional neuroimaging in animals and humans.
- Analyse constituents of tobacco smoke.

5. MINORITY OPINION

None

6. LIST OF ABBREVIATIONS

Ach	Acetylcholine
AM251	N-(piperidin-1-yl)-5-(4-iodophonyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
APA	American Psychiatric Association
ASH	Action on Smoking and Health
BAT	British American Tobacco
BfR	Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment)
BN	Bates Number
BZgA	Bundeszentrale für gesundheitliche Aufklärung (Federal Centre for Health Education)
CAS	Chemical Abstracts Service
CB1	Cannabinoid receptor
CDC	Centers for Disease Prevention and Control

CLD	Cased Leaf Dryer
CMD	Count median diameter
CNRS	Centre national de la recherche scientifique (French National Center for Scientific Research)
CNS	Central nervous system
CO	Carbon monoxide
CSERP	Chemosensory event-related potential
CYP	Cytochrome P450 monooxygenase
DA	Dopamine
DAP	Diammonium hydrogen phosphate
DKFZ	Deutsches Krebsfoschungszentrum (German Cancer Research Center)
DNA	Deoxyribonucleic acid
DSM(-IV)	Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition)
EC	European Commission
ECDC	European Centre for Disease prevention and Control
ECHA	European Chemicals Agency
EEG	Electroencephalography/Electroencephalogram
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EMTOC	Electronic Model Tobacco Control
EU	European Union
FCTC	Framework Convention on Tobacco Control
FDA	(United States) Food and Drug Administration
fMRI	Functional Magnetic Resonance Imaging
FTND	Fagerström Test for Nicotine Dependence
GABA	Gamma (γ)-Aminobutyric acid
Glu	Glutamate
GM	Genetically modified
GYTS	Global Youth Tobacco Survey
HBSC	Health Behviour in School-aged Children
5-HT	5-Hydroxytryptamine
IARC	International Agency for Research on Cancer
IC_{50}	The half-maximal inhibitory concentration
ICD	International Classification of Diseases
ICRP	International Commission on Radiological Protection
i.v.	Intravenous
JTI	Japan Tobacco Inc.
LD_{50}	Median lethal dose
MAO	Monoamine oxidase
mGlu5	Metabotropic glutamate 5

mGlu2/3	Metabotropic glutamate 2/3
MMD	Mass median diameter
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MS	Member State(s)
NAc	Nucleus accumbens
nAChR	Nicotine acetylcholine receptor
NCI	National Cancer Institute
NMDA	N-Methyl-D-aspartate
NNAL	4-N-(Nitrosomethylamino)-1-(3-pyridyl)-1-butanol
NNAL-Gluc	NNAL-Glucuronide
NNK	4-N-(Nitrosomethylamino)-1-(3-pyridyl)-1-butanone
NRT	Nicotine replacement therapy
ONS	Office for National Statistics
pH	Measure of acidity or basicity of a solution
pKa	Dissociation constant – measure of the strength of an acid or a base
PMI	Philip Morris International
ppm	parts per million
PPTg	Pedunculopontine tegmental nucleus
PREP	Pattern reversal evoked potential
QNE	Quantity not exceeded
RECON	Reconstituted tobacco
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (The Netherlands National Institute for Public Health and the Environment)
RYO	Roll your own
SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee n Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
STRATUS	Studies with Rimonabant and Tobacco Use
$T_{1/2}$	Half-life
TNCO	Tar, nicotine and carbon monoxide
TRPM8	Transient receptor potential channel
UK	United Kingdom
US(A)	United States of America
UV	Ultraviolet
VTA	Ventral tegmental area
WHO	World Health Organization

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8. GLOSSARY

Abuse liability	Abuse liability of a drug is the likelihood that its use will result in addiction (dependence) and it can be assessed in laboratories by methods referred to as abuse liability testing.
Additives	The present report uses the term additives for added ingredients or substances. Additives are defined as any substance that is added except water, during the course of manufacture of a tobacco product, including preservatives, humectants, flavours, and processing aids.
Addiction	Addiction is the commonly used term referring to what is technically known as "dependence" and is widely employed to connote severe substance dependence.
Addictiveness	Addictiveness refers to the pharmacological potential of a substance to cause addiction.
Agonist	A ligand for a receptor which induces a response, identical or partial to the response obtained with the endogenous ligand.
Attractiveness	The terms "attractiveness" or "consumer appeal" refer to factors such as taste, smell and other sensory attributes, ease of use, flexibility of the dosing system, cost, reputation or image, assumed risks and benefits, and other characteristics of a product designed to stimulate use.
Break point	Highest number of responses that the animal accomplishes to obtain a single delivery of a drug.
Bronchodilatator	A substance that dilates the bronchi and bronchioles.
Casing	Casing refers to the sauce composed of a variety of ingredients such as humectants, sugars, cocoa, liquorice and fruit extracts which is applied to tobacco during the manufacturing process.
Conditioned cue	Neutral stimulus that associates with a reward. Used in abuse liability testing.
Curing	Curing is the process for drying freshly harvested tobacco with partially or fully controlled temperature and moisture schedules.
CYP2A6	It is an abbreviation of Cytochrome P-450 2A6 (family 2, subfamily A, polypeptide 6), a constitutent of the endoplasmic reticulum P-450 mixed function oxidase system. CYP2A6 is the main enzyme system involved in the oxidative metabolism of nicotine and cotinine, as well as many other xenobiotics and pharmaceuticals. A significant interindividual variability in CYP2A6 and mRNA levels has been observed in humans and other mammals
Denicotinised	The removal or reduction in the nicotine content of tobacco, for example by means of blending genetically-modified tobacco which has been engineered to lack nicotine.
DSM	Diagnostic and Statistical Manual of Mental Disorders. Published by the American Psychiatric Association (USA) provides standard criteria for the classification of mental disorders. It is used in the United States and in varying degrees around the world. It is not exempt of scientific criticism in many countries
EEG	Electroencephalogram.

GABA receptor	An oligomeric class of neuron membrane receptors to which the γ–aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain, binds.
Harman	A beta-carboline that is formed in smoke by interaction between acetaldehyde and tryptophan. It inhibits the enzyme monoamine oxidase (MAO).
Harshness	A chemically induced physical effect associated with a roughness, rawness experience generally localized in the mouth and to a lesser degree in the upper reaches of the throat and the trachea due to inhalation of tobacco smoke. It can cause a drying, rasping, coarse, astringent sensation.
Hyperlocomotive effect	Increase in locomotor activity usually recorded in rodents.
IC50	Inhibitory concentration 50. The concentration of a compound that inhibits 50% a given maximal response (biological, biochemical, etc)
Ingredients	see Additives. The present report uses the term additives for added ingredients or substances.
LD50	Lethal dose 50. Dose of a compound that kills 50% of a group of administered animals (it represents a probabilistic concept).
Manipulandum	Device used in experimental settings in order to transmit an active response. In the present report the device is used to measure self-administration of drugs in experimental animals.
MAO	Monoamine oxidases exist in two forms, A and B. They metabolize monoamines such as noradrenaline, dopamine and serotonin.
Metabolism	The chemical processes occurring within a living cell or organism that are necessary for the maintenance of life. In metabolism some substances are broken down to yield energy while other substances are synthesized.
Narghile or shisha	Expressions for the Oriental waterpipe.
Norharman	Condensation product in smoke that inhibits the enzyme monoamine oxidase (MAO). See also harman.
pH	Measure of the acidity or alkalinity of a solution, numerically equal to 7 for neutral solutions, increasing with increasing alkalinity and decreasing with increasing acidity. The pH scale commonly in use ranges from 0 to 14.
P450 enzyme system	The cytochromes P450 are hemproteins and important constituents of the so-called monooxygenase system
Pyrolysis	Chemical decomposition of condensed substances that occurs spontaneously at high enough temperatures.
Receptor	Protein or protein complexes present in the cell membranes (plasmatic, endoplasmic or nuclear) or the cytoplasm to which physiological signaling molecules, e.g. neurotransmitters, hormones, etc., drugs and xenobiotics specifically, bind.
Reinforcement	Ability of a stimulus to promote behavioural responses in order to obtain (positive reinforcement) or to avoid (negative reinforcement) such a stimulus.
Rewarding	stimuli that have appetitive (desirable) consequences and/or produce a hypothetical pleasurable internal state (hedonia)
Self-administration	Experimental procedures that allow the animal/human to

	administer himself a drug. Self-administration methods are widely used to directly evaluate the reinforcing properties of a drug.
Smoothness	Reduction in the harsh irritation of nicotine-containing tobacco smoke.
Uncharged	Used e.g. for nicotine to describe the free base, that under acidic conditions (lower pH) may be charged (protonated) with one or two protons.