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Influence of Tobacco Additives on the Chemical Composition of Mainstream Smoke*

by

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SUMMARY

Additives used in tobacco product manufacturing are currently in the focus of public discussions with regard to potentially increased consumer health risks on account of certain additives. In addition, a few additives are suspected to enhance the addictiveness of tobacco products. In 2006, the German Federal Ministry for Food, Agriculture and Consumer Protection (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz, BMELV) commissioned a research project intended to provide support for the evaluation of additives and their influence on the composition and properties of cigarette mainstream smoke. In this paper the results of the study are reported. Different amounts of glycerol, cocoa powder and sucrose were added to the tobacco of two kinds of filter-ventilated King size test cigarettes with 'tar' levels of 6 mg and 10 mg per cigarette. The tobacco of the test cigarettes consisted of a commercially available blend made of Virginia, Burley and Oriental tobaccos. Machine smoking was performed according to the applicable ISO smoking regimen. Various smoke components, which are suspected to be harmful for health, were determined in mainstream smoke. Increasing levels of sucrose were correlated with an increase of the amount of formaldehyde but not of acetaldehyde in the mainstream smoke of the test cigarettes. In cigarettes with different levels of added glycerol no substantial change in smoke composition was observed. The addition of cocoa powder to tobacco resulted in a decrease of tobacco-specific *N*-nitrosamines in mainstream smoke. The results obtained in this study can be used as evidence for the toxicological evaluation aimed at approving or banning specific additives for tobacco product manufacturing.
[Beitr. Tabakforsch. Int. 24 (2010) 100–116]

ZUSAMMENFASSUNG

Die bei der Herstellung von Tabakerzeugnissen verwendeten Zusatzstoffe werden im Hinblick auf eine mögliche gesundheitsgefährdende Wirkung öffentlich diskutiert. Zusätzlich stehen einige Zusatzstoffe im Verdacht, die abhängig machende Wirkung von Tabakprodukten zu verstärken. Vom Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (BMELV) wurde 2006 ein Forschungsprojekt in Auftrag gegeben, das die Bewertung von Zusatzstoffen unterstützen und deren Einfluss auf die Zusammensetzung und die Eigenschaften von Tabakrauch untersuchen soll. Diese Arbeit fasst die Ergebnisse des Projektes zusammen.
Dem Tabak von zwei filterventilierten King-size-Testzigaretten mit unterschiedlichen Kondensatwerten (6 mg und 10 mg pro Zigarette) wurden verschiedene Konzentrationen von Glycerin, Kakao (als Puder) und Saccharose zugesetzt. Der Tabak bestand aus einer marktüblichen Mischung aus Virginia-, Burley- und Orienttabak. Der Hauptstromrauch wurde mit Hilfe einer analytischen Rauchmaschine nach dem Abrauchregime der Internationalen Organisation für Normung (ISO) generiert. Verschiedene, als gesundheitsschädigend erachtete Rauchinhaltsstoffe, wurden im Hauptstromrauch der Testzigaretten bestimmt. Während ein steigender Saccharosegehalt zu einem Anstieg von Formaldehyd im Hauptstromrauch führte, konnte diese Korrelation für Acetaldehyd nicht beobachtet werden. Testzigaretten mit unterschiedlichem Glyceringehalt zeigten keine signifikanten Veränderungen in der Zusammensetzung des Tabakrauches im Vergleich zu der Referenzzigarette. Der Tabakzusatz von Kakaopulver resultierte in einer Abnahme von tabakspezifischen Nitrosaminen (TSNA).

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Die Daten dieses Forschungsprojektes können als Entscheidungshilfe für die toxikologische Bewertung und die Zulassung oder das Verbot von Zusatzstoffen bei der Herstellung von Tabakprodukten herangezogen werden. [Beitr. Tabakforsch. Int. 24 (2010) 100–116]

RESUME

Les additifs utilisés dans la fabrication des produits à base de tabac font actuellement l'objet de discussions publiques relatives à l'augmentation éventuelle des risques pour la santé des consommateurs liés à certains additifs. D'autre part, on soupçonne certains additifs d'augmenter la dépendance aux tabacs manufacturés. En 2006, le Ministère fédéral allemand de l'alimentation, de l'agriculture et de la protection des consommateurs (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz, BMELV) a commandé un projet de recherche visant à fournir un soutien pour l'évaluation des additifs et de leur influence sur la composition et les propriétés de la fumée principale de cigarettes. Ce document présente les résultats de cette étude. Différentes quantités de glycérine, de poudre de cacao et de saccharose ont été ajoutées au tabac de deux sortes de cigarettes tests avec filtres ventilées King size avec des niveaux de goudron de 6mg et 10mg par cigarette. Le tabac des cigarettes tests était constitué d'un mélange de tabacs Virginia, Burley et Oriental disponibles dans le commerce. La machine à fumer a été utilisée conformément au régime de fumage ISO en vigueur. Différents composants de la fumée, soupçonnés d'être nuisibles pour la santé, ont été déterminés dans la fumée principale. Des niveaux accrus de saccharose ont été mis en corrélation avec une augmentation de la quantité de formaldéhyde, mais pas d'acétaldéhyde, dans la fumée principale des cigarettes de test. Dans les cigarettes ayant des niveaux différents de glycérine ajouté aucun changement substantiel dans la composition de la fumée n'a été observé. L'ajout de poudre de cacao a entraîné une diminution des *N*-nitrosamines spécifiques au tabac dans la fumée principale. Les résultats obtenus lors cette étude peuvent servir de preuve pour l'évaluation toxicologique visant à améliorer ou à interdire certains additifs lors de la fabrication de produits à base de tabac. [Beitr. Tabakforsch. Int. 24 (2010) 100–116]

INTRODUCTION

Regulatory background in Germany

After the reform of the German Food Law in 1974, tobacco products were no longer considered food but treated as a separate product category under the new German Law on the Commercial Use of Foods, Tobacco Products, Cosmetics and Consumer Goods (Gesetz über den Verkehr mit Lebensmitteln, Tabakerzeugnissen, kosmetischen Mitteln und sonstigen Bedarfsgegenständen LMBG) (1). When a new food regulation came into force in the EU in 2002 (2), the relevant legislation in Germany had to be amended accordingly. Within the framework of the EU regulation, Germany issued, in 2005, the Law on the Reorganization of the Food and Feed Legislation (Gesetz zur Neuordnung des Lebensmittel- und Futtermittelrechts) the Code on Foods, Consumer Protection and Feed (Lebensmittel-, Bedarfsgegenstände- und Futtermittelgesetzbuch LFGB) (3). Regulations pertaining to tobacco products were included in the Provisional Tobacco Law (Vorläufiges Tabakgesetz) (4).

Inter alia, the Provisional Tobacco Law stipulates which tobacco products are allowed to be brought on the market and how they are to be monitored. Furthermore, the law empowers the Federal Ministry of Nutrition, Agriculture and Consumer Protection to set maximum levels for certain smoke constituents and requires manufacturers to inform the surveillance authorities about certain details, particularly the production and composition of tobacco products, the additives used in manufacturing, their function, the toxicity of these substances in burned or unburned form and their influence on the addictiveness of the product and on consumers health.

Since 1977, the use of additives has been regulated by the German Tobacco Ordinance (Tabakverordnung, TVO) (5). It specifies in detail which substances are permitted for manufacturing tobacco products, together with quality requirements, maximum inclusion levels and directions, in which specific tobacco product they may be used (Annex 1 of the Tobacco Ordinance). The Tobacco Ordinance works on the basis of the prohibitory principle: "Everything is prohibited unless it is explicitly allowed". For example, the Tobacco Ordinance defines precisely which plants or parts of plants, fruits, etc., may be used as additives for tobacco products, which solvents are allowed in and for the application of aromatic essences to the tobacco product or which materials may be used for cigarette filters, cigarette papers and adhesives, etc. Another list, a "negative list", defines certain substances, plants or their parts and specific substances that are explicitly prohibited for reasons of health protection. In addition to very precise designations, there are also non-specific terms such as "fruits", "fruit juice", "spirits" and "cocoa products". Moreover, "flavorings" that comply with the requirements of the German Flavoring Ordinance (Aromenverordnung) (6) may be used as additives in tobacco products without restrictions. These non-specific terms make it impossible to pinpoint the exact number of additives approved in Germany.

The German Tobacco Products Ordinance (Tabakprodukt-Verordnung) (7) is based on the EU Tobacco Products Directive 2001/37/EC (8) and came into force in 2002. Among other things, the German Tobacco Products Ordinance - like comparable regulations in other EU member states - places manufacturers and importers of tobacco products under the obligation of making reports on the additives used. Every year, manufacturers and importers have to inform the responsible surveillance authorities of all additives used in the individual tobacco products, compiled in a list organized according to brand name and type and including quantities in descending order by percentage of weight (based on tobacco weight in finished product). Moreover, explanations have to be provided of the reasons for using the additives, together with available toxicological data on the additives, particularly in terms of health and addictive effects. For keeping consumers informed, the contents of the list are published in Germany on the internet in appropriate form by the Federal Office of Consumer Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, BVL).

Design and objectives of the study

Cigarette tobacco may contain a large number of additives. The additives may be individual substances, such as humectants (glycerol, 1,2-propylene glycol), various sugars, aromatic substances like menthol and vanillin, or complex mixtures of aromatic compounds, seasonings, honey, cocoa, licorice, etc. According to Annex 1 of the Tobacco Ordinance and the publications released by the international tobacco industry, it is estimated that up to 500 additives may be used today in the production of cigarettes, as published by DOULL *et al*. for cigarettes made for the U.S. market (9).

The additives for tobacco products are currently in the focus of public discussions with regard to possible health risk and addiction enhancing effects. More than 5000 substances are generated when tobacco burns (10) and according to the International Agency for Research on Cancer (IARC) more than 70 substances in tobacco smoke are classified as carcinogenic or very probably carcinogenic (11). In addition, it cannot completely be ruled out that the burning and pyrolysis processes during smoking generate harmful substances also from additives, thus compounding the risk for the consumer. Due consideration must, therefore, be given to the special aspects of the burning, pyrolysis and pyrosynthesis processes when assessing approved additives.

Two kinds of filter ventilated test cigarettes in King size format with mainstream smoke 'tar' contents of 10 mg and 6 mg (according to ISO 3308 (12)) were used in our study. The cigarettes were made from a commercially available, additive-free blend consisting of Virginia, Burley and Oriental tobaccos. The effects of different levels of the tobacco additives, glycerol, cocoa powder and sucrose, on the yields of several compounds in the mainstream smoke were studied and compared to additive-free reference cigarettes.

The literature data regarding the transfer and pyrolysis of the three additives, glycerol, cocoa powder and sucrose, show that it is not exactly clear which direct influence their addition to tobacco may have on the composition of mainstream smoke (13–24). Furthermore there is only limited comparability of the pyrolysis data because the studies in the literature are based on different experimental conditions which have a major effect on the pyrolysis products and their quantities. Over the last 50 years, numerous studies have been published on the influence of additives on cigarette smoke. A comprehensive review of the effects of tobacco additives on cigarette mainstream smoke was published by PASCHKE, HELLER and SCHERER in 2002 (25). Most publications are studies done by the tobacco industry and not by scientists unconnected to the cigarette industry. For the most part, these refer to studies of additive mixtures actually used in the production of cigarettes. However, the toxicological assessment of an additive has to look at the influence of the individual substance. In other words, the question whether an additive has an effect on the quantity of toxic substances in smoke can only be answered by comparing the smoke of cigarettes with and without the specific additive.

Originally, our intention was to examine the influence of certain additives only on the contents of tobacco-specific nitrosamines (TSNAs) and benzo[*a*]pyrene (B*a*P) in cigarette mainstream smoke. These substances are primarily found in the particulate phase of tobacco smoke, whose composition and toxicity have been discussed in the literature in great detail (11). However, the number of compounds investigated was expanded by the addition of four gas phase components (formaldehyde, acetaldehyde, 1,3-butadiene and isoprene) as the toxicity and biological activity of tobacco smoke is brought about by substances in the gas-phase as well as the particulate phase (26, 27). 1,3-Butadiene and isoprene represent the volatile hydrocarbons in the gas phase. Formaldehyde and acetaldehyde are other toxic components of the gas phase, which were examined in our study because the correlation of their levels in tobacco smoke with the sugar content in tobacco has been discussed in the literature (28).

In summary, the influence of the additives under investigation on the yields of the following components in the mainstream smoke of experimental cigarettes was examined in our study:

- Carbon monoxide (CO)
- Nicotine
- Nicotine-free dry particulate matter (NFDPM), also referred to as 'tar'
- Tobacco-specific nitrosamines (TSNAs)
- Formaldehyde
- Acetaldehyde
- 1,3-Butadiene
- Isoprene
- Benzo[*a*]pyrene (B*a*P).

DESCRIPTION OF THE ADDITIVES EVALUATED IN THE STUDY

Glycerol

Glycerol is used in tobacco product manufacturing as a humectant. Together with other humectants, such as 1,2-propanediol, the total amount in German cigarettes must not exceed the statutory maximum level of 5% of dry tobacco weight. This study was carried out with glycerol. Humectants are used in nearly all tobacco products and glycerol is one of the most frequently used.

A number of questions concerning the fate of glycerol in the tobacco column of a cigarette during smoking are still open. The relevant literature indicates that part of the glycerol added to the tobacco passes unchanged into the mainstream smoke the same way as many other volatile tobacco additives (13). However, the transfer rate depends to a great extent on the design of the cigarette. The share of glycerol found in mainstream smoke increases in proportion to the quantity of glycerol in the tobacco, ranging from 5% to 14% of the glycerol used. Studies with radiolabeled glycerol indicated that a portion could be detected also in the tobacco butt and the filter tip (14–16).

Although some of the glycerol in the tobacco is directly

volatilized and can be detected in the smoke and filter, part of it is burned and pyrolyzed during smoking and suspected to be a major contributor to acrolein and aldehydes in tobacco smoke (29).
It was shown by pyrolizing neat glycerol as a liquid and as a vapor respectively, that the yields of the individual substances formed differ according to the chosen experimental conditions. While an earlier paper found in particular polycyclic hydrocarbons and phenols, together with various aldehydes and carboxylic acids (17), later data mainly revealed aldehydes and low-molecular hydrocarbon compounds as pyrolysis products of glycerol vapor (18). More recent publications of the tobacco industry examining experimental cigarettes containing glycerol report that, while a relatively large amount of the added glycerol is not pyrolyzed or burned (up to 95%), nevertheless small quantities of acrolein and glycolaldehyde are generated (19). In 2010, YIP *et al.* reported that 0.25–0.30% of the added glycerol was converted to acrolein and acetone, detected in cigarette mainstream smoke (20).

Cocoa powder

Cocoa and cocoa products belong to the tobacco additives used as flavorings in so-called American Blend cigarettes. As a rule, they do not exceed 1.5% of the weight of tobacco in cigarettes sold in Germany. One natural component of cocoa is the pharmacologically active substance theobromine (with a share between 7 and 39% (30)). As an additive in tobacco products for smoking, cocoa with its component, theobromine, is alleged to enlarge the pulmonary alveoli. It is speculated that this permits deeper inhalation of the smoke and could augment nicotine absorption in the lungs (29, 31, 32). On the other hand, a report of the Dutch Ministry of Health comes to the conclusion that the exposure of the smoker to the basically psychoactive components, theobromine and caffeine, resulting from the addition of cocoa to cigarettes, is negligible as the uptake is too low to show any effect on the pulmonary system (30). Pyrolysis studies show that cocoa powder and cocoa shell extract generate mainly furfural and phenol by pyrolysis, together with cresol and styrene (21).

Sucrose

Various sugars are frequently added to tobacco by the tobacco industry in considerable quantities. The maximum quantity of sugar added to cigarettes during the production process amounts to approximately 5% of the tobacco weight. According to the tobacco industry, sugar is used to improve the taste of tobacco smoke. However, sugar and sugar compounds are also among the natural components of tobacco. The literature indicates that the total sugar content (mono- and disaccharides) of a Virginia cigarette, i.e. a cigarette without additives, may account for up to 20% of total tobacco weight due to the natural sugar in the tobacco (33). The total sugar content of so-called American Blend cigarettes is about 12%, with the natural sugar content in tobacco accounting for about 8% (28).
Sucrose is not volatile. Experimental data indicate that sucrose does not pass unchanged into mainstream smoke (22). In experimental cigarettes, radioactively labeled sucrose is converted almost completely into various aldehydes, furans and ketones (23). A review article (24) states that polycyclic hydrocarbons predominate as the pyrolysis products of sucrose at higher temperatures (above 800 °C), compared to furanes and low-molecular carbonyl compounds at lower temperatures (300 – 600 °C).

MATERIALS AND METHODS

For our study, the 20 different test cigarettes were produced in the pilot plant of British American Tobacco (BAT) Germany. The production steps in making the cigarettes were comparable to the methods and techniques used in manufacturing factory cigarettes. An additive-free commercial tobacco blend consisting of 50% Virginia tobacco (flue-cured), 20% Burley tobacco (air-cured), 20% tobacco stems and 10% Oriental tobacco (sun-cured) was used for the test cigarettes. Two kinds of filter-ventilated cigarettes in King size format with mainstream smoke condensate levels of 10 mg and 6 mg (according to ISO) were produced for this study. Cigarettes without any additives on tobacco were used as reference.
The three additives were added to the tobacco blend in the following target concentrations: Glycerol at 1.5%, 3.0% and 5.5%; cocoa powder (fat content of 10–12%) at 0.4%, 1.1% and 2.2%; sucrose (purity > 99%) at 1.5%, 2.1% and 4.8%. To obtain the target condensate levels for the two kinds of cigarettes they were designed differently. For the cigarettes with a condensate level of 10 mg a cellulose acetate filter of 22 mm length with 27% ventilation was used, for the cigarettes with 6 mg condensate the length of the filter plug was 27 mm and filter ventilation 47%.
Efforts were made to keep the firmness of the cigarettes constant for both condensate levels independent from the kind and amount of additive used. Therefore, the amount of tobacco burned per puff during machine smoking can be considered comparable for the cigarettes of both condensate levels.
The specified condensate levels of 6 mg/cig and 10 mg/cig were primarily achieved by means of filter ventilation and filter plug length and efficiency. However, small changes in the degree of filter ventilation had to be made to achieve the precise condensate levels depending on the kind and amount of the added additives.
For the 10 mg condensate cigarettes, the filter ventilation of 27% had to be reduced by 3% for samples with sucrose added at 2.1% and 4.8%, and increased by 2% for the samples with a glycerol content of 5.5%. For the 6 mg condensate cigarettes, the degree of filter ventilation of 47% had to be increased by 2% for samples with glycerol added at 5.5%.
The lowest additive concentration in each case corresponds to the typical concentration in commercially available products. The higher additive concentrations were evaluated mainly from an experimental point of view for examining possible dose-effect relationships regarding the composition of mainstream smoke.
The samples with 10 mg condensate had an average tobacco rod weight of 674 mg, compared to 611 mg for 6 mg condensate - a consequence of using filters with two

different lengths.

After conditioning according to ISO 3402 (34) the cigarettes were smoked according to ISO 3308 (12) on a rotary RM20H (Borgwaldt-KC) smoking machine. The nicotine content of mainstream smoke and nicotine-free condensate were determined according to ISO 10315 (35) and ISO 4387 (smoking of the cigarettes on an automatic smoking machine with simultaneous collection of total particulate matter in a glass fiber trap; gravimetric determination of the mass of total particulate matter; extraction of the total particulate matter with isopropanol from the trap for the determination of water and nicotine by gas chromatography (GC) (36). The water content was determined according to ISO 10362–2 (37) and mainstream smoke carbon monoxide according to ISO 8485 (38). The carbon monoxide yield of the total gas phase of the smoke was determined by non-dispersive infrared analysis.

For the determination of formaldehyde and acetaldehyde cigarette mainstream smoke was generated from ten cigarettes. The whole mainstream tobacco smoke was scrubbed of volatile carbonyls by passing each puff through an impinger into a trap containing an acidified solution of 2,4-dinitrophenylhydrazine in acetonitrile. The determination itself was done by high-performance liquid chromatography with UV Detector (HPLC-UV) using diode array detection (DAD) (39).

The tobacco specific *N*-nitrosamines, *N*-nitrosonornicotine (NNN), 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK), *N*-Nitrosoanatabine (NAT) and *N*-nitrosoanabasine (NAB), in mainstream smoke were determined by high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) according to the relevant Official Methods of Health Canada (40). The mainstream smoke of 20 cigarettes was collected on a Cambridge filter and extracted with ammonium acetate solution.

The benzo[*a*]pyrene yield was determined in the mainstream smoke of 20 cigarettes by extracting total particulate matter, collected on the glass-fiber filter pad ("Cambridge filter"), with cyclohexane. The analytical determination by gas chromatography-mass spectrometry (GC/MS) using single ion monitoring detection mode was done according to the relevant Health Canada Method (41).

For the determination of 1,3-butadiene and isoprene the mainstream smoke of 20 cigarettes was collected in cooled methanol traps. The determination was done by GC/MS according to relevant Methods of Health Canada (42).

Considering the high variance resulting from analytical and product-related fluctuations each mainstream smoke component was determined in 10 replicates.

The CORESTA monitor cigarette CM6 and internal monitor cigarettes - all with known, well documented 'tar', nicotine and carbon monoxide mainstream smoke levels - were smoked to assure the quality of the machine smoking procedure.

The glycerol in the tobacco of the test cigarettes was determined by gas chromatography (43). The cocoa content was determined indirectly by high-performance liquid chromatography (HPLC), measuring its theobromine content (44). Sucrose was determined by HPLC according to DIN 10371 (45), measuring glucose, fructose and sucrose in tobacco and tobacco products by HPLC.

Table 1. Targeted and measured levels of glycerol in experimental cigarettes (based on dry tobacco weight)

Glycerol content	6 mg 'tar'		10 mg 'tar'	
	target	actual	target	actual
Reference cigarettes	-	0.2	-	0.2
Test cigarettes	1.5	1.2	1.5	1.2
	3.0	2.9	3.0	3.0
	5.5	5.2	5.5	5.2

Table 2. Targeted and measured levels of cocoa in experimental cigarettes (based on dry tobacco weight)

Cocoa content	6 mg 'tar'		10 mg 'tar'	
	target	actual	target	actual
Reference cigarettes	-	0	-	0
Test cigarettes	0.4	0.36	0.4	0.39
	1.1	1.15	1.1	1.13
	2.2	1.74	2.2	2.18

Table 3. Targeted and measured levels of sucrose in experimental cigarettes (based on dry tobacco weight)

Sucrose content	6 mg 'tar'		10 mg 'tar'	
	target	actual	target	actual
Reference cigarettes	-	0.68	-	0.64
Test cigarettes	1.5	1.5	1.5	1.4
	2.1	2.2	2.1	2.3
	4.8	4.3	4.8	5.1

RESULTS

Characterization of the test cigarettes

The measured concentrations of the additives, glycerol, cocoa powder, and sucrose in the tobacco blend confirmed that the manufacturing targets were met for all three additives to the extent technically feasible.

It should be noted that tobacco as a natural material already contains glycerol and sucrose. The glycerol and sucrose contents of the tobacco in the reference cigarettes (without additives) were determined while the test samples were being produced. The analysis showed an average level of 0.2% for glycerol and 0.7% for sucrose.

The glycerol concentration was chosen to be both within the range of practical use and above the statutory maximum limit (5% in the tobacco dry matter), with target concentrations of 1.5%, 3.0%, and 5.5% (Table 1).

The targets for the total cocoa powder content in the tobacco blend were achieved with good accuracy (Table 2). The sucrose content in the two reference cigarettes amounted to 0.64% and 0.68%, respectively. The sucrose content stated in the table corresponds to the sum of natural sucrose in the initial blend plus added sucrose (Table 3).

During production, the initial tobacco weight per cigarette was to be kept constant within the condensate level. To check this, three cigarettes were weighed per additive for each condensate level together with the reference cigarette. The weight of paper and filter was then subtracted from the overall weight.

Results for the mainstream smoke components under investigation

- Carbon monoxide, nicotine and nicotine-free dry particulate matter

The results obtained by analyzing the test and reference cigarettes are shown in Figure 1 (carbon monoxide), Figure 2 (nicotine in smoke) and Figure 3 (nicotine-free dry particulate matter (NFDPM)).
There were no differences in the basic parameters carbon monoxide, nicotine and nicotine-free dry particulate matter with regard to the various additions of glycerol, cocoa powder and sucrose (Figures 1, 2, and 3). The data show that production of the reference and test cigarettes with the corresponding targets for nicotine-free dry particulate matter was successful when considering the analytical and production-related fluctuations and pursuant to the internationally recognized ISO smoking methods.

- Tobacco-specific nitrosamines (TSNAs)

Our results concerning the influence of the three tobacco additives on the yields of tobacco-specific nitrosamines (TSNAs) in mainstream smoke are shown in the Figures 4–7.
Tobacco-specific nitrosamines (TSNAs) are generated in harvested tobacco by the nitrozation of tobacco alkaloids during curing and fermentation. The final TSNA levels in tobacco depend on the kind of tobacco and on the conditions during cultivation, curing techniques, subsequent storage and processing of the tobacco leaf (46). In our study, the TSNA levels in the mainstream smoke of the test cigarettes with additives are generally reduced compared to the reference cigarettes. With glycerol as an additive a dose-dependent trend is obvious for all four TSNAs.

- Components of the gas / vapor phase

The influence of the three additives on the mainstream smoke yields of formaldehyde and acetaldehyde seen in our study is shown in Figures 8 and 9.
The effect of the three tobacco additives in the cigarette blend on formaldehyde yields in mainstream smoke is shown in Figure 8. The addition of glycerol results in a slight decrease of formaldehyde. The formaldehyde levels in the mainstream smoke of the test cigarettes with cocoa powder as additive are within the range of the reference cigarettes for both condensate levels. As shown by BAKER (47) the sugar content of tobacco correlates directly with the formaldehyde yield in cigarette mainstream smoke. Also in our study, we found a clear increase of formaldehyde in mainstream smoke of the 10 mg condensate cigarettes correlated with the amount of added sucrose to the blend. However, this effect was not equally pronounced in the mainstream smoke of the 6 mg condensate cigarettes.
In the test cigarettes, the inclusion levels of glycerol (1.2%, 2.9%, 5.2%) were substantially higher than those of cocoa powder (0.36%, 1.15%, 1.74%) resulting in more severe reductions of cigarette tobacco weight. In addition, the variability of the analytical method for measuring formaldehyde is generally higher than with the other analytes.
Tobacco burning generates among other compounds various aldehydes that occur primarily in the gas phase of smoke. In our study, the additives under investigation had no essential influence on the levels of acetaldehyde in cigarette mainstream smoke (Figure 9).
Figure 10 shows the effects of the tobacco additives, glycerol, cocoa powder and sucrose, on the yield of 1,3-butadiene found in mainstream smoke.
The 10 mg test cigarettes showed an increase in 1,3-butadiene in mainstream smoke compared to the reference cigarette, while no increase was observed in the 6 mg cigarette samples, regard being given to analytical variability. The addition of sucrose to the 10 mg cigarette resulted in an obvious increase of 1,3-butadiene. It should be noted that the difference between the means of the 10 mg and the 6 mg test cigarettes is larger than the difference between the two additive-free reference cigarettes. This suggests that the measured levels of 1,3-butadiene are too low in the 10 mg reference cigarettes. The relatively large increase in 1,3-butadiene for the 3% addition of glycerol may be due to production-related differences.
The data regarding the influence of the tobacco additives tested on the amount of isoprene in mainstream smoke are shown in Figure 11.
No meaningful differences between the 6 mg condensate test cigarettes and the corresponding reference cigarettes were seen in the yields of the two compounds isoprene and 1,3-butadiene in the gas phase of mainstream smoke.

- Benzo[*a*]pyrene

The effect of the additives on mainstream smoke yields of benzo[*a*]pyrene is shown in Figure 12. In comparison to the benzo[*a*]pyrene levels in the mainstream smoke of the two reference cigarettes there is no substantial difference observed in the test cigarettes. The variations in the benzo[*a*]pyrene levels seen may result from factors related to the production of the cigarettes or variations in the analytical methods.

DISCUSSION

There have been intensive public discussions about the purpose and toxicological effects of additives in tobacco products (29, 31, 32). Criticism was focused in particular on additives with a potential influence on the addictive effects of tobacco smoke or with chemical properties suggesting the formation of toxic compounds when burned and pyrolyzed, this way augmenting the overall toxicity of tobacco smoke.
In our study the effects of the tobacco additives, glycerol, cocoa powder and sucrose, on the level of various selected components in mainstream smoke were evaluated. Most relevant data published to date originated either directly or indirectly from the tobacco industry. This study was com-

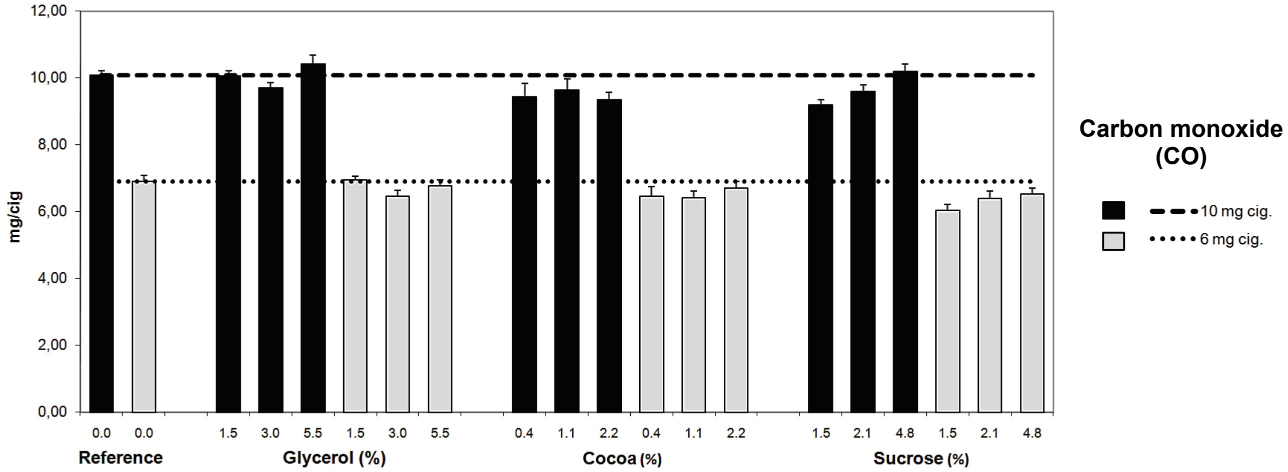

Figure 1. Carbon monoxide (CO) yields in mainstream smoke for different additive levels of glycerol, cocoa, and sucrose in two experimental cigarettes (6 mg, 10 mg nicotine-free dry particulate matter according to ISO smoking regimen). Reference cigarettes without additives. Bars and error indicators represent mean and standard deviation of 10 measurements.

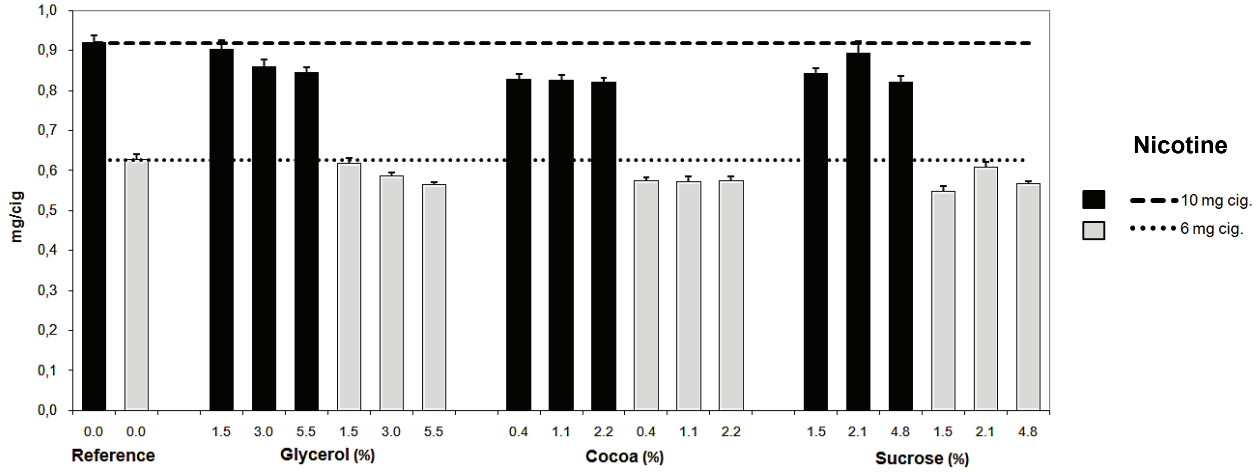

Figure 2. Nicotine yields in mainstream smoke for different additive levels of glycerol, cocoa, and sucrose in two experimental cigarettes (6 mg, 10 mg nicotine-free dry particulate matter according to ISO smoking regimen). Reference cigarettes without additives. Bars and error indicators represent mean and standard deviation of 10 measurements.

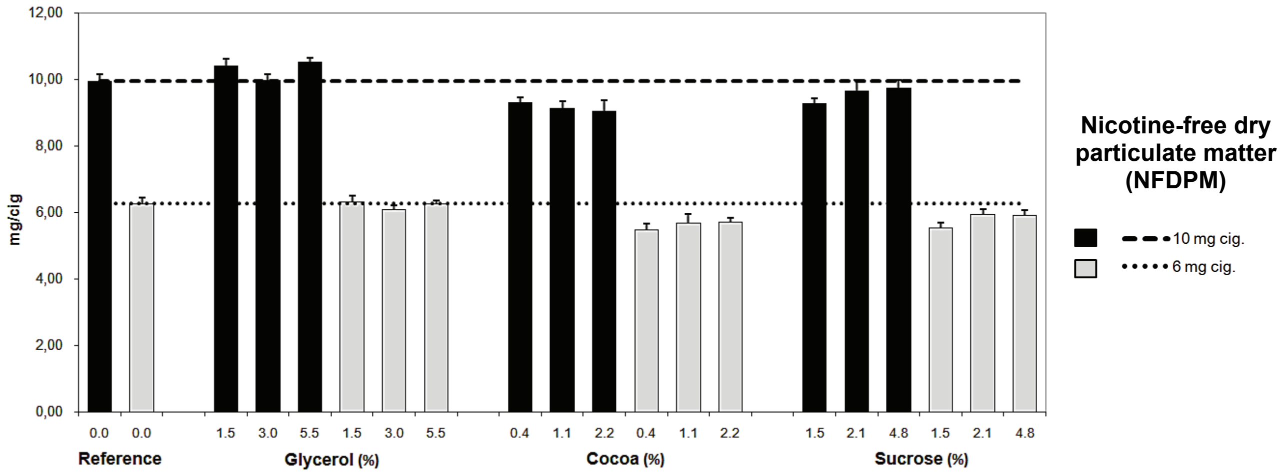

Figure 3. Nicotine-free dry particulate matter (NFDPM) yields in mainstream smoke for different additive levels of glycerol, cocoa, and sucrose in two experimental cigarettes (6 mg, 10 mg nicotine-free dry particulate matter according to ISO smoking regimen). Reference cigarettes without additives. Bars and error indicators represent mean and standard deviation of 10 measurements.

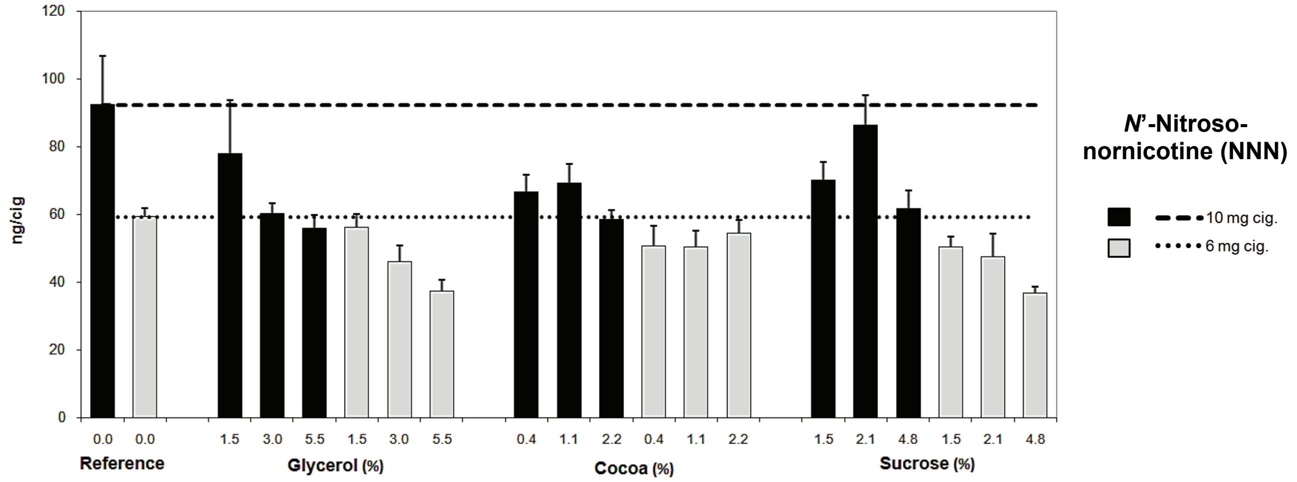

Figure 4. ***N'*-Nitrosonornicotine (NNN) yields in mainstream smoke for different additive levels of glycerol, cocoa, and sucrose in two experimental cigarettes (6 mg, 10 mg nicotine-free dry particulate matter according to ISO smoking regimen). Reference cigarettes without additives. Bars and error indicators represent mean and standard deviation of 10 measurements.**

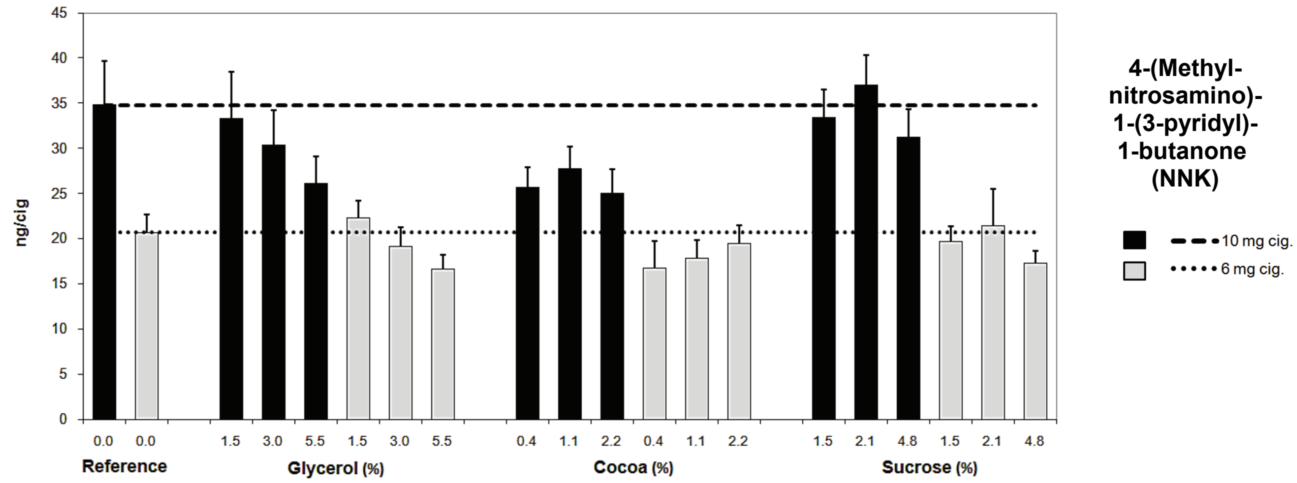

Figure 5. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) yields in mainstream smoke for different additive levels of glycerol, cocoa, and sucrose in two experimental cigarettes (6 mg, 10 mg nicotine-free dry particulate matter according to ISO smoking regimen). Reference cigarettes without additives. Bars and error indicators represent mean and standard deviation of 10 measurements.

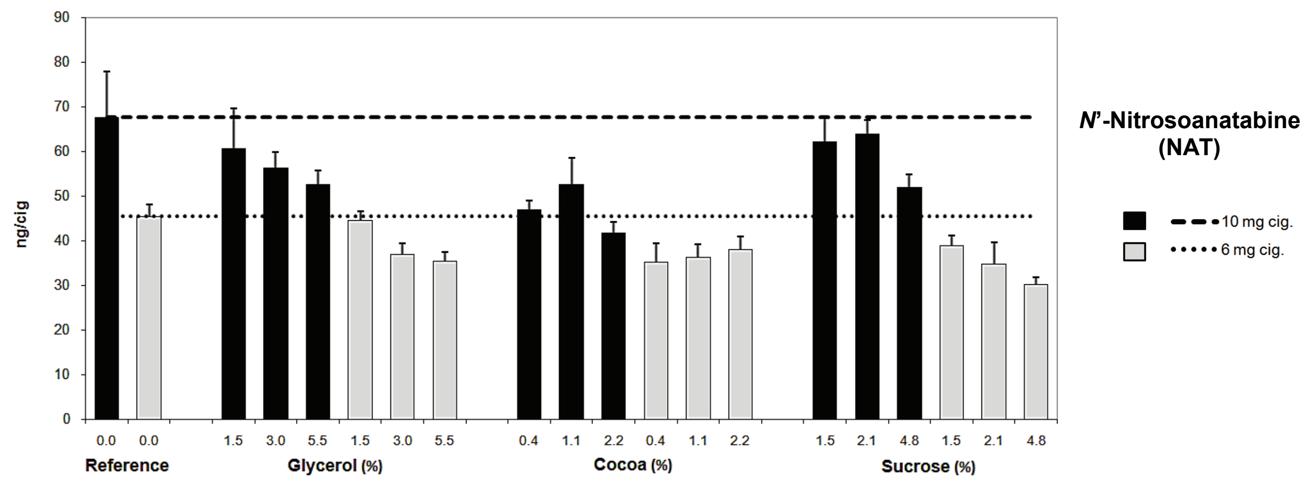

Figure 6. ***N'*-Nitrosoanatabine (NAT) yields in mainstream smoke for different additive levels of glycerol, cocoa, and sucrose in two experimental cigarettes (6 mg, 10 mg nicotine-free dry particulate matter according to ISO smoking regimen). Reference cigarettes without additives. Bars and error indicators represent mean and standard deviation of 10 measurements.**

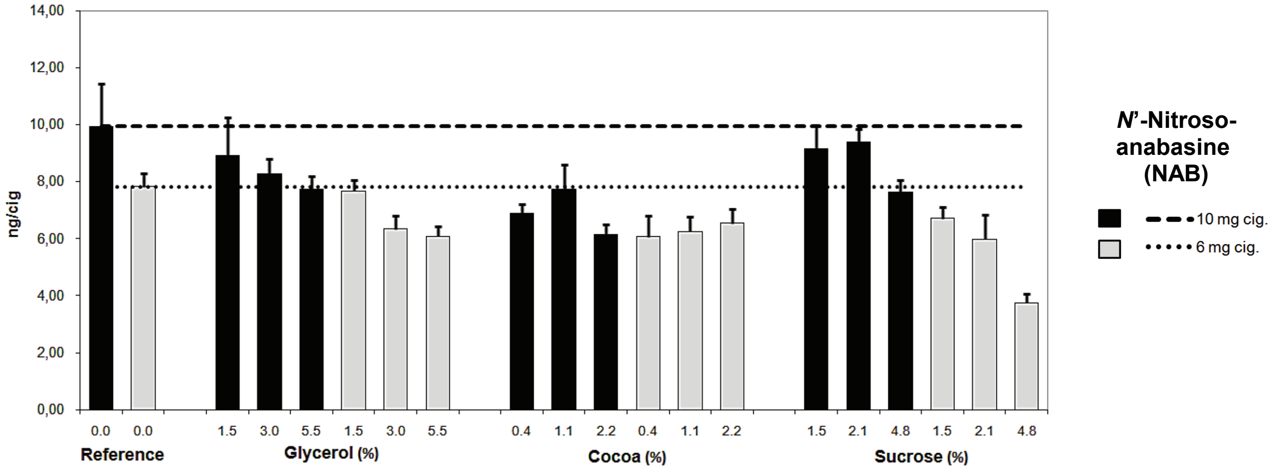

Figure 7. ***N'*-nitrosoanabasine (NAB) yields in mainstream smoke for different additive levels of glycerol, cocoa, and sucrose in two experimental cigarettes (6 mg, 10 mg nicotine-free dry particulate matter according to ISO smoking regimen). Reference cigarettes without additives. Bars and error indicators represent mean and standard deviation of 10 measurements.**

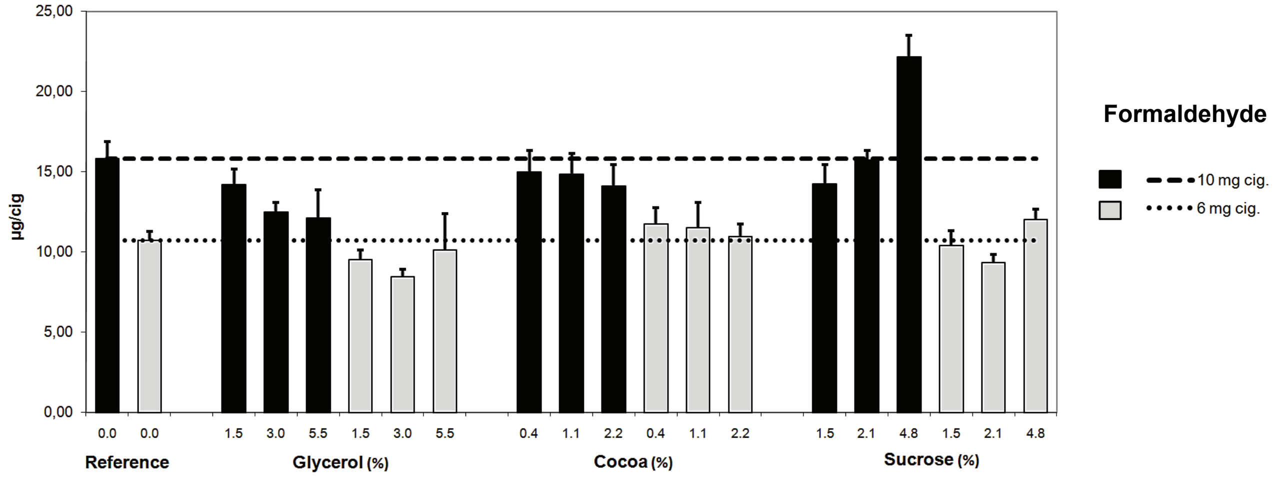

Figure 8. Formaldehyde yields in mainstream smoke for different additive levels of glycerol, cocoa, and sucrose in two experimental cigarettes (6 mg, 10 mg nicotine-free dry particulate matter according to ISO smoking regimen). Reference cigarettes without additives. Bars and error indicators represent mean and standard deviation of 10 measurements.

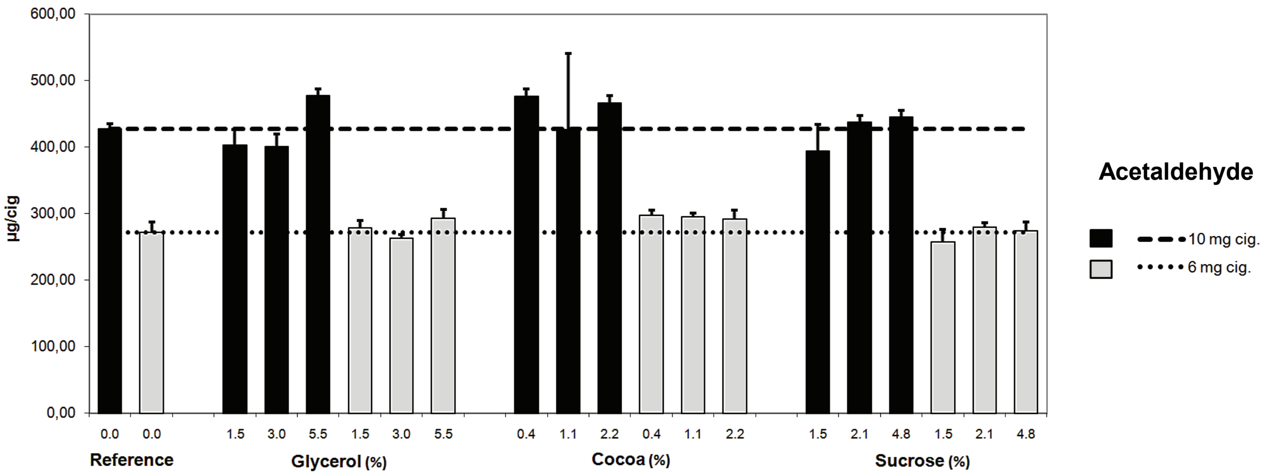

Figure 9. Acetaldehyde yields in mainstream smoke for different additive levels of glycerol, cocoa, and sucrose in two experimental cigarettes (6 mg, 10 mg nicotine-free dry particulate matter according to ISO smoking regimen). Reference cigarettes without additives. Bars and error indicators represent mean and standard deviation of 10 measurements.

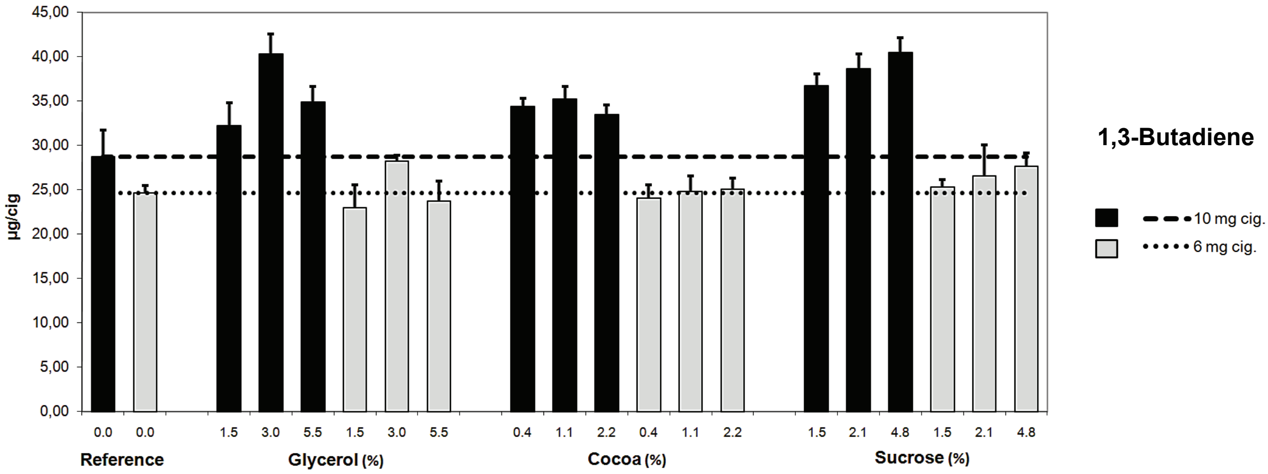

Figure 10. **1,3-Butadiene yields in mainstream smoke for different additive levels of glycerol, cocoa, and sucrose in two experimental cigarettes (6 mg, 10 mg nicotine-free dry particulate matter according to ISO smoking regimen). Reference cigarettes without additives. Bars and error indicators represent mean and standard deviation of 10 measurements.**

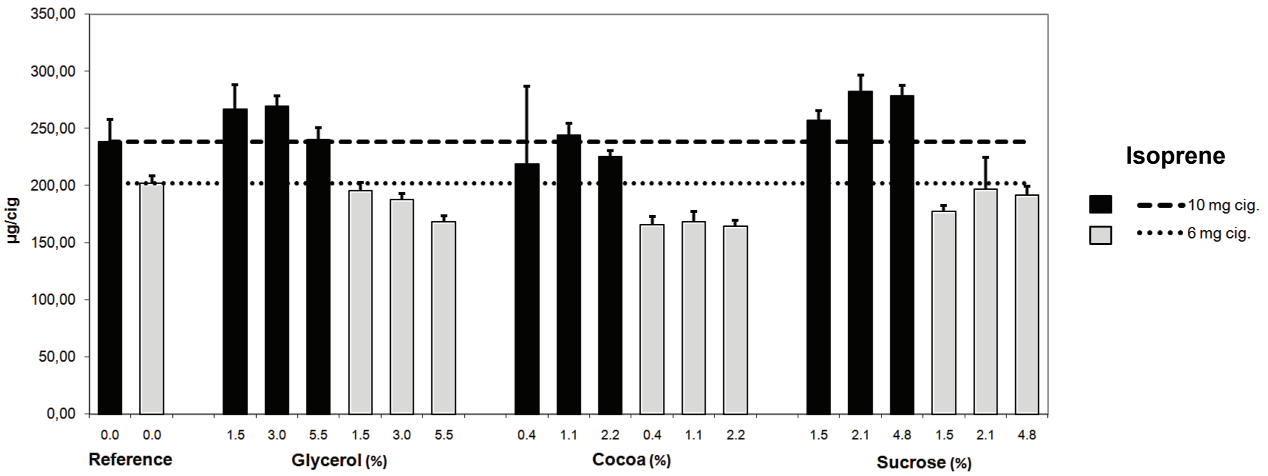

Figure 11. **Isoprene yields in mainstream smoke for different additive levels of glycerol, cocoa, and sucrose in two experimental cigarettes (6 mg, 10 mg nicotine-free dry particulate matter according to ISO smoking regimen). Reference cigarettes without additives. Bars and error indicators represent mean and standard deviation of 10 measurements.**

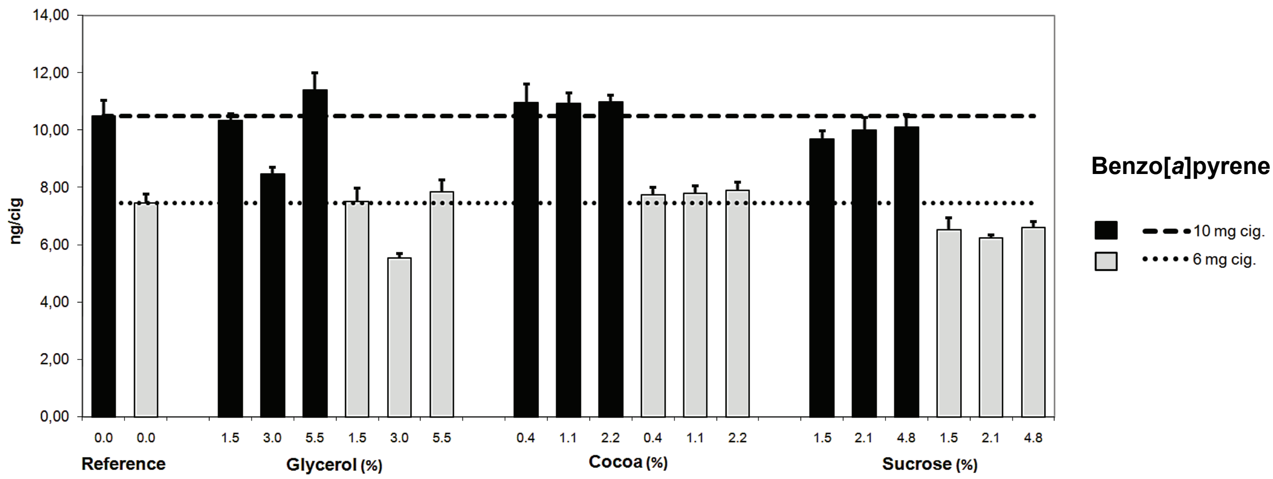

Figure 12. **Benzo[*a*]pyrene yields in mainstream smoke for different additive levels of glycerol, cocoa, and sucrose in two experimental cigarettes (6 mg, 10 mg nicotine-free dry particulate matter according to ISO smoking regimen). Reference cigarettes without additives. Bars and error indicators represent mean and standard deviation of 10 measurements.**

missioned by the BMELV, the German Federal Ministry for Food, Agriculture and Consumer Protection.
Our paper on the effects of tobacco additives on the yields of specific toxic components in cigarette mainstream smoke is one of the few publications released by an independent government-owned monitoring laboratory.

Assessment of the additives under investigation, glycerol, cocoa powder, and sucrose

The chemical analysis of the mainstream smoke of the test cigarettes with glycerol as tobacco additive revealed no essential changes compared to the mainstream smoke of the additive-free reference cigarettes. In particular, no unequivocal dose-effect relationships were observed between the amounts of additives added and the yields of the analytes in question in cigarette mainstream smoke. Glycerol was shown to have no influence on the levels of benzo[*a*]prene, isoprene and acetaldehyde in smoke. A slight decrease was observed in the yields of tobacco-specific nitrosamines and nicotine. Even with the addition of 5% glycerol, the yields of formaldehyde were in the range of analytical variation. Inconsistent increases were observed for 1,3-butadiene with the 10 mg 'tar' test cigarettes. As mentioned before, this may be due to production-related differences for the 3% glycerol test cigarette and an inadequately low level of 1,3-butadiene in the 10 mg reference cigarette.
It has been reported that the levels of tobacco-specific nitrosamines and nicotine in smoke show a tendency to decrease with the addition of glycerol, particularly when the condensate levels increases (19). The addition of glycerol results in a higher proportion of water and, consequently, water containing condensate in the smoke. The resulting "dilution" of the tobacco in the cigarette with glycerol is reflected in lower values for all tobacco-specific components in mainstream smoke. This effect is described in the literature (48, 49) and was confirmed in our study.
Carmines and Gaworski (19) examined 34 mainstream smoke components together with condensate, nicotine and carbon monoxide, following the addition of 5, 10 and 15% glycerol to the tobacco of test cigarettes (the highest level in our study was 5%). They observed no significant effect on benzo[*a*]pyrene yields, while the addition of 10 and 15% glycerol to the tobacco resulted in slight decreases of the formaldehyde and acetaldehyde levels. According to Carmines and Gaworski (19) the addition of about 5% glycerol to cigarette tobacco had no effect on 1,3-butadiene levels in cigarette mainstream smoke. However, unrealistically high levels of glycerol in tobacco (10% and 15%) were noted to cause a significant increase of acrolein by 9%. Acrolein as a constituent of cigarette smoke was not included in our study but should be taken into consideration in future chemical analyses of smoke.
In our study, the addition of cocoa powder produced no substantial effects on most of the investigated analytes. It is noticeable that cocoa powder caused the most pronounced decrease in the tobacco-specific nitrosamines. Cocoa powder may possibly have an influence on the intensity of the burning process, resulting in reduced transfer of TSNAs from tobacco into cigarette mainstream smoke. According to Fischer *et al.* (50) TSNAs in mainstream smoke are transferred directly from tobacco into smoke. However, in 2008, Moldoveanu and Borgerding (51) reported the generation of small amounts of TSNAs from nicotine and nornicotine during smoking. Reduction of the generation of such small amounts of TSNAs by smoking a cigarette cannot explain the decrease of TSNAs observed in our study when cigarettes contained cocoa powder as tobacco additive.
The authors are aware of only one published chemical smoke analysis investigating cocoa or cocoa products as a single-substance tobacco additive. In the 1970s, the American National Cancer Institute carried out a multiple test series to investigate the effect of certain selected tobacco additives on the composition and biological effects of tobacco smoke. The addition of 1% cocoa powder resulted in an increase of phenol, isoprene, indole, cresols, catechol and various fatty acids in mainstream smoke (52). On the other hand, no increase in isoprene was observed in our study.
The addition of sugar compounds to tobacco is assumed to increase the formation of various aldehydes (28).
In our study, only slight increases were observed in some of the analyzed mainstream smoke constituents of test cigarettes with added sucrose. For example, a slight increase was observed for 1,3-butadiene.
On the other hand, sucrose in tobacco has a noticeable influence on the level of formaldehyde in mainstream smoke. In our study a considerable increase was observed for the highest level of added sucrose (5%) in the mainstream smoke of the 10 mg condensate cigarette. In mainstream smoke of the 6 mg condensate cigarette the effect of the sucrose in tobacco on formaldehyde yields was also seen in the test cigarettes with the highest amount of added sucrose though less pronounced than in the 10 mg condensate cigarette. Studies published by the tobacco industry confirm the effect of tobacco sugars on formaldehyde yields in cigarette mainstream smoke (47).
The influence of various types of sugar as tobacco additives was investigated in terms of composition of mainstream smoke, particularly with regard to the yields of various aldehydes. The 10.5% addition of white sugar (consisting of more than 98% sucrose) resulted in a 40% increase of formaldehyde from 30 µg/cig to 42 µg/cig. All these values are in line with the formaldehyde values determined in the mainstream smoke of cigarette brands from the open market, when these cigarettes were machine-smoked according to the ISO smoking regimes (53, 54).
Acetaldehyde has been discussed with regard to its possible addiction enhancing effect in tobacco smoke (28, 55). Levels between 50 µg and 850 µg acetaldehyde were detected in the mainstream smoke of various cigarettes smoked according to standardized ISO methods (53, 54).
In our study, the amounts of acetaldehyde in the mainstream smoke of the 10 mg condensate cigarettes were about 400 µg, for the 6 mg condensate cigarettes about 290 µg. No increase in acetaldehyde yields was observed with the addition of sucrose to tobacco. It was not possible to confirm the assumption that the addition of sucrose results in higher levels of acetaldehyde. However, publications indicate that there is a correlation between the

acetaldehyde level in mainstream smoke and the condensate of a cigarette (56).
With consumer protection in mind, the question arises whether the additives, glycerol, cocoa powder and sucrose, in the presence of tobacco produce an identifiable increase in substances with toxic relevance when a cigarette is being smoked. In this study certain trends were observed, of which some are in agreement with published data, e.g. the decrease in tobacco-specific nitrosamines when glycerol is added. The most significant result is the elevated formation of smoke formaldehyde when a high quantity of sucrose is added to cigarette tobacco. Generally, the data presented in our paper do not indicate any substantial effects of the three tobacco additives on most of the analytes examined.

CONCLUSIONS

In order to create a broad data base for the scientific assessment of the effects of tobacco additives on the toxicity of cigarette mainstream smoke, it is recommended to enlarge the range of additives in a study set up like our investigation. Furthermore, it would be appropriate to include other smoke components in the analytical program for the assessment of additive effects. The so-called "Hoffmann analytes", as mandated for cigarettes by the Canadian authorities (57), could be used as the starting point. These analytes came to be known when, in the late 1990s, a list was drawn up of the supposedly most important toxic substances in tobacco smoke assumed to have particular relevance for smoking-related diseases. This list included about 44 substances referred to as "Hoffmann analytes", named after the scientist DIETRICH HOFFMANN and his ground-breaking work in recent decades in the field of tobacco, tobacco smoke and its toxicity (58, 59, 60).
Generally, there is a need for the systematic evaluation of the additives used in tobacco products. The evaluation should be carried out under standardized conditions using a defined assessment scheme. However, to date there are no binding directives, evaluated or official methods/test systems available to produce a data set, which would permit the overall systematic appraisal of additives for tobacco products.
There is a draft document on the assessment of tobacco additives issued by Deutsches Krebsforschungszentrum Heidelberg (DKFZ, German Cancer Research Center, Heidelberg). It points out that the assessment of tobacco additives should correspond to the assessment of pharmacological products and essentially be based on pyrolysis data of the neat additives (61).
Another advanced option for a toxicological assessment strategy in four stages could be as follows (Figure 13):
Stage 1: A basic prerequisite for the use of an additive in tobacco products is that it is not harmful/toxic in unburned form. Stage 1 should, therefore, consist of the characterization and toxicological evaluation of the additive in unburned form based on already published data provided by national or international expert bodies. If data are not available, the individual additives should be tested using recognized toxicological test methods in consideration of their mode of action (possible methods include, e.g., the OECD Guidelines (Organization for Economic Cooperation and Development) (62).
Stage 2: For additives that are burned in a tobacco product, the toxicological assessment of the unburned substance is not sufficient. Stage 2 of the assessment should include the pyrolysis of the additive as an individual substance based on a standardized method. These methods should mimic the conditions in a cigarette as closely as possible (13, 63). The compounds generated by the pyrolysis of additives provide information on which compounds may be generated from the additive during the smoking of the tobacco product. This is a prerequisite for the chemical analytical evaluation of toxic substances in the smoke of additive containing tobacco products, such as cigarettes.
If components are present in a pyrolysate for which no data on their toxic effects are available, the components should be tested using recognized toxicological test methods, once again considering their mode of action. The OECD Guidelines should be used, as already indicated for stage 1.
Using glycerol, cocoa powder and sucrose as examples, the literature cited in the introduction shows that the chosen experimental conditions of the pyrolysis studies have a major effect on which pyrolysis products are generated and in which quantities. As mentioned above, if pyrolysis studies are to be used as part of an assessment strategy for additives, it would be necessary to develop a standardized, validated method.
However, this method can only illustrate an additive's potential to generate substances or substance classes under the influence of heat. Pyrolysis experiments cannot clarify how an additive behaves in a burning cigarette. Smoking a cigarette is a complex process that consists of a number of different phases, such as combustion, pyrolysis, pyrosynthesis, distillation and sublimation. The thermal decomposition by pyrolysis is controlled by the levels, and variations, of the experimental temperature and the oxygen levels present in the pyrolysis apparatus. It is not possible to mimic the conditions and processes in a cigarette during smoking completely by means of pyrolysis experiments with individual substances.
When tobacco is smoked, it generates a large number of toxic and carcinogenic substances. Up to now, more than 5000 different substances were identified in cigarette smoke (10) and more than 70 substances in tobacco smoke categorized as carcinogenic or very probably carcinogenic by leading national and international organizations and expert bodies (11). Toxic substances are certainly generated when additives or any organic materials are burned and pyrolyzed. During the smoking process, the products generated from additives also react with certain other components of tobacco smoke. In order to take account of the contribution of tobacco additives to the toxic and addictive effects of cigarette mainstream smoke with due consideration of possible synergetic effects and to answer the question whether the additives influence the quantitative share of toxic substances in whole smoke, the pyrolysis of additives itself is not sufficient as an assessment criterion.
Stage 3: In a third stage, all tobacco additives with a share

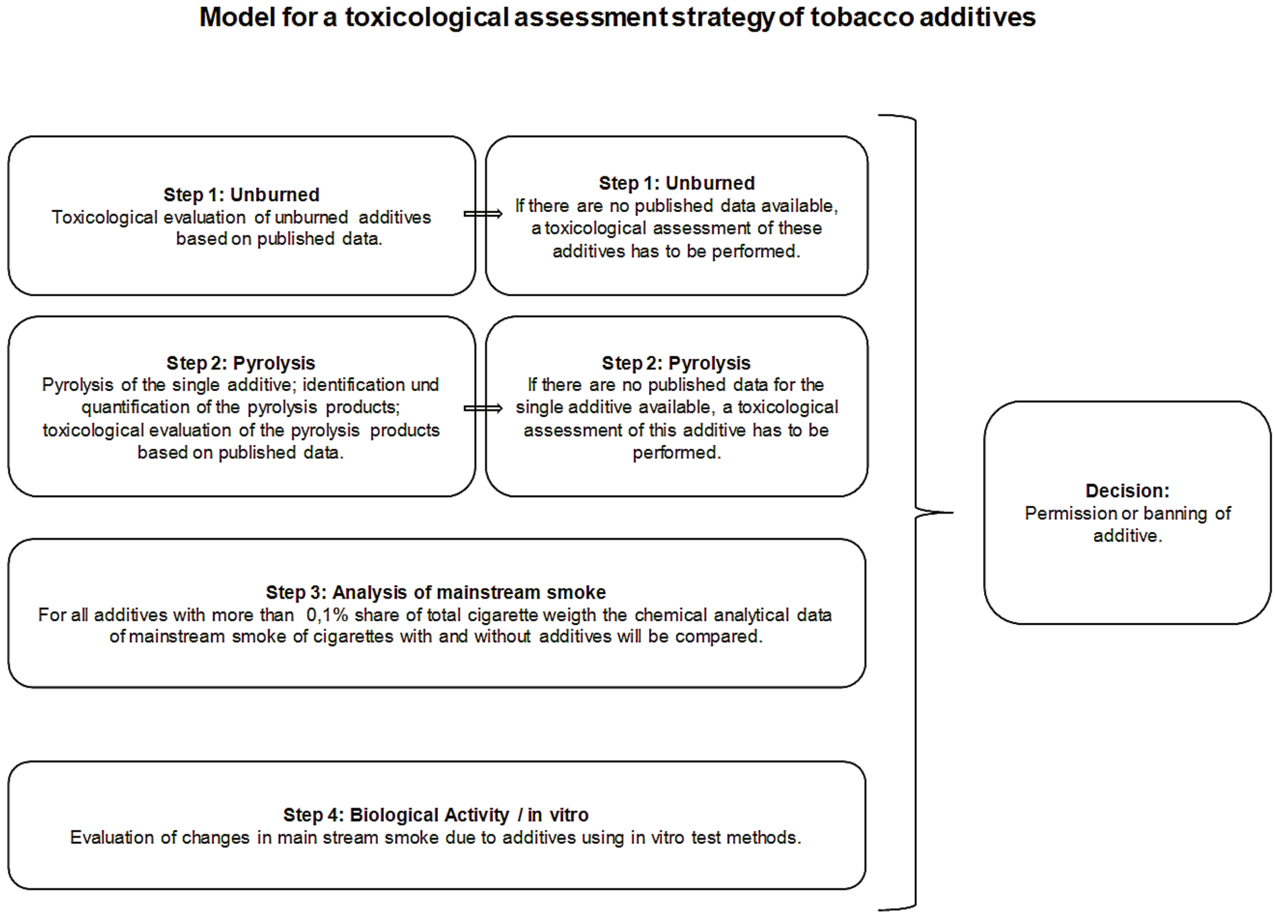

Figure 13. Model for a toxicological assessment strategy in four stages.

of greater than 0.1% of total cigarette weight should be evaluated with respect to their effects on selected compounds in mainstream smoke using cigarettes that contain these additives. Standardized methods with a standardized test cigarette (design, tobacco blend, individual substances and/or their mixtures) should be used. Many methods and techniques needed for this purpose are already established. Mainstream smoke of tobacco products should be compared without and with additives, as was done in our study. Two inclusion levels are appropriate. The “lower” level should be set in a way that 90% of the cigarettes on the market contain the additive in amounts equal to, or lower than, this level. In addition, the additive should be examined at an inclusion level ten times the “lower” level if it is technically feasible. The actual inclusion levels of the various additives used in cigarette manufacturing can be learned by evaluating the additive lists of all brand cigarettes sent each year by the tobacco industry (in Germany) to the Federal Office of Consumer Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, BVL) - or to the respective authorities in the other EU member states. In particular, additives used in larger quantities (≥ 1% in tobacco) should not be tested in additive mixtures but as individual substances.
Stage 4: However, an analysis of smoke that looks at a limited number of smoke constituents only depicts a certain part of tobacco smoke, which is known to contain at least 5,000 different substances. Although smoke analysis is an essential tool in the toxicological assessment of additives, the actual influence of an additive on the overall toxicity of tobacco smoke can only be clarified by *in vitro* or *in vivo* tests using biological systems. Studies that measure changes in the biological response to smoke, therefore, constitute an additional tool for the assessment and regulation of additives.
However, up to now there are no internationally recognized directives, evaluated methods or test systems for the toxicological assessment of additives or tobacco products in general. An initial step towards assessment and in the on-going development of the toxicological testing of tobacco additives was taken by the DIN Report 133 of 2004 titled "Toxikologische Bewertung von Zusatzstoffen für Tabakprodukte - Ein Leitfaden" (Toxicological Assessment of Additives for Tobacco Products – A Guideline). This document is regularly reviewed and currently being revised (64).
If the potential toxic effects of an additive in cigarette mainstream smoke are evaluated in biological test systems it is important that fresh, un-aged smoke is used for the evaluation. Ageing of cigarette smoke results in the decrease of its toxic properties and artifact formation (65). *In vitro* and *in vivo* data obtained with aged cigarette smoke or cigarette smoke condensate do not reflect reality.
Article 11 of the EU Directive 2001/37/EU (8) states that

the Commission shall pay special heed to the on-going development of methods for more realistically assessing and regulating toxic exposure and harm from tobacco products. In the U.S., the Food and Drug Administration (FDA) was given regulatory authority over tobacco products in 2009. The FDA has been given 42 months to draw up test procedures for additives in tobacco products (66). Which test procedures should be used in the EU for the evaluation of tobacco additives remains an open question.

Finally, a basic review on the effects of tobacco additives on mainstream cigarette smoke should take a critical approach. As a matter of fact, the consumer smokes not only additives but a product with approx. 90% tobacco content. To ensure practical consumer protection for the smokers of cigarettes and other tobacco products, an important aspect is looking at which harmful substances are inhaled, and possibly taken up, by the smoker. The measurement and notification of substances with toxicological relevance in mainstream smoke constitutes another possibility for assessing and regulating tobacco products. Furthermore, the introduction of maximum levels for certain smoke components could reduce consumer exposure to harmful substances.

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