National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Risk assessment of tobacco additives and smoke components

A method proposal

RIVM Letter Report 340031001/2012
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Colophon

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This investigation has been performed within the framework of Public Information Tobacco Control (PITOC).

Abstract

Risk assessment of tobacco additives and chemicals in cigarette smoke

Cigarette smoke is a complex mixture of approximate 4000 chemicals. These compounds are generally generated during the burning of tobacco, but can also be generated by the burning of tobacco additives such as those used to alter the taste and smell to make tobacco smoking more attractive. The RIVM has developed a method to assess whether the levels of these substances in the lung pose a health risk, given that a worldwide-accepted method to assess the risk of chemicals in cigarette smoke is not yet available. Understanding the health risks of each chemical in cigarette smoke is important because it can help policy makers select compounds which pose the highest risk to humans in the future. This method was developed under an international project aimed at assessing the health risks of tobacco additives.

The method

In this method an inhalation exposure scenario was developed. In this scenario, the amount of a chemical in cigarette smoke that reaches the lung was estimated (A). Independently, the level at which a chemical in smoke (smoke component) causes irritation to the nose, throat and/or lungs was calculated. For the risk assessment, the highest dose that does not cause nose, throat and/or lung irritation was selected (B) and compared to the amount of a chemical in cigarette smoke that reaches the lung (A). The ratio between these values (B/A) determines the risk; the lower the ratio, the greater the chance of a health risk.

Example

As examples to illustrate the utility of this method, a risk assessment for irritation of the nose, throat and lung was investigated for some chemicals that are present in cigarette smoke; other health effects such as cancer or reproductive toxicity were not assessed. As tobacco additives, glycerol and propylene glycol were selected. For compounds present in cigarette smoke, acetaldehyde, acrolein, formaldehyde and 2-furfural were selected because they may be generated during the burning of tobacco additives. Results showed that a risk for irritation exists for the tobacco additives glycerol and propylene glycol. Similarly, a risk for nose, throat and/or lung irritation was supported by the smoke components acetaldehyde, acrolein and formaldehyde. These results are not representative of all the compounds in cigarette smoke and more research is needed to investigate the health effects of more chemicals in cigarette smoke.

Keywords:
risk assessment, tobacco additives, inhalation exposure scenario, smoke components

Rapport in het kort

Risico beoordeling van tabaksadditieven en rook

Tabaksrook is een complex mengsel van ongeveer 4000 stoffen. Het bevat verbrandingsproducten van de tabak, maar ook van additieven zoals smaakstoffen, die worden toegevoegd om de geur en smaak van het product aantrekkelijker te maken. Het RIVM heeft een methode ontwikkeld om te beoordelen bij welke concentratie in de longen deze stoffen risico's voor de gezondheid veroorzaken. Een dergelijke methode bestond nog niet. Inzicht hierin is belangrijk omdat beleidsmakers hiermee in de toekomst kunnen kiezen op welke schadelijke stoffen zij eventueel kunnen sturen. De methode is voortgekomen uit een internationaal project naar de gezondheidseffecten van tabaksadditieven in brede zin.

De methode
Voor de methode is een inhalatieblootstellingscenario ontwikkeld. Dit werkt als volgt: eerst wordt berekend welke hoeveelheid van de stof tijdens het roken daadwerkelijk de long binnenkomt (A). Daarna wordt berekend in welke hoeveelheid een stof uit de rook (rookcomponent) gezondheidsschade veroorzaakt (irritatie aan de neus, keel en/of long) als hij in de long terechtkomt. Voor de beoordeling is de hoogste dosering die geen neus, keel en longirritatie veroorzaakt van belang (B). Deze uitkomst wordt vervolgens vergeleken met hoeveel van de stof tijdens het roken daadwerkelijk de long binnenkomt. De verhouding tussen deze waarden (B/A) bepaalt de risicobeoordeling: hoe lager de verhouding, hoe groter de kans op een gezondheidsrisico.

Voorbeelden
Als voorbeelden voor de methode is het risico op neus-, keel- en longirritatie onderzocht van enkele stoffen die veel in sigaretten voorkomen; andere gezondheidseffecten, zoals kanker of vruchtbaarheidsproblemen, zijn hier niet in meegenomen. Als tabaksadditieven zijn dat de ammoniumverbindingen, glycerol en propyleenglycol. Voor de stoffen in de rook zijn acetaldehyde, acroleïne, formaldehyde en 2-furfural geselecteerd, omdat ze onder andere kunnen vrijkomen als tabaksadditieven verbranden. Hieruit blijkt dat neus, keel en/of longirritatie ontstaat als de sigaretten de additieven glycerol en propyleenglycol bevatten. Hetzelfde geldt voor de rookcomponenten aceetaldehyde, acroleïne en formaldehyde. Deze uitkomsten zijn niet representatief voor alle stoffen in rook. Meer onderzoek naar gezondheidseffecten van meer stoffen is nodig.

Trefwoorden:
Risico beoordeling, tabaksadditieven, inhalatie blootstelling scenario, rookcomponenten

Contents

Summary

The risk assessment of complex mixtures is very difficult, and even more so for inhalation exposure. A good example of a complex mixture is cigarette smoke where worldwide-accepted methods to assess the risk of compounds in a quantitative fashion are currently not available. The tobacco industry uses many additives in the manufacturing of tobacco products, but the human health effects of these additives or their combustion products in connection to their presence in tobacco smoke have never been thoroughly investigated. In this report a method is proposed on how human health risks from smoke components can be assessed, for instance those generated from the combustion of additives. The proposed method compares the No-Observed-Adverse-Effect Levels (NOAELs) or Lowest-Observed-Adverse-Effect Levels (LOAELs) from relevant studies for local respiratory irritation and systemic effects to an inhalation exposure scenario estimating the concentration of smoke components within the respiratory tract and/or the dose that might be absorbed from the alveoli. The ratio of these two values (the margin of exposure, MOE) determines the risk; the smaller the ratio, the higher the risk. An overall assessment of the risk for local respiratory and systemic effects is performed by taking into account various factors such as less-than-lifetime exposure, interspecies extrapolation (rat to humans), and interindividual variability, among others.

Additives commonly used by the tobacco industry were selected, which included ammonium compounds, glycerol and propylene glycol. The smoke components selected may be generated from the combustion of additives and included acetaldehyde, acrolein, formaldehyde, and 2-furfural. Results from the risk assessment showed that a risk for local respiratory adverse effect exists for acetaldehyde, acrolein, formaldehyde, glycerol and propylene glycol and these are good candidates for lowering in the future. This method can be utilized for the prioritization of smoke components in cigarette smoke on the basis of their ability to induce adverse effects.

1 Introduction

Assessing the risk of complex mixtures is a very challenging field, particularly those whose exposure is via inhalation. There are presently no worldwide-accepted methods to assess the risk of compounds present in complex mixtures such as in cigarette smoke. The emergence of an increased number of additives in cigarettes has posed the question as to whether these could contribute to an increase in adverse effects in smokers. In this report, we describe a method on how human health risks from smoke components can be assessed, particularly those generated from the combustion of additives. The tobacco industry uses many additives in the manufacturing of tobacco products and the health effects of these additives through smoking have never been thoroughly investigated in a quantitative manner. Tobacco additives may increase the consumption rate of tobacco products by making the tobacco smoke more palatable and attractive to the consumer, or by enhancing addictiveness. Therefore, additives may indirectly enhance tobacco smoke-related harm by increasing the inhalation of these toxic products. Many additives give toxic pyrolysis products when burnt. For instance, burning of sugars in tobacco will results in many toxic compounds including aldehydes. Therefore, tobacco additives may directly or indirectly increase the toxicity of the tobacco product.

Additives were selected for those that were most commonly used by the tobacco industry and were assessed by The Public Information Tobacco Control (PITOC) project. The goal of the PITOC project was to generate fact sheets on the toxic and consumption-enhancing effects of additives in tobacco products and to communicate potential risks to consumers. The additives assessed included ammonium compounds, glycerol and propylene glycol. The smoke components selected may be generated from tobacco but also from the combustion of additives and included acetaldehyde, acrolein, formaldehyde, and 2-furfural. From this list, acetaldehyde, acrolein and formaldehyde are toxicants recommended for mandated lowering by the World Health Organization TobReg proposal (Burns et al., 2008) (see Table 1 for description of each chemical and cancer category by the International Agency for Research on Cancer, IARC).

The general procedure to assess human health risks from chemical exposures first requires an adequate insight in the exposure scenario and an assessment of the exposure. The exposure estimate will be compared with an appropriate estimate based toxicological data (human or animal). The second step includes the choice for a toxicological Point of Departure (PoD). This may be an appropriate Human Limit Value (preferably) or a No-Observed-Adverse-Effect Levels (NOAELs) or Lowest-Observed-Adverse-Effect Levels (LOAELs) based on an exposure scenario that closely resembles the human exposure scenario under evaluation. The third step, the actual risk assessment, comprises of a comparison of the human exposure estimate and the toxicological PoD to evaluate whether a human health risk is present or not, taking into account all uncertainties and assumptions involved (See Figure 1).

This procedure forms the basis for the present proposed approach for risk assessment from components in tobacco smoke. The three steps will be discussed in Chapters 2 (exposure assessment), Chapter 3 (choice of PoD) and Chapter 4 (risk assessment). It is noted beforehand that adequate Human Limit

Values (HLVs) are not available for the scenario of cigarette smoking. Thus the risk assessment has to be based on NOAELs or LOAELs and the calculation of a Margin of Exposure (MOE) being the ratio of the toxicological POD and the exposure estimate (see Chapter 4). Furthermore, consideration for both local and systemic effects is desired for a proper risk assessment. As illustration, Figure 1 shows a schematic presentation of this approach for local effects on the respiratory tract.

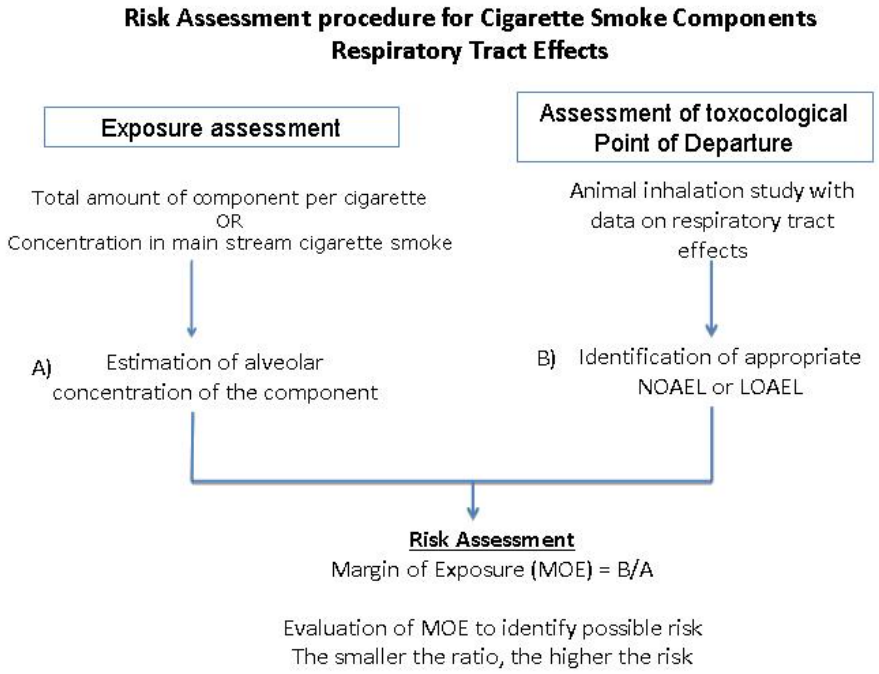

Figure 1 Simplified risk assessment procedure for cigarette smoke components as illustration for the risk assessment of local effects on the respiratory tract. The exposure assessment is described in Chapter 2 and the choice for a PoD in Chapter 3. The final conclusion whether the MOE is or is not sufficient will depend on the characteristics of the animal study (B) in relation to the exposure scenario (A) and can only be made on a ***case-by-case basis*** *(see Chapter 4 for details).*

Exposure to a component in cigarette smoke is by definition a very dynamic process because the exposure time/duration and the concentration in the respiratory tract are continuously varying. The exposure concentration in the respiratory tract is highest immediately after drawing a puff. The alveolar concentration of the smoke component gradually decreases until the next puff because of inhalation of clean air and absorption of the component into the systemic circulation. In addition, the interval between smoking two subsequent cigarettes is irregular, adding to the complexity in assessing the exposure scenario for cigarette smoking. As a conservative scenario, local effects on the respiratory tract epithelium are considered to be determined by the maximum concentration of a smoke component in the lungs (peak or height of exposure). For systemic effects, absorption is key, and for this reason, an estimation of the total amount of smoke component absorbed was made. Local and systemic effects were evaluated separately because of the irregular and dynamic exposure pattern of smoking.

Aim

The present document provides a pragmatic approach to estimate the respiratory exposure to smoke components (*e.g.* generated from the combustion of additives and/or tobacco) during smoking. In addition, a detailed description of the approach used to assess human health risks for local effects in the respiratory tract and lungs, as well as for systemic health effects is provided. The approach is applied to seven compounds present in tobacco smoke (Table 1).

Table 1 Description of compounds

Compound	**Description**	**Carcinogen?**	**IARC Classification**
Acetaldehyde	Smoke component generated during the combustion, e.g. from the many (poly) sugars added to tobacco.	yes	2B: possibly carcinogenic to humans
Acrolein	Smoke component generated during the combustion, e.g. from the many (poly) sugars added to tobacco.	no	-
Ammonium compounds	Added to aid in the formation of reconstituted tobacco, to enhance flavour, and to reduce the harshness and irritation of tobacco.	no	-
Formaldehyde	Smoke component generated during the combustion, e.g. from the many (poly) sugars added to tobacco.	yes	1: Carcinogenic to humans
2-Furfural	Smoke component generated during combustion, e.g. from the many (poly) sugars added to tobacco. In rare cases added to tobacco product as flavour.	no	-
Glycerol	Added as a humectant.	no	-
Propylene glycol	Added as a humectant.	no	-

2 Exposure scenario (Step 1)

The present approach uses a single compound analysis to estimate the health risks of the smoke component in mainstream smoke. As mentioned, exposure to a component in cigarette smoke is a very dynamic process. The concentration in the respiratory tract varies continuously during respiration. Therefore, insight in the respiration process is necessary to adequately estimate the exposure. Figure 2 shows the volumes of air associated with different phases of the respiratory cycle. The Tidal Volume is the volume of air that is inhaled or exhaled during breathing at rest. The Inspiratory and Expiratory Reserve Volume (IRV and ERV) refer to the maximum volume that can be inhaled or exhaled, respectively. Even after complete exhalation some air will be retained in the lungs. This volume of air is called the Residual Volume (RV). The volume of air that remains in the lung at the end of the exhalation phase is the Functional Residual Capacity (FRC), which is equal to the sum of ERV and RV. During a breathing cycle, approximately 30% of the inhaled volume of air will not reach the alveoli where gas exchange takes place. This volume is called the dead space volume. Default values for the relevant parameters in Figure 2 are given in Table 2.

The respiration dynamics are too complex to be fully taken into account in the exposure assessment and often adequate data are lacking. Therefore, a straightforward approach will be described in which the dynamics of the respiration process are included, although in a simplified way. The approach is considered to do sufficiently justice to the dynamics of the respiration process for the present purpose. If appropriate data become available the approach might be adjusted accordingly.

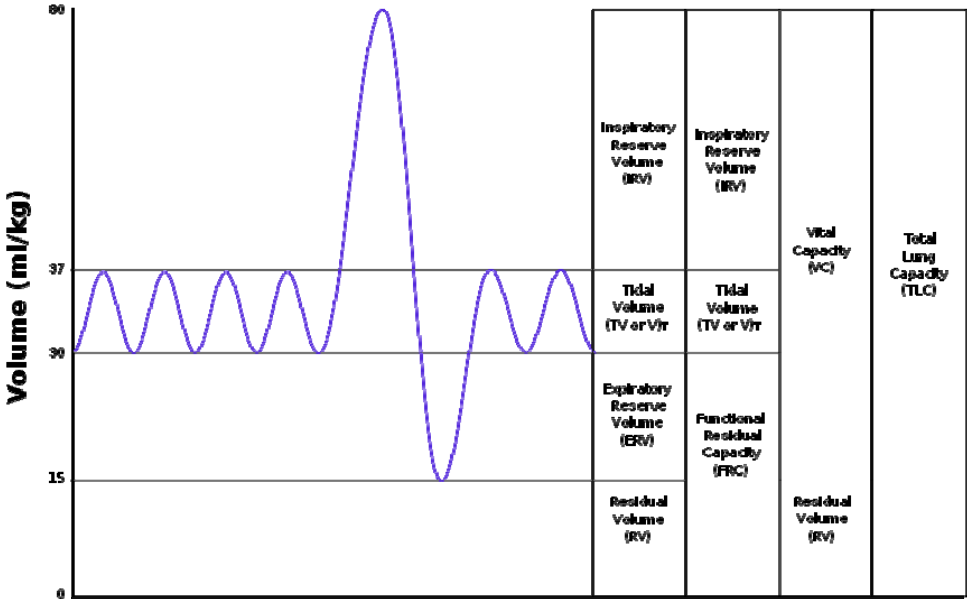

Figure 2 Lung volumes and lung capacities[1]

[1] http://en.wikipedia.org/wiki/Lung_volume

Table 2 Default parameter values for the exposure estimation[2]

Parameter	Default value
Puff volume	50 mL
Puff duration	1.5 sec
Puff interval	20 sec
Number of puffs per cigarette	13
Time to smoke one cigarette	5 min
Functional Residual Capacity	2 L
Tidal volume (rest)	500 mL
Breathing rate	12 min^{-1}
Dead space volume	30%

2.1 Exposure scenario: smoking pattern

The default smoking pattern for the present exposure assessment is based on Djordjevic *et al.* (2000). After drawing a puff and one subsequent breathing-cycle, the interval until the next puff is set at 20 sec. Starting from a breathing-frequency at rest of 12 min^{-1}, there will be four breathing cycles between puffs. A number of thirteen puffs is estimated for the smoking of one cigarette. Smoking one cigarette will then last approximately 5 min. For pragmatic reasons, a conservative approach was taken where a maximum of 40 cigarettes were smoked per day and that these cigarettes were smoked continuously one after another as a continuous 200 min exposure.

2.2 Exposure scenario: concentration of smoke component in the respiratory tract immediately after a puff

It is assumed that a smoker is at rest when smoking a cigarette. A brief puff of 50 mL is drawn and the cigarette smoke is inhaled, together with a volume of clean air (equal to the Tidal Volume at rest of 500 mL). It is assumed that the mixing of cigarette smoke and clean air is complete and, consequently, the concentration of components in cigarette smoke will decrease by a factor of eleven (550 mL/50 mL). It is recognized that smokers may inhale more deeply (*e.g.* the Tidal Volume is increased) following a puff leading to more dilution and thus lower concentrations. Therefore, assuming an inhalation volume equal to the Tidal Volume at rest can be considered to be conservative. Table 2 shows the relevant parameters, including default values, for the exposure assessment of cigarette additives. Because of the many uncertainties involved, the parameters are rounded-off values

During a breathing cycle, approximately 30% of the inhaled volume of air (called the dead space volume) does not reach the alveoli where gas exchange takes place. Thus only 70% of the inhaled 550 mL (*e.g.* 385 mL) reaches the alveoli. The volume of 385 mL is mixed by diffusion with the volume of air present in the lungs (*i.e.* the FRC which equals approximately 2 L) (see Figure 2 and Table 2). Thus, the concentration of cigarette smoke components is diluted further by approximately a factor of maximally six (*i.e.* 385 mL mixed with 2L). This process of diffusion however is not very fast and is probably not completed before the next inhalation of clean air. The component will thus not be evenly distributed within the lungs and the concentration will vary. Therefore,

[2] Sources: Djordjevic *et al*. (2000); http://www.anaesthetist.com/icu/organs/lung/Findex.htm#lungfx.htm; http://en.wikipedia.org/wiki/Lung_volume;

instead of a dilution factor of six, an arbitrary factor of three was utilized for the estimation of the initial alveolar concentration of a component.

In summary, it is estimated that the initial concentration of a component in alveolar air ($C_{alv;initial}$) will be a factor of 33 (11×3) lower than the concentration in mainstream cigarette smoke.

Two extreme scenarios are simulated in a simple way to obtain insight in the course of the alveolar concentration of smoke components in the respiratory tract during the smoking of one cigarette after the first puff. One scenario assumes no or a negligible absorption and a second scenario assumes a rapid absorption into the systemic circulation. As previously stated, starting from a breathing-frequency at rest of 12 min^{-1}, a puff will subsequently be followed by four breathing cycles of clean air during which the alveolar air concentration of the component will decrease. In case of absorption, this decrease will obviously be faster. At the next puff the alveolar concentration will increase again. This pattern will be repeated thirteen times for the smoking of one cigarette. Smoking one cigarette will last for approximately 5 min (300 sec) after which the alveolar air concentration will continuously decrease to zero, unless smoking of a next cigarette will start before a zero concentration has been reached.

Figure 3 shows the two respective alveolar concentration curves during the smoking of one cigarette relative to the maximum alveolar concentration immediately after the first puff ($C_{alv;initial}$). The decrease in alveolar concentration between two puffs is, in case of neglible absorption, only depending on dilution by inhalation of clean air. For the present purpose, a straightforward approach will suffice. It is assumed that with each breath 20% of the alveolar air is refreshed (500 mL (*i.e.* the tidal volume) of a total volume of 2500 mL). This means that the alveolar concentration will decrease by 20% with each subsequent inhalation until the next puff. This pattern is depicted in Figure 2 by the blue line. It is shown that after 5 puffs the maximum alveolar concentration does not further increase and stabilizes at approximately 1.7 times the initial alveolar concentration after the first puff ($C_{alv;init}$).

The pink curve illustrates what happens if a substance is rapidly absorbed. It is assumed that, next to a 20% decrease due to inhalation of clean air, approximately an additional 60% of the amount present in the alveolar air is absorbed. It is shown that in that case the alveolar concentration decreases to a negligible concentration before the next puff.

In conclusion, the maximal alveolar concentration of a tobacco smoke component during the smoking of one cigarette ($C_{alv;max}$) is estimated to be equal to 1.7× $C_{alv;initial}$. This is considered to be a conservative estimate since most compounds will be absorbed to at least some extent.

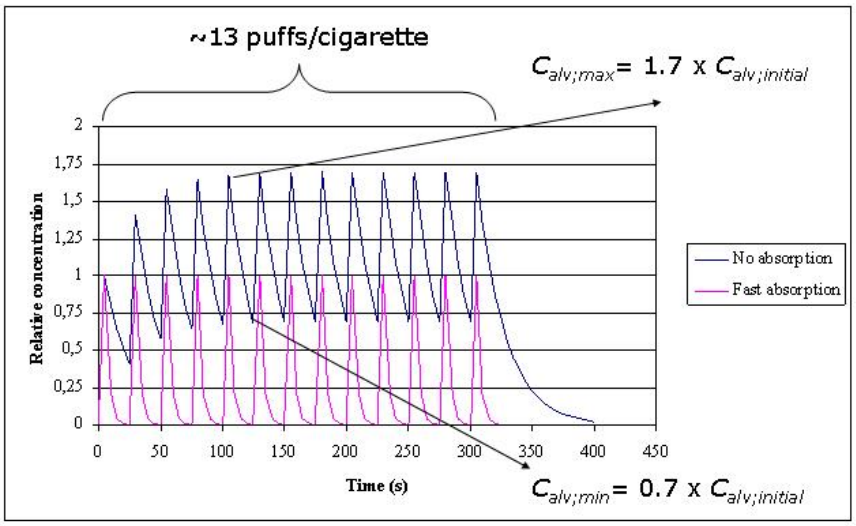

Figure 3 Simulations of alveolar air concentrations of a cigarette smoke component during the smoking of one cigarette in two scenarios, one with negligible absorption (blue curve) and one with substantial absorption (pink curve). Alveolar concentrations are expressed relative to the initial alveolar concentration after the first puff.

2.3 Exposure scenario: absorbed dose

Because of the dead space volume, a maximum of approximately 70% of an inhaled dose reaches the alveoli where gas exchange takes place and the chemical can be absorbed. Thus, maximally 70% of an inhaled dose can become systemically available.

2.4 Exposure assessment (Step 1)

The exposure concentration of a smoke component in the respiratory tract can be estimated from its concentration in mainstream smoke (in mg/L). If this concentration is unknown, it can be estimated from the absolute amount of compound (*e.g.* an additive) per cigarette (in mg/cig, D_{cig}).

2.4.1 Estimation of the concentration in the respiratory tract

I. Calculation based on concentration of compound in mainstream cigarette smoke (C_{smoke})

In section 2.2, it was derived that the maximal initial alveolar smoke component concentration ($C_{alv;initial}$) can be calculated using the following equation:

$$C_{alv;initial} = \frac{C_{smoke}}{(11 \times 3)}.$$

In addition, the maximal alveolar concentration during the smoking of one cigarette then equals (under the assumption of no rapid absorption):

$$C_{alv;\max} = 1.7 \times C_{alv;initial} = 0.052 \times C_{smoke} \quad .$$

II. Calculation based on the total amount of compound per cigarette (mg)

If the concentration of a smoke component in mainstream cigarette smoke is not available, the total amount per cigarette can be used to estimate the initial alveolar concentration. A 100% transfer rate is then assumed unless a transfer rate is known for the regarding component. Further, it will be considered whether other factors contribute to the concentration of the component in mainstream smoke. Apart from used as additive the component may also be a natural constituent of tobacco or be formed by pyrolysis. See also section 2.4.3 for methods to estimate levels of smoke components in mainstream smoke.

It is assumed that the total amount of smoke component in one cigarette (D_{cig} in mg/cig) will be inhaled in a total of thirteen puffs. The total volume of these thirteen puffs equals 0.650 L (13×0.050 L). The average concentration in mainstream smoke can then be calculated by dividing the amount (in mg) of a smoke component in a cigarette by 0.650 L.

Thus to calculate the maximal alveolar concentration ($C_{alv;max}$), C_{smoke} in the previous equation can be replaced by: D_{cig}/0.650 (giving the concentration in mg per L):

$$C_{alv;\max} = 0.052 \times \frac{D_{cig}}{0.650} = 0.08 \times D_{cig}$$

D_{cig} can thus be the **total amount of smoke component** per cigarette in mainstream smoke and this total amount can be:

a) 100% from additive OR
b) additive + other sources (*e.g.* natural tobacco).

If this is not available, D_{cig} can also be calculated based on the **total amount of additive** added to cigarettes

c) assuming 100% transfer rate to mainstream smoke OR
d) with a known transfer rate and estimating the levels in mainstream smoke.

Example of exposure assessment of 2-furfural

The exposure assessment is based on a measured total amount of 0.012 mg in mainstream smoke of one cigarette (Kataoka, Sumida, and Makita, 1997).

Estimation of the concentration in the respiratory tract assuming no rapid absorption (method II).

Calculation based on the total amount per cigarette ((D_{cig}) = 0.012 mg):

$C_{alv;max}$ = 0.08 × D_{cig} mg/L
= 0.08 × 0.012 mg/L
= 0.00096 mg/L
= 0.96 mg/m^3

2.4.2 Estimation of the absorbed dose

The total absorbed dose of a smoke component can be estimated either from its concentration in inhaled smoke (preferable if known), or from the absolute amount of compound (*e.g.* an additive) per cigarette assuming that the total amount is inhaled. The daily absorbed dose is to be expressed as mg/kg body weight.

I. Calculation based on a known concentration of compound in mainstream cigarette smoke (C_{smoke})

The total amount of smoke component inhaled per cigarette (D_{inh}) equals (*Concentration in mainstream cigarette smoke* × *puff volume* × *no. of puffs per cigarette*):

$$D_{inh} = C_{smoke} \times 0.05 \times 13 = 0.65 \times C_{smoke} ;$$

with D_{inh} in mg, C_{smoke} in mg/L and puff volume in L.

Accounting for the dead space volume, *e.g.* only 70% of the inhaled dose reaches the alveoli and can potentially be absorbed, the absorbed dose (D_{abs}) per cigarette can be calculated as (assuming 100% absorption from the alveoli):

$$D_{abs} = 0.7 \times D_{inh} = 0.455 \times C_{smoke} ;$$

with D_{abs} and D_{inh} in mg, and C_{smoke} in mg/L.

The daily dose in mg/kg body weight can be calculated by dividing D_{abs} by a default human body weight of 70 kg.

II. Calculation based on the total amount of compound per cigarette (mg)

If the concentration of a smoke component in mainstream cigarette smoke is not available, the total amount per cigarette can be used to estimate the initial alveolar concentration. A 100% transfer rate is then assumed unless a transfer rate is known for the regarding component. Further, it will be considered whether other factors contribute to the concentration of the component in mainstream smoke. Apart from used as additive the component may also be a natural constituent of tobacco or be formed by pyrolysis. See also section 2.4.3 for methods to estimate levels of smoke components in mainstream smoke.

It is assumed that the total amount of smoke component present in a cigarette (D_{cig}) will be inhaled (taking into account factors as mentioned in the previous paragraph). The total amount of smoke component that can be absorbed per cigarette (D_{abs}) then equals 0.7× D_{cig}. The daily dose in mg/kg body weight can be calculated by dividing D_{abs} by a default human body weight of 70 kg.

$$D_{abs} = 0.7 \times D_{cig}$$

D_{cig} can thus be the **total amount of smoke component** per cigarette in mainstream smoke and this total amount can be:

a) 100% from additive OR
b) additive + other sources (*e.g.* natural tobacco).

If this is not available, D_{cig} can also be calculated based on the **total amount of additive** added to cigarettes

c) assuming 100% transfer rate to mainstream smoke OR
d) with a known transfer rate and estimating the levels in mainstream smoke.

Example of exposure assessment of 2-furfural

Estimation of absorbed dose (method II).

Calculation based on the total amount in mainstream smoke per cigarette (mg) (D_{cig} = 0.012 mg):

- D_{abs} = $0.7 \times D_{cig}$
= 0.7×0.012 mg
= 0.0084 mg
- For a person smoking a maximum of 40 cigarettes/day
= 0.0084 mg × 40 cigarettes/day
= 0.34 mg per 40 cigarettes smoked/day

The amount absorbed per kg body weight then equals (0.34/70) 0.0048 mg/kg bw/d for a person smoking a maximum of 40 cigarettes per day.

2.4.3 Methods for deriving estimates of smoke components

Human smoking behaviour is a complex process influenced by factors such as puff volume, puff duration, inter-puff interval, number of puffs per cigarette and total puff volume (Marian et al., 2009). The total amount of the smoke component from one cigarette can be considered a good estimate for D_{cig}, and these values are generally generated from a smoking machine. Smoking machine generated values are considered as surrogates for values which are expected to be smoked by humans. There is a wide inter-individual variation for smoking behaviour and this varies from commonly used smoking machine regimes such as the International Standardization Organization (ISO) and Health Canada Intense (CI) protocol (Table 3). Normalization of the machine-generated yields per nicotine has been suggested to minimize the variability between methods (Burns et al., 2008). Nicotine yield cannot adequately characterize the distribution of nicotine uptake within a single brand, as evidence suggests that it is the smoker rather than the brand design which determines nicotine dose and smoke exposure (Hammond et al., 2006). Generally, higher estimates are derived from the CI method, in comparison to ISO as illustrated in Table 4. Here, Counts *et al.* (2005) compared the estimates of smoke components between the ISO and CI method and smoke component levels for acetaldehyde, acrolein, ammonia and formaldehyde were on average 2.4 times higher for measurements using the CI method than for the ISO method. For this reason, a conservative approach is used, where CI method values are selected over ISO estimates, when possible.

Table 3 Differences in parameters between smoking machine and humans

Regimen	**ISO**	**CI**	**HM (n=51)***	**HM^{A} (n=56)****	**HM^{B} (n=77)****
Puff Volume (mL)	35	55	53.3	48.6	44.1
Puff Duration (sec)	2	2	1.4	1.5	1.5
Puff Frequency (sec)	60	30	33.2	21.3	18.5
Puffs/minute	1	2	1.8		
Average Puffs/cigarette	13	13	11.5	12.7	12.1
Total Puff Volume (mL)	455	715	613	617 (566-668)	534 (487-561)
Ventilation holes	Open	100% Blocked	--	21% Blocked	30% Blocked
Reference	(Marian et al., 2009)	(Marian et al., 2009)	(Hammond et al., 2006)	(Djordjevic, Stellman, and Zang, 2000)	(Djordjevic, Stellman, and Zang, 2000)

*HM, human mimic study where participants (mean age 37.1 years, mean cigarette per day 19.3) smoked a total of 5409 cigarettes (17 brands with ISO tar yields 9-15 mg) (Hammond et al., 2006).

**HM^{A} participants between 19-59 years, smoking cigarettes with ISO nicotine yields ≤ 0.8 mg of nicotine/cigarette; HM^{B}0.9-1.2 mg of nicotine/cigarette (Djordjevic, Stellman, and Zang, 2000).

Table 4 Smoking machine values using the ISO and the CI method in Counts et al. (2005)

Compound	**ISO (µg/cigarette)**	**CI (µg/cigarette)**
Acetaldehyde	574	1448
Acrolein	46.3	122.4
Ammonia	13.4	31.1
Formaldehyde	26.4	60.5

3 Toxicological Point of Departure (Step 2)

In order to assess the risk of local respiratory and systemic effects, HLVs that reflect smoking are needed. Unfortunately, there is currently no methodology to derive HLVs for the specific and complex exposure scenario such as cigarette smoking. Therefore, in the absence of adequate HLVs, a Margin of Exposure (MOE) approach is proposed. The MOE is calculated as the ratio of an appropriate toxicological point of departure (PoD) and the exposure estimate. The MOE is evaluated considering factors associated with relevant extrapolation and uncertainty issues (see Chapter 4).

Preferably, the exposure scenario on which the PoD is based on should resemble the exposure scenario under evaluation as close as possible. These data will not be available for the smoking scenario (intermittent exposures over a day to varying concentration during an exposure period). Therefore, the PoD will be derived from the study with the most appropriate exposure scenario. The impact of the differences between this scenario and the smoking scenario on the outcome of the evaluation can be weighed during the risk assessment step. It is noted that the reasons for choosing for a specific endpoint and dataset should always be well-founded in a transparent way.

For the seven compounds described in Table 1, selected data sources were scanned to retrieve an appropriate toxicological PoD. No-Observed-Adverse-Effect Levels (NOAELs) or Lowest-Observed-Adverse-Effect Levels (LOAELs) from relevant studies that most closely resembled the smoking exposure scenario in study design and duration were selected from the following sources:

- Occupational Exposure Limits (OELs). Examples are the Dutch values, the TLVs derived by the American Conference of Governmental Industrial Hygienists (ACGIH), or the German MAK-values
- The Agency for Toxic Substances and Disease Registry (ATSDR) Toxicity Profiles (http://www.atsdr.cdc.gov/toxprofiles/index.asp)
- The WHO Air Quality Guidelines.
- The Integrated Risk Information System (IRIS) database of the US Environmental Protection Agency (EPA) (http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showSubstanceList)
- Health Canada reports (http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/index-eng.php)

In addition, comprehensive evaluation reports of chemical substances, such as the EU Risk Assessment Reports (http://esis.jrc.ec.europa.eu/index.php?PGM=ora) and the WHO Environmental Health Criteria documents (http://www.who.int/ipcs/publications/ehc/en/) and the Acute Exposure Guideline Levels-Technical Support Documents (http://www.epa.gov/oppt/aegl/) were searched for relevant toxicity data.

4 Risk assessment (Step 3)

The MOE approach is employed to assess the risk of local respiratory and systemic effects for the seven compounds. The MOE is calculated as the ratio of an appropriate toxicological PoD (*e.g.* NOAEL or LOAEL from the most relevant study) and the human exposure estimate; the lower the ratio, the higher the risk. Several factors are to be considered in the evaluation of a calculated MOE that are associated with extrapolation and uncertainty issues related to differences between the conditions under which the PoD was derived and the conditions of the smoking exposure scenario. These factors include, among others, interspecies differences, interindividual variability, differences in exposure conditions on which the PoD is based and the smoking scenario and whether the PoD reflects a NOAEL or LOAEL. Not all of these factors need to be considered in every risk assessment or not to the same extent. Therefore, a default minimum value for the MOE to conclude that no risk is present cannot be derived. Whether or not a calculated MOE indicates a health risk is to be evaluated on a **case-by-case basis**.

A tiered approach is used to assess the health risks from respiratory exposure to smoke components.

In the first tier the MOE is calculated under conservative assumptions:

- If a calculated MOE for a smoke component is considered to be sufficiently large (taking all relevant factors into account), it can be concluded that no health risk from inhalation of the additive is to be expected.
- If a calculated MOE for a smoke component is considered to be too small (taking all relevant factors into account), it can be concluded that a health risk from the smoke component exists or cannot be excluded.

In some instances, it cannot unequivocally be concluded that a MOE is or is not sufficiently large in which case a more detailed assessment will have to be made in the second tier. Smoke components were '**risk cannot be excluded**' fall in this category. The level of conservatism for each assumption in the exposure assessment as well as of the factors to be considered in the MOE evaluation will be judged and their impact on the evaluation of the MOE will be assessed. Further, a more detailed (literature) search for additional data to replace default assumptions or refine the risk assessment process may be useful. If necessary and possible, important data gaps will be identified and/or filled in. A final conclusion will be drawn taking all the uncertainties into account.

4.1 Step 3a: Risk assessment for local effects

In the first tier it will be assumed that the exposure scenario includes a 200 min continuous exposure to a concentration equal to $C_{alv;max}$, as calculated under I or II in section 2.4.1. If it is known that the chemical is absorbed rapidly into the systemic circulation, $C_{alv;initial}$ might be used as the maximal exposure concentration. This concentration will be compared to an appropriate toxicological PoD for respiratory tract irritation. The MOE will be evaluated considering relevant factors related to extrapolation and uncertainty issues (Figure 4).

Exposure assessment				Hazard assessment
	Local respiratory tract effects			
I.	Known concentration in cigarette smoke (C_{smoke})	→	$C_{alv;max} = 0.052 \times C_{smoke}$	Point of Departure (PoD):
	Or			NOAEL or LOAEL from most relevant study
II.	Total amount per cigarette (D_{cig})	→	$C_{alv;max} = 0.08 \times D_{cig}$	
Risk assessment → Margin of exposure (MOE) = NOAEL or LOAEL / $C_{alv;max}$				

*Figure 4 Scheme of the risk assessment process for **local respiratory effects** D_{cig} can be obtained from the **total amount of smoke component** per cigarette in mainstream smoke and this total amount can be: a) 100% from additive OR b) additive + other sources (e.g. natural tobacco). If this is not available, D_{cig} can also be calculated based on the **total amount of additive** added to cigarettes c) assuming 100% transfer rate to mainstream smoke OR d) with a known transfer rate and estimating the levels in mainstream smoke.*

In the second tier, the impact of the assumptions are made related to the exposure pattern (for example assuming continuous exposure instead of intermittent peak exposure) and the choice of the PoD on the outcome of the risk assessment, needs to be further explored, transparently assessed and discussed. A detailed (literature) search for additional data to replace the assumptions or refine the risk assessment process may be useful. A final conclusion on possible risks for respiratory tract irritation will be provided.

Human risk assessment of local effects on the respiratory tract can only be performed by comparing the exposure scenario with a PoD based on respiratory toxicity data. If respiratory data are absent, a risk assessment for local effects on the respiratory tract cannot be performed.

4.2 Step 3b: Risk assessment for systemic effects

In the first tier the MOE is calculated as the ratio of a PoD (being the absorbed daily dose per kg body weight derived from an appropriate study) with the absorbed dose (D_{abs}) per kg body weight as calculated under I or II in section 2.4.2. It is noted that if appropriate respiratory data are not available, data derived from oral toxicity studies might be useful for the risk assessment of systemic effects under certain conditions. For instance, the absence of route-specific effects should be confirmed or their relevance should be considered.

In the second tier, the impact of the assumptions made (for example assuming continuous instead of intermittent exposure, level of absorption) and the adequacy of the PoD for the present risk assessment needs to be further explored, transparently assessed and discussed. A (literature) search for additional data to replace the assumptions or refine the risk assessment process may be useful. A final conclusion on possible health risks for systemic effects will be provided (Figure 5).

Exposure assessment			Hazard assessment
Systemic effects			
I. Known concentration in cigarette smoke (C_{smoke})	→	$D_{abs} = 0.455 \times C_{smoke}$	Point of Departure (PoD):
Or			NOAEL or LOAEL from most relevant study
II. Total amount per cigarette (D_{cig})	→	$D_{abs} = 0.7 \times D_{cig}$	
Risk assessment → Margin of exposure (MOE) = NOAEL or LOAEL[a] / $(D_{abs}/70)^b$			

*Figure 5 Scheme of the risk assessment process for **systemic effects***
a: For calculation of the MOE the NOAEL or LOAEL should be expressed as internal dose (mg) per kg body weight.
*b: The human exposure is expressed as internal dose (mg) per kg body weight ($D_{abs}/70$). D_{cig} can be obtained from the **total amount of smoke component** per cigarette in mainstream smoke and this total amount can be: a) 100% from additive OR b) additive + other sources (e.g. natural tobacco). If this is not available, D_{cig} can also be calculated based on the **total amount of additive** added to cigarettes c) assuming 100% transfer rate to mainstream smoke OR d) with a known transfer rate and estimating the levels in mainstream smoke.*

5 Risk assessment acetaldehyde

5.1 Amount of acetaldehyde in cigarette smoke

The mainstream smoke from an average cigarette contains 1.448 mg of acetaldehyde when the cigarette is smoked on a smoking machine using the Canadian Intense method (Counts et al., 2005); maximum levels of acetaldehyde reported to be present in inhaled smoke can reach up to 2.1 mg per cigarette, depending on the puff profile intensity, method of detection and/or tar level (Seeman, Dixon, and Haussmann, 2002; Talhout, Opperhuizen, and van Amsterdam, 2007). These values were used for the exposure assessment.

5.2 Pyrolysis and reaction products in cigarette smoke

Acetaldehyde can condense with small molecules such as amino acids (*e.g.* tryptophan and tryptamine), which are present in tobacco as well as other with other molecules present throughout the body. Harman is formed from the reaction of acetaldehyde with tryptophan and tryptamine at levels of ranging from 0.1-5.8 µg of harman per cigarette (Talhout, Opperhuizen, and van Amsterdam, 2007). Although produced in lower amounts than other acetaldehyde metabolites, combustion products and possible degradation products, harman, together with these other by-products are hypothesised to have important biological effects in the brain stimulating addictiveness to cigarette smoking (Talhout, Opperhuizen, and van Amsterdam, 2007).

5.3 Non-carcinogenic effects: Local respiratory effects and systemic effects

The main effects reported following acetaldehyde exposure occurred in the respiratory tract which included mild to severe changes in the olfactory epithelium of rats exposed to 1365 mg/m^3, 6 hours per day, 5 days per week for 112 weeks (Woutersen et al., 1986). A similar study investigating changes in the olfactory epithelium of rats exposed for 6 hours per day, 5 days per week for 4 weeks, reported a NOAEL of 273 mg/m^3 (Appelman et al., 1986). No systemic effects were reported in these studies.

5.4 Risk assessment of local respiratory effects and systemic effects

5.4.1 Step 1: Exposure assessment

Smokers are exposed to acetaldehyde through inhalation with the respiratory tract being the first site of exposure. A cigarette generates on average 1.448 mg acetaldehyde upon combustion with maximum levels in mainstream smoke reaching 2.1 mg; these values are used as estimates for D_{cig} for the exposure assessment. The maximum alveolar concentration ($C_{alv;max}$) was then calculated to be 116 mg/m^3 from average levels present in mainstream smoke (1.448 mg) and 168 mg/m^3 from maximum levels in mainstream smoke (2.1 mg).

5.4.2 Step 2: Point of Departure

Two studies with rats were retrieved that could serve as PoD (the most relevant study/ies that most closely resemble the smoking exposure scenario in study design and duration) for risk assessment purposes. A 28-month study (6 hours per day, 5 days per week) yielded a LOAEL of 1365 mg/m3 (Woutersen et al., 1986). The main effects reported following acetaldehyde exposure occurred in the respiratory tract which included mild to severe changes in the olfactory epithelium of rats exposed for 112 weeks. A second, 4-week, study (6 hours per

day, 5 days per week) provided a NOAEL of 273 mg/m3 (Appelman et al., 1986). Both studies were used as PoD values for further risk assessment. Please refer to Table 5 for MOE calculation.

5.4.3 Step 3a: Risk for local effects

28-month study

The MOE for morphological changes in the olfactory epithelium was 12 based on average levels present in mainstream smoke and 8 for maximum levels (Table 5). Factors to be taken into account for evaluation of the MOE included the use of LOAEL as PoD, interspecies extrapolation (rats to humans) and interindividual variability.

Considering the MOE and the factors to be accounted for, it is concluded that a risk of effects on the respiratory tract epithelium from acetaldehyde cannot be excluded.

4-week study

The rounded-off MOE for morphological changes in the olfactory epithelium was 2 for analysis in average levels present in mainstream smoke and 2 (rounded up) for maximum levels in mainstream smoke (Table 5). Factors to be taken into account for evaluation of the MOE include less-than-lifetime exposure, interspecies extrapolation (rat to humans), and interindividual variability.

Considering the MOE and the factors to be accounted for, it is concluded that a risk for effects on the respiratory tract epithelium due to acetaldehyde exists.

Table 5 Summary of the studies used as a PoD, the Calv;max from measurements in cig cigarette smoke and the MOE analysis

Description	**Selected study 1**	**Selected study 2**
Critical endpoint	Changes in olfactory epithelium	Degeneration of olfactory epithelium
Study	(Woutersen et al., 1986) http://www.epa.gov/iris/subst/0290.htm	(Appelman et al., 1986) http://www.epa.gov/iris/subst/0290.htm
Species	Wistar rats	Wistar rats
Exposure regimen	6 h per day, 5 days/week	6 h per day, 5 days/week
Concentrations tested (mg/m^3)	0, 1365, 2730, 5460	0, 273, 913
Duration of exposure	28 months	4 weeks
NOAEL (mg/m^3)	not available	273
If no NOAEL, then value for LOAEL	1365	-
$C_{alv;max}$ (mg/m^3)	116	116
Source of $C_{alv;max}$ (mg/m^3)	from average levels present in mainstream smoke	from average levels present in mainstream smoke
MOE_1	***12***	***2***
$C_{alv;max}$ (mg/m^3)	168	168
Source of $C_{alv;max}$ (mg/m^3)	From maximum levels in mainstream smoke	From maximum levels in mainstream smoke
MOE_2	***8***	***2***

Combining both evaluations it is concluded that a risk of effects on the respiratory tract epithelium due to acetaldehyde exists.

It is recognized that several assumptions have been made and that the risk assessment can be refined reconsidering these assumptions but such a refinement is beyond the scope of the present analysis.

5.4.4 *Step 3b: Risk for systemic effects*

No thorough assessment on systemic effects was made. Nevertheless, no systemic effects were reported in the inhalation studies described here, which is expected given that acetaldehyde is very reactive and will therefore exert its effect at the first site of exposure.

6 Risk assessment for acrolein

6.1 Amount of acrolein in cigarette smoke

Studies have reported a sales-weighted mean for 74 tested brands of 0.11 mg acrolein per cigarette (Phillips and Waller, 1991), with the sales weighted mean being the mean amount of acrolein in the smoke produced per cigarette adjusted to the difference in composition of the brands most commonly sold. A study in which the average acrolein smoke content of 48 different brands of filtered cigarettes from various international markets (Philip Morris commercial brands) was analysed by a smoking machine using the Canadian Intense method, the amount of acrolein in mainstream smoke was found to be 0.122 mg per cigarette (Counts et al., 2005). This study was used for the exposure assessment.

6.2 Pyrolysis and reaction products in cigarette smoke

Acrolein is the pyrolysis product of the combustion of most of the (poly)sugars contained in tobacco (this is not the case with just tobacco) and is a highly reactive compound that will interact with numerous smoke components thereby forming several secondary pyrolysis products. However, there is insufficient information on these reaction products to make a meaningful conclusion on the contribution of acrolein. Acrolein is also a toxic pyrolysis product of glycerol, which is added to cigarettes as a humectant.

6.3 Non-carcinogenic effects: Local respiratory effects and systemic effects

Because of the high reactivity of acrolein, it binds primarily to the respiratory tract (the application site) of smokers during smoking. This has been observed in non-lethal studies in experimental animals; an indication that the respiratory system is a target organ for acrolein toxicity. It has also been demonstrated that acrolein is a potent irritant at relatively low concentrations and short exposure durations. The main source of exposure of the general population to acrolein is through tobacco smoke. The main effects reported following acrolein exposure occurred in the respiratory tract and this included changes in the olfactory epithelium of rats exposed to 0.9, 3.2 and 11 mg/m^3, 6 hours per day, 5 days per week for 13 weeks (Feron et al., 1978). This study for acrolein gave the lowest value to be used in the risk assessment (*e.g.* point of departure (PoD)) with a LOAEL of 0.9 mg/m^3. No systemic effects were reported in this study.

6.4 Risk assessment of local respiratory effects and systemic effects

6.4.1 Step 1: Exposure assessment

An estimate of the human exposure to acrolein is made from smoking machine data due to the difficulty in determining the actual exposure to a given consumer. As aforementioned, mainstream smoke resulting from one cigarette is reported to contain 0.122 mg of acrolein, and this value is used as estimate for D_{cig} for the exposure assessment. The maximum alveolar concentration ($C_{alv;max}$) was then calculated to be 9.8 mg/m^3.

6.4.2 Step 2: Point of Departure

The LOAEL of 0.9 mg/m^3 in a 13-week rat study (Feron et al., 1978) was chosen as the best PoD (the most relevant study/ies that most closely resemble

the smoking exposure scenario in study design and duration) value for effects on the respiratory tract. See Table 6 for MOE calculation.

6.4.3 *Step 3a: Risk for local effects*

The MOE for changes in the nasal epithelium is 0.09 (Table 6). Factors to be taken into account for evaluation of this MOE included the use of a LOAEL as PoD instead of NOAEL, less-than-lifetime exposure, interspecies extrapolation (rat to humans), and interindividual variability.

Considering the MOE and the factors to be accounted for, it is concluded that a risk of effects on the respiratory tract epithelium due to acrolein exists.

It is recognized that several assumptions have been made and that the risk assessment can be refined reconsidering these assumptions. Although such a refinement is beyond the scope of the present analysis, considering the low MOE, it remains to be seen if further refinement will alter the conclusion.

6.4.4 *Step 3b: Risk for systemic effects*

No thorough assessment on systemic effects was made. No systemic effects were reported in the inhalation study, which is expected given that acrolein is very reactive and will therefore exert its effect at the first site of exposure.

Table 6 Summary of the study used as a PoD, the Calv;max from measurements in cigarette smoke and the MOE analysis

Description	**Selected study**
Critical endpoint	Structural changes in nasal epithelium and nasal lesions
Study	http://www.epa.gov/iris/subst/0364.htm (Feron et al., 1978)
Species	Wistar rats
Exposure regimen	6 h per day, 5 days/week
Concentrations tested (mg/m^3)	0, 0.9, 3.2, 11
Duration of exposure	13 weeks
NOAEL (mg/m^3)	-
If no NOAEL, then value for LOAEL	0.9
$C_{alv;max}$ (mg/m^3)	9.8
Source of $C_{alv;max}$ (mg/m^3)	from average levels present in mainstream smoke
MOE	***0.09***

7 Risk assessment for ammonium compounds

7.1 Amount of ammonium compounds added to cigarettes

Assuming an average tobacco weight of 700 mg per cigarette (Counts et al., 2004), a commercial cigarette may contain a total amount (naturally present and added) of ammonium compounds in the range of 0.6 to 2.4 mg per cigarette (Willems et al., 2006). In the Netherlands, tobacco manufacturers rarely report ammonium compound addition (analysis of data delivered to Dutch regulators in 2010 via the Electronic Model Tobacco Control (EMTOC, 2010)), nevertheless ammonium compounds are still naturally present in tobacco.

7.2 Pyrolysis and reaction products in cigarette smoke

Ammonium compounds undergo pyrolysis during cigarette smoking to produce ammonia. The average ammonia content from 48 brands of filtered cigarettes obtained from various international markets (Philip Morris commercial brands) was analysed by a smoking machine using the Canadian Intense method and the amount in mainstream smoke was found to be 0.0311 mg per cigarette (Counts et al., 2005). This study was used for the exposure assessment.

7.3 Non-carcinogenic effects: Local respiratory effects and systemic effects

Ammonia is the major pyrolysis product generated from ammonium compounds during cigarette smoking. The European Union Scientific Committee on Occupational Exposure Limits (SCOEL) derived an Indicative Occupational Limit Value for ammonia in 1992 based on human data (SCOEL, 1992). They concluded that the critical effect of ammonia is irritation of the eyes, skin and upper respiratory tract. Human volunteer studies indicated that subjective symptoms start to occur at approximately 36 mg/m^3 for exposures up to 6 hours, which was considered to be a LOAEL. No systemic effects were reported.

7.4 Risk assessment of local respiratory effects and systemic effects

7.4.1 Step 1: Exposure assessment

Mainstream smoke of one cigarette was reported to contain 0.0311 mg of ammonia per cigarette, which is used as estimate for D_{cig} for the exposure assessment. The maximum alveolar concentration ($C_{alv;max}$) was then calculated to be 2.5 mg/m^3.

7.4.2 Step 2: Point of Departure

The LOAEL of 36 mg/m^3 for upper respiratory tract irritation as derived by the SCOEL from human volunteer data was used as PoD (the most relevant study/ies that most closely resemble the smoking exposure scenario in study design and duration) value for ammonia (SCOEL, 1992). Please refer to Table 7 for MOE calculation.

7.4.3 Step 3a: Risk for local effects

The MOE for respiratory tract irritation is 14 (Table 7). Factors to be taken into account for evaluation of this MOE included LOAEL as PoD instead of NOAEL, less-than-lifetime exposure and interindividual variability. Considering the MOE and the factors to be accounted for, it is concluded that a risk of effects on the respiratory tract epithelium due to ammonia cannot be excluded.

It is recognized that several assumptions have been made and that the risk assessment can be further refined reconsidering these assumptions. Although such a refinement is beyond the scope of the present analysis, it remains to be seen if further refinement will alter the conclusion.

Table 7 Summary of the study used as a PoD, the Calv;max from measurements in cigarette smoke and the MOE analysis

Description	**Selected study**
Critical endpoint	Irritation of eyes, skin and upper respiratory tract
Study	http://www.ser.nl/documents/43839.pdf
Species	Human volunteers
Exposure regimen	Up to 6 h per day
Concentrations tested (mg/m^3)	Not mentioned, based on various human studies
Duration of exposure	Up to 6 h (Ferguson et al., 1977)
NOAEL (mg/m^3)	-
If no NOAEL, then value for LOAEL	36
$C_{alv;max}$ (mg/m^3)	2.5
Source of $C_{alv;max}$ (mg/m^3)	from average levels present in mainstream smoke
MOE	***14***

7.4.4 Step 3b: Risk for systemic effects

No thorough assessment on systemic effects was made. Nevertheless, no systemic effects were reported in the inhalation studies described here.

8 Risk assessment for formaldehyde

8.1 Amount of formaldehyde in cigarette smoke

A study in which the average formaldehyde smoke content of 48 different brands of filtered cigarettes from various international markets (Philip Morris commercial brands) was analysed by a smoking machine using the Canadian Intense method, the amount of formaldehyde in mainstream smoke was found to be 0.0605 mg per cigarette (Counts et al., 2005). This study was used for the exposure assessment.

8.2 Pyrolysis and reaction products in cigarette smoke

Formaldehyde breaks down into methanol and carbon monoxide at temperatures above 150°C. However, uncatalysed decomposition of formaldehyde is slow at temperatures below 300°C (SIDS, 2002). A study showed that norharman was formed in high levels in mainstream cigarette smoke as a result of the pyrolysis of tryptophan and subsequent reaction with formaldehyde (Herraiz, 2004). In total, approximately 0.1-5.8 µg of norharman was found to be present in one cigarette (Talhout *et al.*, 2007).

8.3 Non-carcinogenic effects: Local respiratory effects and systemic effect

Inhalation is the principal route of exposure to formaldehyde. The main effects reported following formaldehyde exposure occurred in the respiratory tract. Personal breathing zone air concentrations ranged from 0.05 to 0.5 mg/m^3 (median 0.3 ± 0.16 mg/m^3) for the chemical workers in a mean durations of employment of 10.4 years (SD 7.3, range 1–36 years) (Holmstrom, Wilhelmsson, and Hellquist, 1989). Clinical symptoms of mild irritation of the eyes and upper respiratory tract and mild damage to the nasal epithelium were observed in workers exposed for 10.4 years (range 1-36 years) to a median 8-hour-time-weighted median concentration of 0.3 mg/m^3 (range: 0.05 to 0.5 mg/m^3) (Holmstrom, Wilhelmsson, and Hellquist, 1989). This concentration of 0.3 mg/m^3 is considered the lowest LOAEL by the Agency for Toxic Substances and Disease Registry (ATSDR) (http://www.atsdr.cdc.gov/toxprofiles/tp111-a.pdf). Studies showed that only a very small proportion of the population experienced symptoms of irritation following exposure to less than 0.12 mg/m^3 formaldehyde (http://www.atsdr.cdc.gov/toxprofiles/tp111-a.pdf). Mucociliary clearance in the anterior portion of the nasal cavity and histopathological effects in the nasal epithelium were observed at 0.30 mg/m^3 both in clinical studies in human volunteers and in cross-sectional studies of formaldehyde -exposed workers (Holmstrom, Wilhelmsson, and Hellquist, 1989). No systemic effects were reported in these studies.

8.4 Risk assessment of local respiratory effects and systemic effects

8.4.1 Step 1: Exposure assessment

Human exposure to formaldehyde was estimated from smoking machine data to be 0.0605 mg per cigarette which is used as an estimate for D_{cig} for the exposure assessment. The maximum alveolar concentration ($C_{alv;max}$) was then calculated to be 4.84 mg/m^3.

8.4.2 Step 2: Point of Departure

In this assessment, focus remained on non-cancer endpoints. The main effects reported following formaldehyde exposure occurred in the respiratory tract. A LOAEL of 0.3 mg/m3, based on occupational data, (Holmstrom,

Wilhelmsson, and Hellquist, 1989) was considered to be the best PoD (the most relevant study/ies that most closely resemble the smoking exposure scenario in study design and duration) value for further risk assessment. Please refer to Table 8 for MOE calculation.

8.4.3 *Step 3a: Risk for local effects*

The MOE for changes in the nasal epithelium was 0.06 (Table 8). Factors to be taken into account for evaluation of this MOE included LOAEL as PoD instead of NOAEL and interindividual variability (which might be larger in the general population than in the worker population).

Considering the MOE and the factors to be accounted for, it is concluded that a risk of effects on the respiratory tract epithelium due to formaldehyde exists.

Table 8 Summary of the study used as a PoD, the Calv;max from measurements in cigarette and the MOE analysis

Description	**Selected study**
Critical endpoint	Clinical symptoms of mild irritation of the eyes and upper respiratory tract and mild damage to the nasal epithelium
Study	(Holmstrom, Wilhelmsson, and Hellquist, 1989) (http://www.atsdr.cdc.gov/toxprofiles/tp111-a.pdf)
Species	Human
Exposure regimen	Occupational exposure.
Concentrations measured (mg/m^3)	Personal breathing zone air concentrations ranged from 0.05 to 0.5 mg/m^3 (median 0.3 ± 0.16 mg/m^3) for chemical workers.
Duration of exposure	Mean durations of employment: 10.4 years (SD 7.3, range 1–36 years).
NOAEL (mg/m^3)	-
If no NOAEL, then value for LOAEL (mg/m^3)	0.3
$C_{alv;max}$ (mg/m^3)	4.84
Source of $C_{alv;max}$ (mg/m^3)	average levels present in mainstream smoke
MOE	***0.06***

It is recognized that several assumptions have been made and that the risk assessment can be refined reconsidering these assumptions. Although such a refinement is beyond the scope of the present analysis, considering the low MOE, it remains to be seen if further refinement will alter the conclusion.

8.4.4 *Step 3b: Risk for systemic effects*

No thorough assessment on systemic effects was made. Nevertheless, formaldehyde is classified as a group 2A carcinogen, which indicates that it is probably carcinogenic to humans (IARC).

9 Risk assessment for 2-furfural

9.1 Amount of 2-furfural in tobacco smoke

In the Netherlands, tobacco manufacturers report the addition 2-furfural in amounts of 0.03% (w/w) tobacco, which is 0.21 mg based on a cigarette containing 700 mg tobacco (analysis of data delivered to Dutch regulators in 2010 via the Electronic Model Tobacco Control (EMTOC, 2010)). Mainstream cigarette smoke is reported to contain, on average, 12 (±1) µg of 2-furfural based on a study conducted using four commercial filter cigarettes differing in tar and nicotine content and measurements with a laboratory smoking machine and ISO puffing conditions) (Kataoka, Sumida, and Makita, 1997). This study was used for the exposure assessment.

9.2 Pyrolysis and reaction products in cigarette smoke

2-Furfural is a pyrolysis product of many of the (poly)sugars added to tobacco, in particular fructose, sugar containing additives like liquorice, and other cigarette components. Sugars, being a major component of tobacco, lead to the generation of high levels of 2-furfural in cigarette smoke. However, there is insufficient information on the possible reaction products of 2-furfural.

9.3 Non-carcinogenic effects: Local respiratory effects and systemic effect

PubMed and the European Union Risk Assessment Report for 2-furfural (http://esis.jrc.ec.europa.eu/doc/existing-chemicals/risk_assessment/SUMMARY/2furaldehydesum050.pdf) were used to select a suitable study for the point of departure (PoD; the most relevant study/ies that most closely resemble the smoking exposure scenario in study design and duration) for 2-furfural after inhalation exposure. In hamsters exposed for 6 h per day, five days per week, over 13 weeks, a NOAEL was established at a level of 77 mg/m^3 (Feron and Kruysse, 1978). Rats, however, were more susceptible, showing histopathological nasal changes at the lowest concentration tested in a 28-day study (6 hours/day, 5 days/week), 20 mg/m^3 (Arts et al., 2004). This study also reported NOAELs of 640 mg/m^3 (3 hour/day) or 320 mg/m^3 (6 hour/day) for systemic effects. Arts *et al.* (2004) report the same systemic dose of 92 mg/kg for both exposures. Rats were found to be more susceptible than mice, suggesting that species specificity should be taken into account in human risk assessment, as this could have profound effects on results. Concentrations used in the animal study were equivalent to the concentrations to which workers were reported to be exposed. This exceeds 8 mg/m^3, the established 8-h TWA* (SER, 1992) and these concentrations were reported to have caused respiratory tract irritation in these workers (Di Pede et al., 1991).

9.4 Risk assessment of local respiratory effects and systemic effects

9.4.1 Step 1: Exposure assessment

Mainstream smoke of one cigarette is reported to contain on average 12 ± 1 µg of 2-furfural (measured with a laboratory smoking machine) (Kataoka, Sumida, and Makita, 1997). The maximum alveolar concentration ($C_{alv;max}$) was

* 8-h TWA, time-weighted average concentration to which a worker can be repeatedly exposed for 8 hours per day, 5 day per week for 40-years, without experiencing adverse effects.

then calculated to be 0.96 mg/m^3 per cigarette. Similarly, for the assessment of systemic inhalation toxicity, the maximal absorbed dose (D_{abs}) per kg body weight was calculated to be 0.0048 mg/kg when smoking 40 cigarettes per day.

9.4.2 *Step 2: Point of Departure (PoD)*

The main effects reported following 2-furfural exposure occurred in the respiratory tract which included histopathological changes in the nasal epithelium of Fischer 344 rats exposed to 20 mg/m^3 (the lowest concentration tested) , 6 hours per day, 5 days per week for 4 weeks (Arts et al., 2004). This study with a LOAEL for respiratory tract irritation of 20 mg/m^3 for 2-furfural was considered to be the best PoD value for further risk assessment. In addition, two NOAELs for systemic inhalation toxicity were reported, 320 mg/m^3 for 6 hour exposure per day and 640 mg/m^3 for 3 hour exposure per day. The systemic dose of 92 mg/kg is chosen as PoD for systemic effects. Please refer to Tables 9 and 10 for MOE calculations.

9.4.3 *Step 3a: Risk assessment for local effects*

The MOE for changes in the nasal epithelium was estimated to being 21 (Table 9). Factors to be taken into account for evaluation of this MOE included LOAEL as PoD instead of NOAEL, less-than lifetime exposure, interspecies extrapolation (rat to humans), and interindividual variability.

Table 9 Summary of the study used as a PoD, the Calv;max from measurements in cigarette and MOE analysis

Description	**Selected study**
Critical endpoint	Histopathological changes in nasal epithelium
Study	http://esis.jrc.ec.europa.eu/doc/existing-chemicals/risk_assessment/SUMMARY/2furaldehydesum050.pdf (Arts et al., 2004)
Species	Fischer 344 rats
Exposure regimen	6 h per day, 5 days/week
Concentrations tested (mg/m^3)	0, 20, 40, 80, 160, 320, 640,1280
Duration of exposure	4 weeks
NOAEL (mg/m^3)	-
If no NOAEL, then value for LOAEL	20
$C_{alv;max}$ (mg/m^3)	0.96
Source of $C_{alv;max}$	from average levels present in mainstream smoke
MOE	***21***

Considering the MOE and the factors to be accounted for, it is concluded that risks of effects on the respiratory tract epithelium from 2-furfural cannot be excluded.

It is recognized that several assumptions have been made and that the risk assessment can be further refined reconsidering these assumptions.

9.4.4 *Step 3b: Risk assessment for systemic effects*

The MOE for changes for systemic inhalation toxicity was estimated to being 19,167 for a person smoking a maximum of 40 cigarettes per day (Table 10). Factors to be taken into account for evaluation of this MOE included less-than-lifetime exposure, interspecies extrapolation (rat to humans), and interindividual variability.

Table 10 Summary of the study used as a PoD, the Calv;max from measurements in cigarette smoke and the MOE analysis

Description	**Selected study**
Critical endpoint	Systemic inhalation toxicity
Study	http://esis.jrc.ec.europa.eu/doc/existing-chemicals/risk_assessment/SUMMARY/2furaldehydesum050.pdf (Arts et al., 2004)
Species	Fischer 344 rats
Exposure regimen	3 h or 6 h per day, 5 days/week
Concentrations tested (mg/m^3)	0, 160, 320, 640, 1280 (3 h per day) 0, 20, 40, 80, 160, 320, 640, 1280 (6 h per day)
Duration of exposure	4 weeks
NOAEL (mg/kg)	92
If no NOAEL, then value for LOAEL	-
D_{abs} (mg/kg, per 40 cigarettes/day)	0.0048 (lower bound of range)
Source of D_{abs}	from average levels present in mainstream smoke
MOE	***19167***

Considering the MOE and the factors to be accounted for, it is concluded that the risk of systemic inhalation toxicity due to 2-furfural can be excluded.

10 Risk assessment for glycerol

10.1 Amount of glycerol added to cigarettes

Glycerol is contained in casing materials, cigarette paper and the tobacco itself. Therefore, the amount of glycerol present in the final product depends on the materials used in the manufacturing process and varies greatly per brand. The amount present is relatively high in comparison to other constituents of cigarettes. In Scandinavia, the total amount of glycerol present in tobacco was reported to be 4.5% (w/w), which corresponds to 31.5 mg based on a cigarette containing 700 mg tobacco (DCS, 2007a). In the Netherlands, the average amount added as reported by the manufacturers is 1.0% (w/w) tobacco, with a maximum of 4.4% (w/w), corresponding to an average of 7.1 mg and a maximum of 30.8 mg per cigarette based on the same weight of tobacco in a cigarette (analysis of data delivered to Dutch regulators in 2010 via the Electronic Model Tobacco Control (EMTOC, 2010)). The transfer rate of glycerol to mainstream smoke in filtered cigarettes has been reported to be 12% (Paschke, Scherer, and Heller, 2002). Thus, the estimated levels in mainstream smoke assuming a 12% transfer rate were 3.8 mg from average levels in Scandinavia, 0.9 mg from average levels in The Netherlands and 3.7 mg from maximum levels in The Netherlands. These values were used for the exposure assessment.

10.2 Pyrolysis and reaction products in cigarette smoke

It has been reported that almost all glycerol present in tobacco is transferred to the pyrolysate in its pure form (Baker and Bishop, 2004). Acrolein is a toxic pyrolysis product of glycerol, which is highly reactive and causes irritation in the respiratory tract. The relationship between added glycerol and acrolein formation is unclear and warrants investigation (Carmines and Gaworski, 2005).

10.3 Non-carcinogenic effects: Local respiratory effects and systemic effects

The addition of glycerol may result in increases in the total amount of acrolein present in cigarette smoke. This is of concern because acrolein is toxic and can induce mild to moderate irritation in the respiratory tract (refer to Chapter 6 regarding the risk assessment of acrolein). The main effects reported following glycerol exposure were local irritant effects to the upper respiratory tract observed when rats were exposed to 662 mg/m^3, 6 hours per day, 5 days per week for 13 weeks, with no toxic effects observed at 165 mg/m^3 (Renne, 1992). No systemic effects were reported in this study or in a study with rats exposed to concentrations of 1000, 1930 and 3910 mg/m^3, 6 hours per day, 5 days per week for 14 days (Renne, 1992).

10.4 Risk assessment of local respiratory effects and systemic effects

10.4.1 Step 1: Exposure assessment

The amount of glycerol in mainstream smoke was reported to be 3.8 mg in Scandinavia and on average 0.9 mg in the Netherlands with a maximum of 3.7 mg. These values were used as estimates for D_{cig} for the exposure assessment. The maximum alveolar concentrations ($C_{alv;max}$) calculated from these values are 302 mg/m^3 in Scandinavia and on average 69 mg/m^3 with a maximum of 296 mg/m^3 in the Netherlands.

10.4.2 *Step 2: Point of Departure*

The NOAEL of 165 mg/m^3 in a 13-week rat was the best PoD (the most relevant study/ies that most closely resemble the smoking exposure scenario in study design and duration) value for further risk assessment (Renne, 1992). Please refer to Table 11 for MOE calculation.

10.4.3 *Step 3a: Risk for local effects*

The MOE for respiratory tract irritation based on average glycerol values was 0.5 and 2.3, and 0.6 when based on Dutch maximum glycerol values (Table 11). Factors to be taken into account for evaluation of this MOE included less-than-lifetime exposure, interspecies extrapolation (rat to humans), and interindividual variability.

Considering the MOE and the factors to be accounted for, it is concluded that the risk of effects on the respiratory tract epithelium due to glycerol exists.

It is recognized that several assumptions have been made and that the risk assessment can be refined reconsidering these assumptions. Although such a refinement is beyond the scope of the present analysis, considering the low MOE, it remains to be seen if further refinement will alter the conclusion.

Table 11 Summary of the study used as a PoD, the Calv;max from measurements in cigarette and the MOE analysis

Description	**Selected study**
Critical endpoint	Local irritant effects to the upper respiratory tract
Study	(Renne, 1992) http://www.inchem.org/documents/sids/sids/56815.pdf
Species	Rat
Exposure regimen	6 h per day, 5 days/week
Concentrations tested (mg/m^3)	0, 33, 165, 662
Duration of exposure	13 weeks
NOAEL (mg/m^3)	165
If no NOAEL, then value for LOAEL	-
$C_{alv;max}$ (mg/m^3)	304 (Scandinavian average)
Source of $C_{alv;max}$ (mg/m^3)	from average levels present in mainstream smoke
$MOE_{Scandinavian\ average}$	***0.5***
$C_{alv;max}$ (mg/m^3)	72 (Dutch average)
Source of $C_{alv;max}$ (mg/m^3)	from average levels present in mainstream smoke
$MOE_{Dutch\ average}$	***2***
$C_{alv;max}$ (mg/m^3)	296 (Dutch maximum)
Source of $C_{alv;max}$ (mg/m^3)	from maximum levels present in mainstream smoke
$MOE_{Dutch\ maximum}$	***0.6***

10.4.4 Step 3b: Risk for systemic effects

No thorough assessment on systemic effects was made.

11 Risk assessment for propylene glycol

11.1 Amount of propylene glycol added to cigarettes

The typical amount reported in European cigarettes is 0.2-2.4% (w/w) tobacco, corresponding with 1.4 to 14 mg considering a cigarette with 700 mg tobacco (DCS, 2007b). In the Netherlands, the average amount is reported to be 1.3% (w/w) cigarette, with a maximum of 5% (analysis of data delivered to Dutch regulators in 2010 via the Electronic Model Tobacco Control (EMTOC, 2010)). Less than 10% of the manufacturers report an amount exceeding 2.0% (w/w) cigarette. Dutch manufacturers report that propylene glycol is added to the filter material as well as tobacco. Assuming a 9.9% transfer rate of propylene glycol to mainstream smoke in cigarettes (Paschke, Scherer, and Heller, 2002), the estimated levels in mainstream smoke were 0.14 to 1.4 mg from average levels in Europe (1.4-14 mg), 0.91 mg from average levels in The Netherlands (9.1 mg) and 3.5 mg from maximum levels in The Netherlands (35). These values were used for the exposure assessment.

11.2 Pyrolysis and reaction products in cigarette smoke

Propylene glycol gives rise to propylene oxide at levels ranging from 12-100 ng in the smoke of U.S. cigarettes smoked on a smoking machine (Hoffmann, Hoffmann, and El-Bayoumy, 2001). Additionally, pyrolysis of propylene glycol results in formation of small amounts (<10%) of 1,3-propylene glycol, acetol or acetic anhydride, and pyruvaldehyde (Baker and Bishop, 2004).

11.3 Non-carcinogenic effects: Local respiratory effects and systemic effects

The main effects reported following propylene glycol exposure were an increased number of goblet cells in the respiratory tract and nasal hemorrhaging observed when rats were exposed to 160 mg/m^3 (the lowest concentration tested), 6 hours per day, 5 days per week for 13 weeks (Suber et al., 1989). Effects such as nasal burning, stinging and throat irritation were attributed to exposure to propylene glycol as part of a pharmaceutical formulation inhaled 2 times a week by patients suffering from allergic rhinitis for 1-4 weeks. However, these effects were significantly less following a change in the content of propylene glycol in the formulation from 20% to 5% (The Health Council of the Netherlands (2007)). Minor systemic effects were observed only in female rats which included body weight reduction and changes in leukocyte profile. These systemic effects on body weight and leukocyte profile have not been found consistently in other studies indicating that gender differences in susceptibility to propylene glycol's adverse effects in the rat, but other studies do not provide additional evidence for this. Short-term exposure levels to amounts of propylene glycol in artificial mist cause eye and throat irritation in healthy human subjects (The Health Council of the Netherlands (2007)).

11.4 Risk assessment of local respiratory effects and systemic effects

11.4.1 Step 1: Exposure assessment

The estimated levels in mainstream smoke were 0.14 to 1.4 mg from average levels in Europe, 0.91 mg from average levels in The Netherlands and 3.5 mg from maximum levels in The Netherlands. These values are used as estimates for D_{cig} for the exposure assessment. The maximum alveolar concentration ($C_{alv;max}$) calculated from these values are 11.2-112 mg/m^3 from

the average European levels, 72.8 mg/m^3 from average amounts in The Netherlands and 280 mg/m^3 from estimated maximal levels in The Netherlands.

11.4.2 Step 2: Point of Departure

The LOAEL of 160 mg/m^3 from a 13-week rat study (Suber et al., 1989) was the best PoD (the most relevant study/ies that most closely resemble the smoking exposure scenario in study design and duration) value for further risk assessment. Please refer to Table 12 for MOE calculation.

11.4.3 Step 3a: Risk on local effects

The MOE for respiratory tract irritation was 1.4 to 14 from a range of levels present in mainstream smoke in Europe, 2.2 from average levels in The Netherlands and 0.6 from maximum levels in The Netherlands (Table 12). Factors to be taken into account for evaluation of this MOE included LOAEL as PoD instead of NOAEL, less-than-lifetime exposure, interspecies extrapolation (rat to humans), and interindividual variability.

Considering the MOE and the factors to be accounted for, it is concluded that a risk of effects on the respiratory tract epithelium due to propylene glycol exists.

It is recognized that several assumptions have been made and that the risk assessment can be refined reconsidering these assumptions. Although such a refinement is beyond the scope of the present analysis, considering the low MOE, it remains to be seen if further refinement will alter the conclusion.

11.4.4 Step 3b: Risk on systemic effects

No thorough assessment on systemic effects was made.

Table 12 Summary of the study used as a PoD, the Calv;max from measurements in cigarette and the MOE analysis

Description	**Selected study**
Critical endpoint	Increased number of goblet cells in the respiratory tract and nasal hemorrhaging
Study	(Suber et al., 1989) http://www.atsdr.cdc.gov/toxprofiles/tp189.pdf
Species	Rat
Exposure regimen	6 h per day, 5 days/week
Concentrations tested (mg/m^3)	0, 160, 1000, 2200
Duration of exposure	13 weeks
NOAEL (mg/m^3)	-
If no NOAEL, then value for LOAEL	160
$C_{alv;max}$ (mg/m^3)	11.2 (lower bound of range Europe)
Source of $C_{alv;max}$ (mg/m^3)	from lower range levels present in mainstream smoke
$MOE_{lower\ bound\ Euorpe}$	***14***
$C_{alv;max}$ (mg/m^3)	112 (upper bound of range Europe)
Source of $C_{alv;max}$ (mg/m^3)	from upper range levels present in mainstream smoke
$MOE_{upper\ bound\ Europe}$	***1***
$C_{alv;max}$ (mg/m^3)	72.8 (average levels in The Netherlands)
Source of $C_{alv;max}$ (mg/m^3)	from average levels present in mainstream smoke
$MOE_{average\ levels\ Netherlands}$	***2***
$C_{alv;max}$ (mg/m^3)	280 (maximum levels in The Netherlands)
Source of $C_{alv;max}$ (mg/m^3)	from maximum levels present in mainstream smoke
$MOE_{maximum\ levels\ Netherlands}$	***0.6***

12 Summary of results and conclusions

The risk assessment for local respiratory effects is summarized in Table 13. From this summary it is clear that a risk for local respiratory effects exists for acetaldehyde, acrolein, formaldehyde, glycerol and propylene glycol, and a risk cannot be excluded for ammonia and 2-furfural. As to systemic effects, no toxicological data were available or risks could be excluded.

Table 13 Summary of the risk assessment of local respiratory effects

Compound	**$MOE_{average\ levels}$**	**$MOE_{maximum\ levels}$**	**Assessment**
Formaldehyde	0.06	-	Risk exist
Acrolein	0.09	-	Risk exist
Glycerol	0.5-2.3	0.6	Risk exist
Propylene glycol	1.4-14	0.6	Risk exist
Acetaldehyde	2.4-12	1.6-8	Risk exist
Ammonia	14	-	Risk cannot be excluded
2-Furfural	21	-	Risk cannot be excluded
	Increased risk		

13 Future research

This method has demonstrated to be very useful in prioritizing the health effects of smoke components (*e.g.* generated from the combustion of additives and/or tobacco) and this assessment can be expanded to for instance smoke components that are mandated for lowering by the WHO Tob Reg proposal which include: 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), *N'*-nitrosonornicotine (NNN), benzene, benzo[a]pyrene, 1,3-butadiene and carbon monoxide. In addition, the current risk assessment focused only on local respiratory and systemic effects and future analysis can be extended to include multiple endpoints to provide an overall risk of the smoke component to be used to make further prioritization decisions. The current assessment relied on NOAEL or LOAELs and future analysis should incorporate proper dose-response assessments using approaches such as the benchmark dose approach (Hernandez *et al.*, 2011). The method would also benefit from toxicity data obtained with animal studies with exposure designs that more closely resemble the smoking scenario. This method is useful not only for prioritization of smoke components but also for monitoring the shift in the MOE ratio as levels of smoke components are reduced with new tobacco regulations that may come into place in the future. The integration of this method to a 'strategy for toxicity evaluations of tobacco additives and their regulation' (DKFZ, 2012) may be a good starting point for the reduction of harmful smoke components in cigarette smoke.

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