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Review

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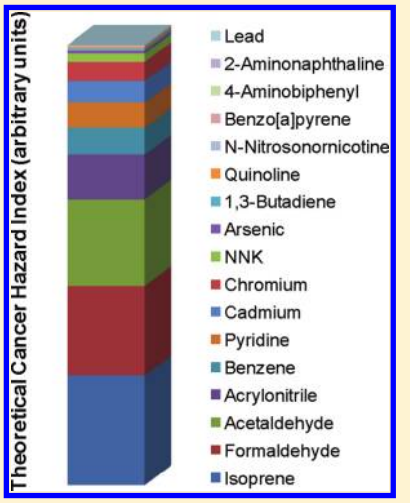
Use of Hazard Indices for a Theoretical Evaluation of Cigarette Smoke Composition

Hans-Juergen Haussmann*

Toxicology Consultant, Ueberhoefer Feld 36a, 51503 Roesrath, Germany

ABSTRACT: The chemical composition of cigarette mainstream smoke (MS) has been quantitatively analyzed in multiple studies, often with the objective to toxicologically evaluate and compare various types of MS. Increases and decreases in yields of constituents between MS types can only be consolidated if these yields are compared on the basis of toxicological properties of the individual constituents. For the risk assessment of various complex mixtures including MS, a hazard index (HI) approach has been used that requires weighing of the exposure to individual MS constituents by cancer and noncancer potency values. The objective of the current study is to review the past uses of the HI concept for MS and smokeless tobacco and discuss strengths and limitations of using this concept. Published information as well as information made available on the Web was used. The HI concept has been applied to MS for determining and comparing theoretical lifetime risks, for consumer communication, for the prioritization of constituents for reduction, for ingredient assessment, and for the selection of constituents for regulation. The limitations of this approach are associated with the limited number of MS constituents with available yield data, the gaps and uncertainties in available potency values, the application to relatively high exposure concentrations, and the default assumption of additivity. The derived theoretical noncancer index is dominated by acrolein to an extent that there seems to be not much advantage in using the HI concept for noncancer assessments. The derived theoretical cancer index is dominated by genotoxic carcinogens of the MS vapor phase and may thus complement currently used toxicological assays in a tiered evaluation approach. As is the case for every other assay and interpretation model, the HI concept needs to be applied with its limitations and weaknesses in mind. Its best application is for comparative purposes. It should be kept in mind that the HI concept is a theoretical concept and does not provide actual risk information.



CONTENTS

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1. INTRODUCTION

Cigarette smoking can be the cause of various serious diseases, such as cancer, chronic obstructive pulmonary disease (COPD), or cardiovascular disease (CVD).[1] Cigarette smoke is a complex mixture of constituents, and the disease pathologies induced by this mixture are complex, too. While various parameters of the pathogenesis of these diseases have been established, such as the relationship of the disease risk to smoking duration and dose or the decreasing disease risk after smoking cessation, the actual mode of action and the etiology of these disease pathologies are less well understood.[2] Improved knowledge of the role of the various smoke constituents in the pathogenesis of smoking-related diseases could inform product development, in particular for novel products with the potential for reduced risk, product evaluation, e.g., for the toxicological assessment of ingredients or manufacturing processes, product regulation, and consumer communication.

Chemical−analytical characterizations of cigarette mainstream smoke (MS) have been performed in many studies and for various objectives by corporate, academic, and governmental researchers, e.g., for market benchmarking,[3−7] the evaluation of ingredients and manufacturing processes,[8−10] or the comparative evaluation of novel tobacco products or technologies.[11−15] In addition, chemical−analytical data of the smoke of marketed brands are requested by various regulatory bodies,[16] and further regulatory requirements are shaping.[17−20] Available MS composition data related to the evaluation of cigarette ingredients are required for submission to the European Union.[21] In most cases, from the MS of a given cigarette type several dozens of constituents are being analyzed, and the selection of these has not solely been based on toxicological reasoning. In turn, quite often, no toxicological assessment of MS chemistry data sets has been performed due to the lack of generally acceptable approaches.

The assessment of the disease risk of aggregate and cumulative exposures has been a challenge beyond the subject of cigarette smoking, e.g., for occupational or environmental exposures. Besides a relatively early guidance document with sustained impact,[22] more recent documents have provided more clear definitions and approaches for the risk assessment of complex exposures.[23−26] Aggregate exposure is defined by combined exposure to one chemical, which can be from multiple sources or pathways (single compound, all routes). Cumulative risk assessment considers the combined health risks from multiple route exposures to multiple agents or stressors, including the possibility of aggregate exposures to individual agents. Risk assessments can be performed for the individual constituents but need to be combined for the cumulative assessment. In all applicable guidance documents, the standardized default way of assessing a mixture based on its constituents is by applying the hazard index (HI) concept. The prevalence of each individual constituent in a mixture is weighed by the potency of this constituent for a given toxicological hazard to obtain the so-called hazard quotients (HQ), and the individual HQs are combined to the mixture hazard index or HI. This combination by default is additive, but interactions between constituents, whether independent, additive, synergistic, or antagonistic, need to be considered. The resulting mixture HI can be related to acceptable risk values, such as a *de minimis* lifetime cancer risk, chronic reference exposure levels (RELs), or occupational threshold limit values, to determine whether risk management actions are required. The mixture HI can also be compared to that of similar mixtures, e.g., for prioritizing risk management actions. The HI for a mixture of i constituents (only one source and one route of exposure) is calculated as follows:

$$\text{Hazard Index (HI)} = \sum_i HQ_i = \sum_i (\text{Prevalence}_i \times \text{Potency}_i)$$

Prevalence data can be concentrations or doses of constituents i. Potency values can be cancer unit risks or reference exposure levels (as inverse potency values), depending on the actual hazard under investigation. HIs can be derived for various hazards or even more detailed toxicological activities of the same mixture. Incremental lifetime cancer risks (ILCR) may be calculated using this approach, if more detailed exposure data, such as daily and lifetime duration of exposure, are available. However, for comparative purposes, more simple approaches by just weighing the exposure concentration with the cancer potency value can be used, if other exposure variables are similar or the same for the exposure scenarios to be compared. RELs are defined as a safe level; therefore, for an individual constituent i, the respective HQ should not exceed 1 for the exposure scenario to be considered safe. The same logic is applied to the combined exposure to several constituents i: As long as the combined HI does not exceed 1, no significant chronic noncancer risk can be expected to result from the exposure to the respective mixture.

In the current review, the past uses of the HI concept for cigarette smoke and smokeless tobacco are reviewed and provide the basis of a discussion of strengths and limitations of using this concept and for a recommendation for selectively applying it to MS. For this purpose, published information as well as information made available on the Web was comprehensively used. In the particular case of cigarette smoke and different from the applications to most other complex exposures, the calculated theoretical lifetime risks can also be compared to epidemiologically observed risks. Nevertheless, it is emphasized that the application of the HI concept to smoke will always result in theoretical estimates of risks rather than any representation of true risks. To acknowledge this, this review will use the terms theoretical cancer hazard index (TCHI) and theoretical noncancer hazard index (TNHI).

2. SELECTION OF SMOKE CONSTITUENTS FOR ANALYSIS AND REGULATION

More than 5000 constituents have been identified in cigarette smoke.[27] It is not possible to routinely analyze all of these. Therefore, selections were made by various researchers and organizations that mostly included approximately 30 to 50 constituents with the intention to cover the potentially most relevant constituents for smoking-related disease risk. There is considerable overlap between these selections.[16] Recently, expanded selections of approximately 100 constituents were suggested for the regulatory evaluation of MS,[18,19] while the

dx.doi.org/10.1021/tx200536w | Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

WHO Study Group on Tobacco Product Regulation (TobReg) settled for less and suggested regulating and monitoring only nine MS constituents, respectively.[17,28]

Only few of these selections for routine assessment of cigarette smoke used risk assessment-based methods. In a relatively early study, MS constituents associated with a significant theoretical risk for cancer and noncancer effects were identified and ranked by calculating the respective HQs.[29] A significant health risk was defined if acceptable levels for the lifetime cancer (1×10^{-6}) or noncancer risk (HI = 1) would be exceeded by the uptake of the individual constituents from smoking a certain number of cigarettes per day. The theoretical cancer and noncancer HQs (TCHQs and TNHQs, respectively) for these constituents were assessed for a smoker of three packs of cigarettes per day by using cancer unit risks and chronic reference concentrations as potency values. Because of the limitations in available MS yield data and respective potency values, TCHQs and TNHQs could only be produced for 25 and 13 constituents, respectively (Table 1). Acetaldehyde was determined to contribute both to the theoretical cancer and noncancer risks from smoking, with TCHQs and TNHQs exceeding the significant risk levels for cancer and noncancer risks by approximately 4000- and 500-fold, respectively. The TCHQs were dominated by 1,3-butadiene, which was estimated to contribute 2.5-fold more than the next major constituent, i.e., acetaldehyde. The theoretical noncancer risk was dominated by the TNHQ of acrolein, which contributed 40 times as much as the next highest constituent, i.e., hydrogen cyanide. The list of constituents identified in this study was suggested to be analyzed for the risk assessment of cigarette ingredients. This assessment was updated for the Massachusetts Tobacco Control Program with regard to MS constituent yield databases and potency values two years later[30] with results similar to those in the previous version.

Table 1. Selection of Mainstream Smoke Constituents with Significant Theoretical Cancer and Noncancer Hazard Quotients Identified Using a Risk Assessment Framework and Suggested for Use in Ingredient Analyses[a]

constituent	ILCR	TNHQ
acetaldehyde	4×10^{-3}	467
acetonitrile		9
acrolein		21,000
acrylonitrile	1×10^{-3}	23
4-aminobiphenyl	4×10^{-5}	
ammonia		4
arsenic	7×10^{-4}	
benzene	5×10^{-4}	2
benz[*a*]anthracene	1×10^{-5}	
benzo[*a*]pyrene	5×10^{-5}	
benzo[*b*]fluoranthene	3×10^{-6}	
benzo[*j*]fluoranthene	3×10^{-6}	
benzo[*k*]fluoranthene	2×10^{-6}	
1,3-butadiene	1×10^{-2}	
cadmium	1×10^{-4}	
chromium (hexavalent)	1×10^{-3}	105
dibenz[*a,h*]anthracene	6×10^{-6}	
dibenz[*a,i*]anthracene	5×10^{-5}	
formaldehyde	2×10^{-3}	83
hydrazine	3×10^{-4}	
hydrogen cyanide		500
hydrogen sulfide		270
hydroquinone		252
indeno[1,2,3-*cd*]pyrene	3×10^{-6}	
nickel	2×10^{-4}	8
N-nitrosodiethanolamine	4×10^{-5}	
N-nitrosodimethylamine	3×10^{-3}	
N′-nitrosonornicotine	2×10^{-3}	
N-nitrosopyrrolidine	9×10^{-5}	
phenol		11
o-toluidine	1×10^{-5}	
vinyl chloride	2×10^{-6}	

[a]Ref 29; significant risk thresholds for cancer and noncancer effects were set to 10^{-6} and 1, respectively.

The WHO TobReg group calculated TCHQs and TNHQs for a series of constituents, which they called toxicant animal carcinogenicity and toxicant noncancer response index, respectively.[17,28] These indices provided one of several arguments for the selection of the constituents recommended for regulation. For the calculation of TCHQs, this group did not use potency values issued by an authoritative body, such as the California Environmental Protection Agency (CalEPA) (available at http://www.oehha.ca.gov/air/hot_spots/index.html) or the United States Environmental Protection Agency (USEPA) (available at http://www.epa.gov/iris/), but used the daily dose T25 eliciting a tumor incidence of 25% above the control in animal bioassays (mostly oral exposures, collected in the Cancer Potency Database (CPDB)[31]) obtained by linear extra- or interpolation (according to a method described elsewhere[32]). For noncancer potency values, they used CalEPA RELs. MS constituent yields were taken from databases covering a selection of international cigarette brands of mixed types.[17] TobReg decided to use constituent yields obtained under Health Canada intense (HCI) smoking conditions.[17,28] Constituent yields were normalized to a nicotine basis in order to shift emphasis from the quantity of MS generated per cigarette toward product characterization by the quality of MS. TobReg recognized that for comparative purposes, a machine-smoking standard is required, although this may not represent actual human smoking topography. Using this data set, HQ constituent rankings were obtained, which were quite similar to those discussed above. Exceptions were isoprene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which were not included in some other evaluations of this kind due to the lack of appropriate cancer potency values but received relatively high TCHQs in the TobReg study.

TobReg did not rely solely on the HI-based concept of selecting MS constituents for their final list of constituents recommended for regulation.[28] Other arguments used were

- the known animal and human toxicity of the constituents, e.g., based on classifications by the International Agency for the Research on Cancer;
- a large variation in constituent yields across brands, which would suggest there are means of reducing these yields toward the lower levels of the distribution;
- the potential availability of technologies to reduce specific constituents;
- the need to include constituents representing both the particulate and the vapor phase of the smoke aerosol;
- the need to include different chemical classes of constituents; and

dx.doi.org/10.1021/tx200536w | Chem. Res. Toxicol. XXXX, XXX, XXX–XXX

Table 2. Selection Criteria for Regulation of Smoke Constituents by the World Health Organization Study Group on Tobacco Product Regulation[a]

constituent	TCHQ (AU)	TNHQ (AU)	reasoning for regulation	criteria for determining a ceiling level based on market benchmark data
Recommended for Mandated Lowering				
NNK	3.4		TCHQ	median value of the data set
N′-nitrosonornicotine	0.29		TCHQ	median value of the data set
acetaldehyde	6.1	67.1	TCHQ and TNHQ	125% of the median value of the data set
acrolein		1099	TNHQ	125% of the median value of the data set
benzene	2.6	0.64	TCHQ	125% of the median value of the data set
benzo[*a*]pyrene	0.0086		TCHQ	125% of the median value of the data set
1,3-butadiene	9.9	2.4	TCHQ and TNHQ, human carcinogen	125% of the median value of the data set
carbon monoxide		1.3	TNHQ, mechanistically related to CVD	125% of the median value of the data set
formaldehyde		19.8	TNHQ	125% of the median value of the data set
Recommended for Disclosure and Monitoring				
acrylonitrile	1.4	2.1	TCHQ and TNHQ	
4-aminobiphenyl			human carcinogen	
2-aminonaphthalene	0.00068		TCHQ	
cadmium	1.7	2.6	TCHQ and TNHQ	
catechol	0.58		TCHQ	
crotonaldehyde			aldehyde with reactive structure	
hydrogen cyanide		17.2	TNHQ	
hydroquinone	1.2		TCHQ	
nitrogen oxides		3.1	TNHQ	

[a]Ref 17. AU: arbitrary unit. Note that isoprene was not considered in spite of a TCHQ of 3.7.

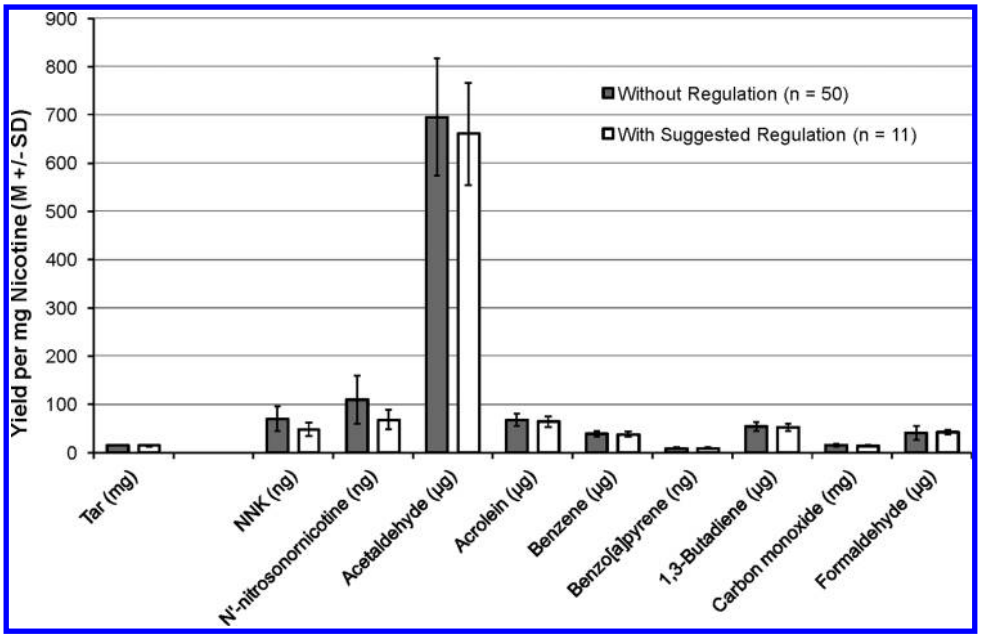

Figure 1. Yields (per mg nicotine) for tar and the nine mainstream smoke constituents recommended for mandated lowering generated at Health Canada intense machine-smoking conditions for a data set of international cigarette brands[3] without and with concurrent application of the suggested regulatory ceiling levels.[28]

- the need to include constituents that would reflect not only cancer as a disease end point but also respiratory and cardiovascular disease end points.

Last but not least, TobReg intended to recommend a relatively short list of constituents in order to make regulation feasible for both manufacturers and the public health community.[28] TobReg recommended the mandated lowering of a first group of nine constituents as well as the monitoring and disclosure of a second group of nine constituents (Table 2). The group was aware of the possibility that while one regulated constituent might be lowered by a certain technology, other constituents, either regulated or not, may in fact increase in yield due to the application of this technology. In order to deal with such unintended consequences, TobReg recommended using the sum of the TCHQs, i.e., the TCHI, of all constituents included in the current evaluation applied to all brands remaining on the market undergoing the regulation as a measure for the effectiveness of the regulation.

TobReg also recognized that the HI-based concept would best be used in a comparative way between constituents or between brands in a given market and that it would not be

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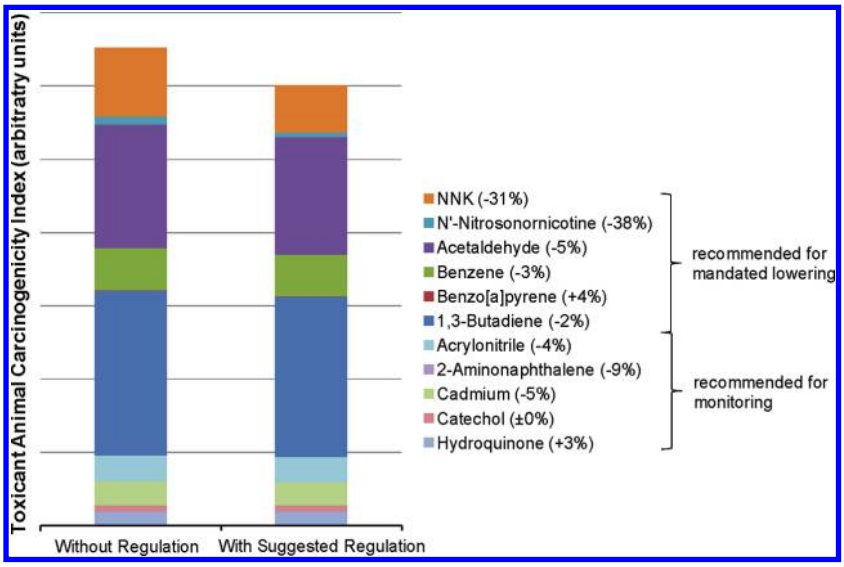

Figure 2. Application of the cancer hazard index concept (toxicant animal carcinogenicity index) to the 18 mainstream smoke constituents recommended for mandated lowering and for monitoring without and with concurrent application of the suggested regulatory ceiling levels[28] for a data set of international cigarette brands[3] (potency values as used in ref 28). Values in parentheses give percent changes in the constituent's toxicant animal carcinogenicity index.

appropriate to derive quantitative estimates about the actual human cardiovascular and respiratory toxicity or carcinogenicity from smoking.[28] Likewise, as TobReg considered the use of HI-based evaluations appropriate for precautionary means, the justification of a product health claim based on success in reducing the theoretical HIs was not considered appropriate. Overall, it seems that TobReg used the HI-based evaluation of cigarette MS in a quite careful manner by recognizing some of its limitations. On the contrary, it seems that the setting of the rules for deriving mandated ceiling levels (Table 2) was less deliberate: in the absence of further product modifications, the concurrent application of these rules to the nine constituents of one of the data sets used for deriving TCHQs and TNHQs, which is meanwhile approximately ten years old,[3] would result in banning 39 of the 50 brands of this data set from the market (data were provided by Japan Tobacco International, Geneva, Switzerland), as these ceiling levels would hit the various brands differentially. The number of brands in this data set that would be banned based on NNK, *N*′-nitrosonornicotine (NNN), acetaldehyde, acrolein, benzene, benzo[*a*]pyrene, 1,3-butadiene, carbon monoxide, and formaldehyde would be 25, 25, 5, 7, 4, 5, 4, 4, and 11, respectively. As an example, exclusion of one brand because of one constituent that would exceed the recommended ceiling level would result in missing the yields of the other eight constituents that may have been below their respective ceiling levels and thus eventually increase rather than decrease the average yield for these constituents by the remaining brands. Another brand would be excluded because of another constituent, with the described effect *vice versa*. Often, brands are excluded because of more than one constituent exceeding the recommended ceiling levels, though, and mostly but not always, the two tobacco-specific *N*-nitrosamines (TSNAs) go in parallel. As a result of these differential effects, though, the recommended regulation would fail to significantly reduce mean yields of all but the TSNAs in the remaining compared to the total data set (Figure 1). Average TSNA yields would be reduced because of the relatively more stringent definition of the ceiling levels compared to the other constituents (Table 2). Moreover, if the TCHQs for the 18 constituents recommended for regulation are calculated, according to the suggestions of TobReg for checking for potential unintended consequences of regulation,[28] there is little reduction of the TCHI, i.e., the sum of the TCHQs, for the data set representing the cigarettes that would remain on the market after the suggested regulation compared to the total set of cigarettes without regulation (Figure 2). The 8% reduction obtained for this theoretical risk estimate is driven by the reduction of NNK yields but does not seem to be significant in view of relative standard deviations of approximately 30% for the range of NNK yields observed in this study.[3] The application of the recommended regulation to other published benchmark data sets, e.g., from the UK[5] or the 2004 Canadian data set published by TobReg,[17] results in similar percentages of banned products. Thus, careful assessment and implementation of any regulations seem to be warranted, and the use of a HI-based approach for the control of unintended consequences as suggested by TogReg[28] might be helpful.

Most recently, a comprehensive analysis of the published literature was performed to establish a database of MS constituents and their yields, if available.[19] The constituents in this database were associated with potency values for cancer and noncancer effects as much as available. The potency values were taken from the International Toxicity Estimates for Risk Assessment (TERA) (available at http://www.tera.org/ITER/index.html) database, which provides a synopsis of potency values established by various authoritative bodies. Potency values were available for 98 MS constituents of this database, including 60 for cancer and 48 for noncancer effects. All constituents with available potency value were selected and proposed for regulatory purposes and to direct activities toward the progressive reduction in the yields of toxic MS constituents. No weighing of constituent yields by the collected potency factors was performed that could have been used for prioritization within this list. No recommendation was given as to whether the associated potency values could also be used for the assessment of MS types.

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3. IDENTIFICATION OF POTENTIAL RISK REDUCTION TARGETS

The HI concept has also been used to identify MS constituents as possible targets for reduction in the context of tobacco harm reduction. The earliest study of this type was conducted on behalf of the New Zealand Ministry of Health.[33] It used chemical analytical data of 95 constituents, which were obtained from various sources, and potency values from USEPA and CalEPA. TCHQs for 39 constituents could be calculated. The highest ranking constituents were 1,3-butadiene, chlorinated dioxins, acrylonitrile, arsenic, acetaldehyde, and benzene. It was recognized that polycyclic aromatic hydrocarbons ranked relatively low compared to the public perception of their role in smoking-related carcinogenesis. In a later publication by the same main author, chlorinated dioxins and furans were excluded from the assessment because a threshold would be expected for their carcinogenicity due to their receptor-mediated mode of action.[34] A later analytical study confirmed that the uptake of dioxin equivalents from smoking would indeed not exceed the Tolerated Daily Intake set by the World Health Organization.[35]

TNHQs were calculated in the New Zealand study separately for different disease areas, depending on the end point determining the setting of the chronic REL.[33] If a given compound elicited effects for several noncancer end points, RELs derived for the lowest observed adverse effect level were used. For CVD, hydrogen cyanide and arsenic ranked highest with HQs exceeding 1. For respiratory disease, acrolein outweighed all other constituents, but acetaldehyde still was calculated to pose a significant risk. A third TNHI was developed for reproductive and developmental effects, which was led by arsenic as the sole constituent with significant HQ. This latter end point was omitted from the follow-up publication with no reason given.[34]

The stated purpose of this follow-up publication was to provide a plausible and objective framework for the hazard prioritization of MS constituents within a strategy of harm reduction.[34] MS chemistry data for 158 constituents were collected from various sources, and cancer and noncancer potency values were available for 40 and 17 of these constituents, respectively. Vapor phase constituents dominated both the TCHI and TNHI.

A very systematic application of the HI approach to cigarette smoke relied on a selection MS chemistry data with priority for the University of Kentucky standard reference research filter cigarette 1R4F as a representative of concurrent American-blend cigarettes, rather than using data from benchmarking studies that suffer from inherent heterogeneity.[36] Only if data were missing for the 1R4F, other sources were used to widen the database to the extent possible, including more historic data on nonfilter cigarettes, finally arriving at 140 constituents entering the HI analysis. Two major types of cancer potency values were compared. First, the tumorigenic dose for 50% tumor incidence in animal bioassays (TD_{50}) was used from CPDB. The ten highest ranking constituents for TCHI were formaldehyde, crotonaldehyde, acetaldehyde, 1,3-butadiene, ethyl carbamate, isoprene, nitrobenzene, NNK, benzene, and catechol. This is a slightly different list from the ones described so far due to the comprehensive sourcing of MS chemistry data and the use of rodent-specific cancer potency values. In a second attempt,[36] the authors used cancer unit risks collected from various sources including CalEPA and USEPA. ILCRs for 52 MS constituents were calculated assuming a certain smoking intensity and duration. Of these, yields for 18 constituents were only available for nonfilter cigarettes, including high ranking constituents, such as ethyl carbamate, 2-nitropropane, and ethylene oxide. It is thus not clear whether these constituents would rank similarly using the MS of filter cigarettes nowadays. NNK, for which no cancer unit risk had been located by the authors, could not be included in this second TCHI analysis. Otherwise, TCHI ranking was similar as in other analyses of this type, i.e., with the highest contribution from 1,3-butadiene and acetaldehyde. As a difference to other analyses, acrylamide and quinoline were also ranking relatively high. For the TNHI, average daily concentrations were calculated based on smoking 20 cigarettes per day and averaging to a daily respiratory volume of 20 m^3.[36] These concentrations were weighed by using mainly USEPA Reference Concentration (RfC) values. The resulting ranking of 24 MS constituents was of course led by far by acrolein, which was followed by acetaldehyde and hydrogen cyanide.

An additional weighing was performed to obtain an HI using Permissible Exposure Levels (PELs) from the US Occupational Safety and Health Administration (OSHA).[36] The PEL list is rather unspecific regarding the categorization for carcinogens and noncarcinogens, while many recognized carcinogens are exempted from the PEL list. For a difference, there are also PEL values for nicotine and carbon monoxide, which have mostly not been considered in the HI-based assessment of MS because of missing chronic potency values in the respective databases used. Sixty constituents were ranked relative to uptakes under PEL conditions. Nicotine ranked highest and was the only constituent for which smoking would yield an uptake beyond that of working under PEL conditions. Next highest ranking constituents were acrolein and carbon monoxide. These were followed by methyl isocyanate and formic acid, two constituents for which only yield data from nonfilter cigarettes were available, similar to 15 others.

In a follow-up of the New Zealand study discussed above, MS constituents were additionally categorized according to their main appearance in the particulate or vapor phase of the MS aerosol.[37] In addition, in order to derive an overall HI per cigarette, the calculated theoretical HIs for cancer, cardiovascular, and respiratory effects were weighed by the relative contribution of the respective disease group (cancer 39%, cardiovascular 26%, and respiratory 25%) to the estimated smoking-related mortality in New Zealand and added up. Of the constituents entering this assessment, the three most significant were acrolein, 1,3-butadiene, and hydrogen cyanide. These three constituents accounted for 65% of the overall theoretical HI identifiable by this approach. The 13 most significant constituents tested accounted for 81% of the TCHI, 99.5% of the theoretical cardiovascular HI, 99.9% of the theoretical respiratory HI, and 83% of the overall theoretical HI. The authors concluded that it is a relatively small number of constituents, which account for most of the theoretically calculated HIs and which would therefore be the main targets for reduction and regulation. Focusing on 13 smoke constituents out of several thousand would make regulation technically feasible.

These authors further concluded that the major risk contribution based on the three indices (80% of the overall theoretical HI) was from vapor phase constituents, which they suggested would call for the use of effective charcoal filters in cigarettes.[38] Charcoal filters used in cigarettes marketed in New

Zealand at the time of this study were reported to be little effective. The authors also investigated a test-marketed cigarette with much more effective charcoal filtration and determined an overall theoretical HI of 4% relative to a British Columbia market benchmark for the respective index (termed the Relative Toxic Emissions score), if smoked under International Organization for Standardization (ISO) conditions (Figure 3).[39] This index was based on nine MS constituents only,

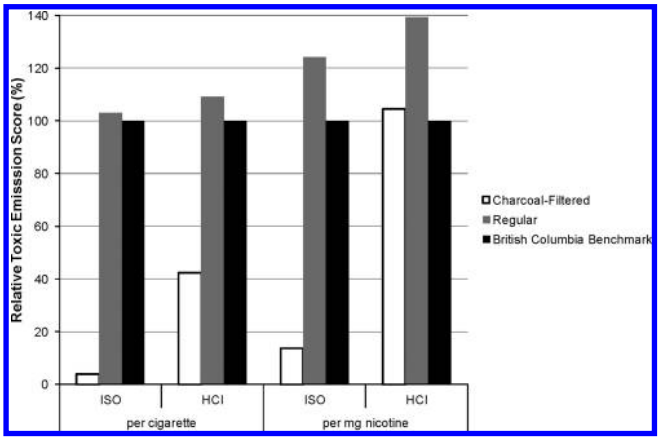

Figure 3. Development of a relative toxic emission score by applying the hazard index concept to the mainstream smoke of a test market cigarette with efficient charcoal filtration and a regular market cigarette, both relative to a Canadian benchmark mainstream smoke composition.[39] Two machine-smoking conditions were used, i.e., International Organization for Standardization (ISO) and Health Canada Intense (HCI).

namely, carbon monoxide, formaldehyde, acetaldehyde, acrolein, hydrogen cyanide, 1,3-butadiene, benzene, acrylonitrile, and the cresols. While the previous applications of the classic HI approach mostly evaluated MS composition on a per cigarette basis, these authors went on and calculated constituent yields and theoretical HIs relative to nicotine yields in an attempt to adjust for differing smoking behaviors. On this basis, the theoretical charcoal test market cigarette HI was still at 10% of the benchmark under ISO conditions. However, if smoked under HCI conditions, the charcoal filtration was less effective leading to theoretical HIs of 42 and 104% of the Canadian benchmark, if calculated on a per cigarette or on nicotine yield basis, respectively.

A probabilistic risk assessment approach was used to prioritize MS constituents based on analytical data from a benchmark study of cigarettes sold in China.[40] ILCRs, HQs for noncancer effects, and Margins of Exposure (MoEs) were calculated. In order to account for uncertainty and variability in exposure parameters, their distribution was incorporated using a Monte Carlo simulation. Cut-off values for determining significant risks were an ILCR of 10^{-4}, a TNHQ of 1, and a MoE of 10^4. Highest ILCRs beyond 10^{-4} were calculated for isoprene, formaldehyde, acetaldehyde, and acrylonitrile (Figure 4). Acrolein led a group of 14 constituents with TNHQs larger than 1 (Figure 5). The lowest MoE was determined for hydrogen cyanide, followed by 16 other constituents with MoE $<10^4$. Acetaldehyde, acrylonitrile, benzene, cadmium, formaldehyde, and pyridine were associated with significant risks in all three categories.

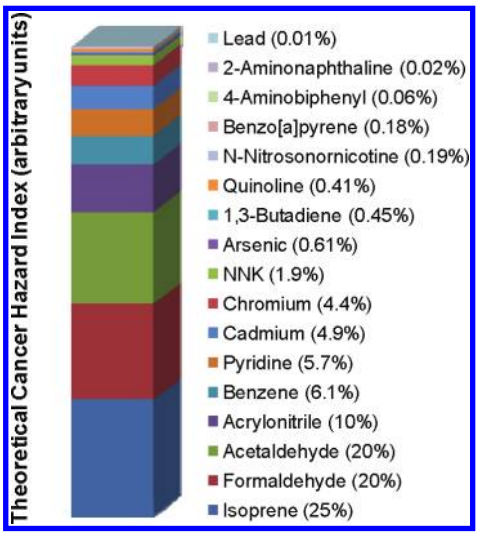

Figure 4. Theoretical cancer hazard index according to the calculated incremental lifetime cancer risk values developed in a Chinese mainstream smoke benchmark study.[40] Percent contributions to the overall index based on available data are notified.

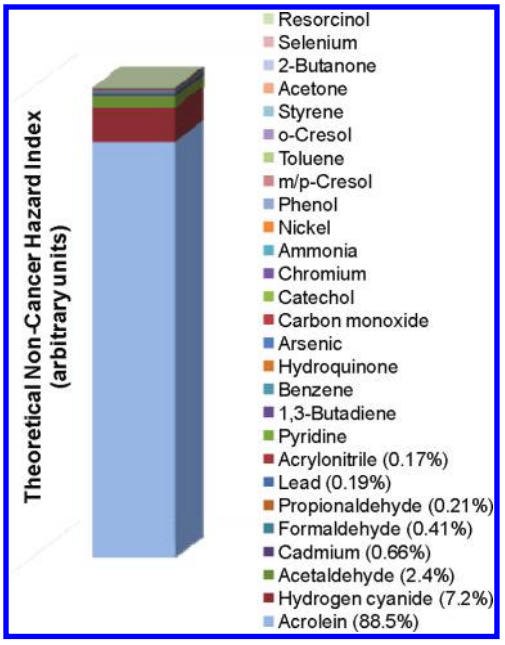

Figure 5. Theoretical noncancer hazard index according to the calculated hazard quotient values developed in a Chinese mainstream smoke benchmark study.[40] Percent contributions greater than 0.1% to the overall index based on available data are notified.

4. COMPARISON OF SMOKING CONDITIONS AND CIGARETTE DESIGNS

Apart from the modulation of charcoal filtration efficacy, the actual impact of varying machine-smoking conditions on MS composition and the related HIs was investigated in another study, which used the reference research cigarettes 1R4F and 1R5F.[41] A total of 34 smoke constituents were available for the assessment of the TCHI, while 16 were available for the TNHI, which is probably the most consistent data set with both MS yield and potency values from single sources each. On the basis of this analysis, 1,3-butadiene, acrylonitrile, benzene, and several aldehydes were identified as major contributors to the HIs for theoretical cancer and noncancer risks and thus as

dx.doi.org/10.1021/tx200536w | Chem. Res. Toxicol. XXXX, XXX, XXX–XXX

targets for reduction in a tobacco harm reduction context. The priorities for potential risk reduction determined by this method were the same when the MS was generated under three different smoking conditions.

MS constituent yields for the research reference cigarettes 1R4F and 1R5F, for a novel cigarette prototype, as well as for eight market brands were determined, and weighed with cancer and noncancer risk potency values obtained from CalEPA.[42,43] In MS from an electrically heated cigarette smoking system, the yields of most constituents were significantly reduced compared to that of the conventional cigarettes, while a few were increased. Application of the HI approach allowed one to integrate across increases and decreases and demonstrated an overall reduction in the TCHI and TNHI for the electrically heated smoking system compared to the conventional cigarettes. The limitations of the approach were recognized, but the use of this approach was considered as a possible supplement to the evidence obtained from experimental toxicological investigations for the overall evaluation of new cigarette designs.

5. CONSUMER COMMUNICATION

An HI-based approach was also suggested for improved consumer communication regarding the composition of cigarette MS. The concentrations of 28 MS constituents were preferably weighed by the 15-min short-term exposure level defined by the American Conference on Governmental and Industrial Hygienists to derive a Relative Exposure Index (REI).[44,45] Of the MS constituents entering this assessment, acrolein was reported to contribute most to the REI (26%), followed by benzo[*a*]pyrene and formaldehyde. Eight constituents contributed 89% of the REI under the conditions of this study. The authors pointed out that different styles of cigarettes could be differentiated by this approach, i.e., American-blend (reference cigarette 1R4F) from commercial Virginia-style cigarettes or a novel tobacco-heating cigarette from the conventional cigarettes. In view of the many inherent limitations of the approach, the authors suggested that further research would be required.

6. INGREDIENT ASSESSMENT

The risk assessment-based approach and in particular the potency values used for the selection of MS constituents for ingredient assessment discussed above[29] were applied to MS yield data obtained in a study to assess ingredient mixtures.[10] Three mixtures of ingredients were applied to research cigarettes at low and high application levels each, and potential changes in MS composition were evaluated in comparison to that of a control cigarette with none of the ingredients. Several statistically significant increases and decreases were found for the TPM-based yield data in the ingredient mixture vs control groups, which called for a more integrative evaluation to reach a conclusion on the safety of the ingredient mixtures relative to the background of intrinsic MS toxicity. Of the 50 MS constituents analyzed, cancer- and noncancer-related potency values were available for 23 and 10 constituents, respectively. In order to account for potential changes in TPM yield between the research cigarettes, constituent yields relative to the respective TPM yields were used. Application of the HI approach produced estimates that were between 80 and 94% and 87 and 104% of the TCHI and TNHI, respectively, of the control group. The slight increases beyond 100% were within the accuracy of the chemical−analytical methods.

7. QUANTITATIVE RISK ESTIMATES IN COMPARISON TO OBSERVED RISKS

Most of the above-discussed applications of the HI-based risk evaluation for cigarette MS expressively excluded the calculation of quantitative or absolute risk values and their comparison to risks observed in epidemiological studies. This was because of several inherent limitations, such as the direct use of animal bioassay data for the assessment of human diseases without any effort for adjustments due to species extrapolation or the mere gaps in available MS constituent yield data and potency values. In addition, comparative analyses between cigarette types and ranking of MS constituents for prioritization do not require specific information or assumptions for smoking behavior, such as the number of cigarettes smoked per day or the smoking intensity, and arbitrary units for TCHQs and TNHQs were sufficient for these purposes. Nevertheless, quantitative evaluations using the HI concept have been performed for various reasons.

The earliest quantitative use of an HI-based evaluation of cigarette smoking-related cancer was conducted in order to evaluate the extent to which theoretical cancer risk assessments based on standard USEPA methodologies would produce results consistent with data from epidemiological studies of exposed populations.[46] An average exposure to 30 cigarettes per day over a period of 35 years was assumed in order to calculate lifetime average daily doses per kg body weight for 130 constituents. Inhalation cancer potency values were available for about half of the constituents from various USEPA databases. The resulting calculated theoretical cancer risk was 5×10^{-2}. The authors compared the calculated risk with the epidemiologically observable risk of 8×10^{-2} and concluded that the results were very similar. 1,3-Butadiene, acetaldehyde, and 2-nitropropane accounted for 70, 6, and 6%, respectively, of the overall calculated theoretical risk. Although these authors found a good accordance between theoretical and observable cancer risks, the theoretical evaluation included many uncertainties and limitations. For example, the cancer unit risk used in this study for 1,3-butadiene (0.28 m^3/mg) was 10-fold higher than the one USEPA is suggesting today (0.03 m^3/mg).[47] The cancer potency value of 2-nitropropane at that time (2.7 m^3/mg) was 500-fold higher than the one currently used (0.0056 m^3/mg).[48] In addition, the study assumed a 2-nitropropane yield of 1.2 μg per cigarette, which is 100-fold higher than more recently determined yields.[49] Thus, even within the framework of the HI concept, the theoretical cancer risk contribution by 2-nitropropane was overestimated by 50,000-fold, while that of 1,3-butadiene was overestimated by approximately 10-fold compared to more recent data. Overall, the similarity of calculated theoretical and observed cancer risks by smoking seems to be incidental considering the many uncertainties that went into these calculations.

Quantitatively calculated theoretical cancer risks were also determined in an attempt to assess potentially reduced exposure products (PREPs).[50] Yield data for both conventional and several PREP prototypes as well as cancer potency values were collected for 13 MS constituents. In spite of their diversity, all yield data from the PREPs were averaged. ILCRs per pack-year of smoking were calculated for overall and lung cancer risks separately. A subset of constituents, i.e., formaldehyde, acrylonitrile, arsenic, and cadmium, were specifically

dx.doi.org/10.1021/tx200536w | *Chem. Res. Toxicol.* XXXX, XXX, XXX−XXX

Table 3. Application of the Hazard Index Concept to Smokeless Tobacco[a]

constituent	CAS no.	GothiaTek limit and TSNA assortment (ng/g dry weight)	bioavailability (%)	oral cancer slope factor (mg/(kg × day)$^{-1}$)	theoretical ILCR	relative contribution to theoretical ILCR (%)
NNK	64091-91-4	1500	85	49	3.8×10^{-3}	90
NNN	16543-55-8	4600	85	1.4	3.4×10^{-4}	8
N′-nitrosoanatabine	71267-22-6	3600	85			
N′-nitrosoanabasine	37620-20-5	300	85			
benzo[*a*]pyrene	50-32-8	20	36	12	5.3×10^{-6}	0
cadmium	7440-43-9	1000	6	15	5.5×10^{-5}	1
lead	7439-92-1	2000	6	0.0085	6.2×10^{-8}	0
arsenic	7440-38-2	500	36	1.5	1.7×10^{-5}	0
chromium	18540-29-9	3000	36	0.42	2.8×10^{-5}	1

[a]Selection of constituents, GothaTek limits, bioavailability, and assumptions for exposure were used according to Ayo-Yusuf and Connolly.[55] The GothaTek limit for TSNAs was proportionally assorted to individual constituents according to published average constituent concentrations.[60] Oral cancer slope factors were taken from CalEPA[56,88] according to the CAS numbers specified.

considered for evaluating lung cancer risk, while others were only considered for total cancer risk, such as benzo[*a*]pyrene, NNN, and NNK. The calculated ILCR for lung cancer from conventional cigarettes (ILCR(conv)) was only approximately 2% of the epidemiologically observable lung cancer risk using this approach,[50] which according to the authors demonstrated the current inability to account for the observed health risks by smoking. However, this result is not surprising with only four constituents entering the calculations and considering all other weaknesses of applying this concept to absolute risk terms. The authors further suggested measuring the efficiency of PREPs compared to conventional products by the following (slightly simplified) "science-based" equation (CR, observed lung cancer risk):[50]

$$\text{Efficiency (PREP)} = \frac{\text{ILCR (conv)} - \text{ILCR (PREP)}}{\text{observed CR}} \times 100\%$$

If the ILCR(PREP) would be low, then the PREP efficiency would be high and *vice versa*.[50] However, this equation does not allow a PREP efficiency beyond 2% even if all exposure to smoke would have stopped by quitting: if one would assume quitting smoking as the most advanced 'PREP', then the ILCR('PREP') should be zero. With the above equation and the authors' limitation to just four constituents with relatively low theoretical cancer risk contribution, this would still only result in an efficiency or rather a reduction of the lung cancer ILCR by maximally 2%, which is not in line with the epidemiological findings of a reduction of lung cancer risk with years after smoking.[51] While it is certainly correct that there is a lack of etiological understanding for smoking-induced carcinogenesis, the above equation that led the authors to their conclusion is inappropriate. The better conclusion would have been that with only four constituents entering this analysis and theoretically contributing only 2% of the observed lung cancer risk, no meaningful evaluation of PREP efficiency can be made. In a later study on the same subject and by the same principal author, benzo[*a*]pyrene, NNN, and NNK were suddenly also considered as lung carcinogens.[52] NNK was calculated to contribute 0.2 to 1% to the male and 0.7 to 21% to the female lung cancer risk from smoking. Estimates for NNN and benzo[*a*]pyrene were one and 2 orders of magnitude lower.

Epidemiological and animal bioassay data on cadmium were used to estimate the contribution of this constituent to smoking-related lung cancer.[53] The use of epidemiological data revealed contributions of 0.2 to 1.6% (upper confidence limits (UCLs) of 1.6 to 8.8%) to the estimated smoking-related lung cancer mortality, while animal bioassay data revealed contributions of 13 to 47% (UCLs 23 to 81%). Considering the large number of carcinogens in smoke, the authors thought that extrapolation from animal bioassay data would overestimate human risks. Using the same approach, the contribution of benzene to smoking-related total leukemia and acute myeloid leukemia was estimated[54] at 8 to 48% (UCLs 20 to 66%) to smoking-induced total leukemia deaths and from 12 to 58% (UCLs 19 to 121%) to the estimated smoking-related acute myeloid leukemia deaths, considering smoking intensities of up to 40 cigarettes per day. Other mathematical models resulted in much lower attributable fractions at <1%.[54]

8. APPLICATION TO SMOKELESS TOBACCO

Quantitative lifetime cancer risks were also calculated for various types of smokeless tobacco as well as for a theoretical smokeless tobacco product with the constituent level limits defined by the GothaTek standard.[55] This analysis was built on the contribution of four constituent classes, i.e., TSNAs, cadmium, lead, and toxic equivalents of benzo[*a*]pyrene. CPDB TD_{50} values were used as cancer potency values. Four different TSNAs were added up by mass without consideration of differences in cancer potency. Using CPDB TD_{50} values, NNK and NNN have very similar potency, while according to the oral cancer slope factors listed by CalEPA, NNK is 35-fold more potent than NNN.[56,57] NNK was also more potent in direct comparison to NNN in a laboratory animal bioassay,[58] which is surprisingly not reflected in the CPDB TD_{50} values. *N*′-Nitrosoanatabine and *N*′-nitrosoanabasine were recently determined not to be classifiable regarding their carcinogenicity to humans (group 3),[59] and their yields should not be included in the TSNA sum used in this model calculation. Nevertheless, *N*′-nitrosoanatabine on average contributed 36% to the total TSNA yield in the analytical data used in this study (Table 2a in ref 60) and was considered in the authors' calculations with the same cancer potency as NNK and NNN.[55] NNK on average contributed approximately 15% to the overall TSNA yields. In a model calculation on the basis of the CalEPA instead of the TD_{50}-based cancer potency data and using all other assumptions made regarding dosing and bioavailability,[55] the theoretical ILCR for NNK under conditions of the GothaTek standard would be 3.8×10^{-3}, i.e., by incidence

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not much different from the 5.3×10^{-3} reported by the authors for the sum of the TSNAs.[55] Using CalEPA oral cancer slope factors also for the other constituents of this analysis, NNK by itself would contribute 90% of the overall theoretical lifetime cancer risk calculated on the basis of these constituents (Table 3). For all other constituents, a theoretical ILCR below the concern level of 10^{-4} set by the authors[55] would be obtained. Thus, the application of the suggested TCHI approach to smokeless tobacco seems to be more or less an exercise without added value compared to a simple chemical−analytical monitoring of NNK yields.

Within the scope of applying the hazard index concept to smokeless tobacco and among those constituents selected for analysis by these authors,[55] NNK may be the sole constituent with relevant contribution. Nevertheless, this theoretical evaluation *per se* does not confirm any actual role of NNK in smokeless tobacco-related disease pathogenesis. For the judgment of this relationship, all available epidemiological and mechanistic information needs to be considered.[59,61] By example, replacing the oral cancer slope factor for NNK from CalEPA with another one published[61] (see also Table 4), a theoretical ILCR of 6.7×10^{-6} would result for this constituent using all others of the above parameters. In particular, the higher metabolic activation rate by α-hydroxylation in tissues of the rodent species used for determining cancer slope factors compared to human tissues needs to be considered.[62]

Table 4. Variation in Published Oral Cancer Unit Risks for NNK

unit risk $(mg/(kg \times day)^{-1})$	cancer type	reference
49	all cancers	CalEPA (2001)[56]
28	lung cancer	CalEPA (2001)[56]
19	lung cancer	Naufal et al. (2009)[89]
10	all cancers	Ayo-Yusuf and Connolly (2011)[55]
0.086	all cancers	Nilsson (1998)[61]

9. ALTERNATIVE CONCEPTS FOR RISK-BASED PRIORITIZATION OF SMOKE CONSTITUENTS

In an independent approach to derive risk prioritizations for MS constituents, the relevant cancer and noncancer literature was evaluated and used to determine MoEs against the smoking-related uptake of these constituents.[63] For example, the cancer-related MoEs determined for acetaldehyde on the basis of nasal tumors observed in a rat chronic inhalation study (140 to 1300) were below an acceptable margin of 10^4, as were the noncancer MoE determined on the basis of histopathological changes in the respiratory tract of rats in a subchronic acetaldehyde inhalation study (approximately 1300). The authors concluded that acetaldehyde was a medium priority MS constituent in a harm reduction framework. On the basis of MoEs of 1 and 11 for noncancer effects in rat inhalation studies, acrolein was ranked the top priority. Acrylonitrile, cadmium, ethylene oxide, formaldehyde, and isoprene were also given some priority, while vinyl chloride and benzo[*a*]pyrene were determined to be of lower priority due to MoEs higher than 10^4. For ethylene oxide, only data from unfiltered cigarettes were available. On the basis of mouse lung bioassay data, 1,3-butadiene was classified as a high priority MS constituent; however, the authors pointed out that there is uncertainty because the lesions found in mice are at odds with the lack of epidemiological evidence for lung tumors in occupationally exposed humans. MoEs could be derived for NNK and NNN, however, scattered above and below the cutoff of 10^4, and none based on inhalation studies or even on epidemiological data. The authors thus felt unable to rank the TSNAs in this concept. Future efforts would include the use of physiologically based toxicokinetic models and the evaluation of the mode of action of the respective constituent for the investigation of potential interactions with other constituents, which was already initiated for a group of MS aldehydes.[64] In the context of the current review, MoEs could also be used as inverse potency factors for deriving HQ or HI and applied to comparative assessments of MS constituents or cigarette types.

Other approaches to rank MS constituents by their toxic potential investigated particular activities, such as the potency to react with sulfhydryl groups[65−67] or *in vitro* cytotoxicity.[68] Elsewhere, various characteristics related to carcinogenesis were assessed by subjective scores, and the sum of these scores determined the level of relevance for smoking-related cancer risk.[69]

10. LIMITATIONS

As with every model, the current concept of a risk-based weighing of constituents to assess the composition of smoke has a number of limitations and weaknesses. The most notorious limitation stems from the number of constituents that can only enter the evaluations because of gaps in both analytical and potency data availability.

10.1. Limited Number of Smoke Constituents in Routine Analysis. Of the more than 5000 MS constituents identified so far,[27] quantitative analytical data are routinely only obtained for a master list of up to 50, which is a selection of just one percent. Although this selection has historically been based on some toxicological considerations, e.g., regarding carcinogenic or ciliatoxic activities,[70] it has been conducted at times when only a limited understanding of the etiology of smoking-related diseases was available and when the scope of the analytical characterization of smoke was still limited, too. This master list has not been dramatically modified after new research results have become available over the years. Two recent suggestions of extended lists are exceptions to this rule, as these lists comprise approximately 100 MS constituents.[18,19] However, the rationale for selecting additional constituents for either of the more extended lists is rather poor, too. For example, the mere availability of potency factors is the major determinant for selecting constituents for one extended list,[19] and rather limited toxicological information has been used to justify the extension for the other suggested list.[71,72]

For model calculations, it could be assumed, though, that both the more historical list of up to 50 constituents or the more recent suggestions of approximately 100 constituents would be fair representations of the toxicologically relevant chemical classes of constituents and that changes in the composition of the constituents included in a given analysis would be representative for relevant overall changes in the composition of the MS types under investigation. This assumption would limit the use of the HI-based assessment concept to comparative evaluations, if quantitative data on the same representative selection of MS constituents would be available for the set of MS types to be compared. In a next approximation step, a comparative assessment could also be considered meaningful for semiquantitative data obtained in

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fingerprint analyses, although the selection of MS constituents analyzed in fingerprint analyses is mainly driven by the possibilities of the analytical equipment rather than by toxicological considerations. The interpretation of fingerprint analyses could be further optimized by applying a threshold-of-toxicological concern (TTC) approach to minor constituents of the mixture.[19,73]

A clear weakness of the HI-based approach is evident when lifetime cancer risks are determined on the basis of a limited number of constituents entering the evaluation. This seems to be only justifiable if one would assume that the selection of 50 or 100 constituents would indeed determine a major fraction of the smoking-related disease risk. Such justification has been attempted by comparing the risk-based lifetime cancer risk to that observable in epidemiological studies. Depending on the input data, the calculated risk was found to be in fair agreement with the epidemiologically observed risk, thus justifying this concept,[46] or it was found to result in only a fraction of the observed risk, thus questioning the concept.[34] The most questionable application used only a few constituents to find that the associated lifetime cancer risk only accounted for a small percentage of the observed risk, took that for granted, and concluded regarding the evaluation of PREPs that "there would be little reason to be confident that such removal would by itself lead to any observable reduction in smoking related lung cancer".[50]

Approaches to compare calculated theoretical risks with observable epidemiological risks have not been conducted for noncancer effects related to smoking.

10.2. Appropriateness of Available Constituent Yield Data. Smoking behavior is highly variable between smokers, and there is a limitation to the number of machine-smoking conditions that can be used in a given study. Neither of these conditions can be considered representative. For the determination of absolute ILCRs and the comparison of markets or certain populations of smokers, this might be an issue. For comparative analyses, this is of minor impact. The relative contribution of MS constituents to TCHIs and TNHIs did not appreciably change when different machine-smoking regimens were compared, although the constituent-specific HQs and the HIs increased with increasing yields depending on the smoking regimen.[41] Besides smoking topography, other parameters of smoking behavior, such as smoking intensity or duration, would not be important for comparative assessments as long as it can be assumed that all of these parameters would be similar for the smoke types to be compared. This may not be necessarily the case if novel products are being compared with conventional products; studies with an electrically heated smoking system have shown higher numbers of cigarettes consumed per day for the novel compared to the conventional products.[74]

10.3. Limitations in Availability of Potency Values. A further limitation is that there is not a sufficient number of potency values available, not even for the limited number of constituents analyzed. The broadest coverage of routinely assessed constituents with potency numbers is for carcinogenic activity, but for other smoking-related diseases, such coverage is much worse. Several authors have attempted to combine potency values from different sources, although this may introduce additional heterogeneity. There are several listings of carcinogenic potency values from various authoritative sources, including inhalation-related unit risks. These potency numbers may either be derived from laboratory animal carcinogenicity studies or, if available, from epidemiological studies. Most often, these potency numbers are used to determine a general cancer risk, although the potency numbers are derived from various target sites and tumor types. This practice of combining heterogenic potency values has been considered defensible because similar general mechanisms are being assumed to be critical for the carcinogenic process irrespective of the target tissue.[75]

For CVD and COPD potencies, chronic reference exposure levels have been used if these were derived from cardiovascular or respiratory end points, respectively, as the lowest observed adverse effect level. Thus, in most if not all cases, it is not the disease itself that determines the noncancer potency but rather a study end point that can mechanistically be linked to the disease. This is more indirect as for carcinogenicity. The qualitative representativeness of the particular end points for smoking-related CVD and COPD symptoms remains vague, but even more so the relevance of the quantitative potency value determined for any of these end points in determining the risk for these multifaceted disease classes. For reproductive and developmental toxicological activity, there is a list of Maximum Allowable Dose Levels for a number of compounds,[76] which could be considered to represent inverse potencies; however, only four commonly measured MS constituents are included in this list.

Thus, the limitation stemming from the analytical−chemical characterization of MS is potentiated by the limitation in potency values, which is most severe for all smoking-related diseases other than cancer.

10.4. Uncertainty of Available Potency Values. There are a number of sources of uncertainty for individual potency values. Cancer potency numbers can be derived from laboratory animal or epidemiological data, dependent on availability. The selection of the study to be used and the modeling approach to interpret the study data already determines one source of uncertainty for potency values. As a worst case, tumor incidence data from single laboratory animal studies, such as those collected in CPDB, were inter- or even extrapolated to derive tumorigenic doses for 25 or 50% tumor incidences (T25 or TD_{50} values).[17,28,36,55] This method of deriving potency values seems to be inferior to the current standards of data modeling, such as the benchmark dose concept for the evaluation of laboratory animal carcinogenicity data.[77] A compilation of published oral cancer unit risks for NNK is given in Table 4.

Potency values derived from epidemiological studies also bear intrinsic uncertainties. The example of 1,3-butadiene demonstrates that there is an uncertainty of various orders of magnitude even if the same disease data are used, just by assuming different exposure scenarios and dose−response models (Table 5). The unit risk values listed for 1,3-butadiene also show a temporal development. Potency values are being refined with the availability of additional data or modeling tools. This temporal refinement leads to heterogeneity in the quality and comparability of potency values even within a given authoritative source. Therefore, even if one would assume that there are common methodologies applied within one authoritative body, it cannot be assumed that the potency values for all compounds listed by this authority were derived by the same approach or on the same level of data quality. If the development of potency values is already heterogeneous within one authoritative source, the question arises whether it is indeed required to stay within one source for a given analysis or whether potency values from various sources can justifiably be

Table 5. Variation in Published Inhalation Cancer Unit Risks for 1,3-Butadiene[a]

unit risk $(mg/m^3)^{-1}$	data basis	reference
0.28	mouse inhalation, lung tumor data	USEPA (1985)[90]
0.17	mouse inhalation, lung tumor data	CalEPA (2005)[91]
0.03	human occupational epidemiological data (1995 status)	USEPA (2002)[47]
0.0003	human occupational epidemiological data (2000 status)	Sielken and Valdez-Flores (2001)[92]
0.0005	human occupational epidemiological data (2004 status)	Grant et al. (2009)[93]
0.00004	human occupational epidemiological data (2004 status)	Sielken at al. (2007)[94]
0.00003	human occupational epidemiological data (2004 status)	Sielken and Valdez-Flores (2011)[95]

[a]All human occupational epidemiological data are based on the University of Alabama at Birmingham study of leukemia in North American male workers in the styrene–butadiene rubber industry, which is considered to provide the best available data.[47] This data set has sequentially been updated. Differences between those studies using the same data set are based on various methods of exposure assessment and dose–response modeling.[95]

combined into one analysis in order to increase the number of constituents that can be included in this analysis.

10.5. Uncertainties in Applying Available Potency Values to Smoke. Most of the potency values have been developed to evaluate exposures at environmental (low) risks, much lower than the risk for smoking-related diseases in smokers. For environmental exposures or food-related exposures, cancer risks at 10^{-5} or 10^{-6} are considered acceptable, while the risk for smoking-related cancer is at approximately 10^{-1}.[78] Using the potency-based weighing of MS constituent yields, lifetime cancer risks for some constituents (NNK, 1,3-butadiene) were calculated to be as high as 10^{-2}.[29,50]

The HI concept assumes linear dose–response relationships for both cancer and noncancer end points. From a mechanistic point of view, it is questionable whether a potency value set at a safe value can indeed be used to assess exposures as high as that from smoking, at least for MS constituents with relatively high yields per cigarette, such as acrolein: CalEPA is listing an RfC value of 0.35 $\mu g/m^3$ for acrolein.[79] Under HCI machine-smoking conditions, an average acrolein yield per cigarette of 120 μg has been estimated, which is taken up by approximately 9 puffs per cigarette.[3] With a puff volume of 55 mL, this results in an acrolein concentration of 240,000 $\mu g/m^3$ in the undiluted puff or 27,000 $\mu g/m^3$ if inhaled with a breath volume of 0.5 L. Assuming complete retention of inhaled acrolein, the uptake under RfC conditions is 7 μg/day (assuming a daily breath volume of 20 m^3), while that from smoking under the above conditions would be 2,400 μg/day at 20 cigarettes per day. Thus, a large extrapolation is necessary to apply potency values to the exposure by some smoke constituents, and it is highly questionable whether the same mode of action and thus potency would apply at such different concentration or dose levels.

Potency values may have been derived from adverse health effects that are not related to the major smoking-related diseases. For instance, the chronic reference exposure level for toluene was derived from a neurological end point as the lowest observed effect level.[80] Many cancer potency values may be derived from liver cancer, which is a relatively frequent malignancy in laboratory rodents but has not been classified as a smoking-related type of cancer.[1] Aromatic amines may play a significant role in smoking-related bladder cancer,[2] but in the overall assessment of a smoking-related TCHI, they only play a minor role. 1,3-Butadiene is a multiorgan carcinogen in mice, and the most susceptible organ is the lung.[47] However, when classifying 1,3-butadiene as a human carcinogen, this was based on its potential to induce leukemia. Correspondingly, the current cancer potency value for 1,3-butadiene is based on the epidemiological data on the occupational risk for developing leukemia from 1,3-butadiene exposure. The lungs were not identified as target organ in these epidemiological studies. Nevertheless, cancer target organ specificity may change between relatively low exposure levels and those under conditions of smoking. In addition, the constituents are taken up in the smoke matrix, and the fate of a given smoke constituent may be changed on the toxicokinetic and toxicodynamic level.

The cancer potency values that were determined from laboratory animal bioassays only cover those that act as complete carcinogens. In such cases, a genotoxic mechanism can be assumed to initiate the carcinogenic process. The doses of the carcinogen have to be high enough not only to set a genotoxic insult but also to trigger some promoting activities in order to act as a complete carcinogen. For smoking-related cancer, in particular lung cancer, a major role for the promotion phase in carcinogenesis has been proposed,[81] which may also be related to the reduction of the relative risk of developing lung cancer after smoking cessation. Most MS constituents with major contribution to the TCHI are genotoxic carcinogens, whereas constituents that have once been considered to be promoters or cocarcinogens do rarely show up, mainly because they are not carcinogenic in the classic laboratory animal bioassay and thus do not have a potency value associated.

10.6. Limitations in Applying the Hazard Index Concept to Smoke. The application of the HI concept by itself is of course also associated with some limitations, in particular its default assumption of additivity among constituents of a mixture. In general, potency values have been derived in order to give guidance for assessing and managing occupational and environmental risks. There is no systematic research nor guidance on how potency values would change upon mixed exposures, but literature reviews suggest that interactions on toxicokinetic or toxicodynamic parameters seem to be rare.[75,82] While this lack of significant interaction seems to be reasonable at low exposure concentrations or doses, e.g., at the levels of threshold limit values or no observable effect levels, there might be more interactions upon higher exposure levels and longer durations of exposure, as they are achieved with smoking. Smoke exposure can modulate metabolic activation, e.g., by inducing cytochrome P450-dependent reactions, as well as detoxification or DNA repair, and thus one constituent may indeed be able to interact with the potency of another constituent. Moreover, the smoking-related disease syndromes are rather complex, which would argue against simple etiologies. For example, experimental and epidemiological data suggest the importance of a promotional phase in smoking-induced carcinogenesis in addition to a genotoxic initiation component.[81,83] Interactions between MS constituents were also described for effects observed in *in vitro* studies.[84]

The contribution by acrolein to the TNHI is overwhelming. The impact of other MS constituents is almost entirely within

dx.doi.org/10.1021/tx200536w | *Chem. Res. Toxicol.* XXXX, XXX, XXX–XXX

the analytical variability of acrolein determinations. Thus, there does not seem to be any further advantage of using the HI-approach for noncancer effects as long as acrolein is playing such a dominant role, except for demonstrating just that. The number of MS constituents, which are covered by the HI approach with potentially relevant end points in the specific noncancer smoking-related diseases, is very limited.

Most listings of noncancer potency values do not list nicotine. However, nicotine is listed by some authorities with occupational threshold limit values, such as the PELs issued by US OSHA. If PELs would be used as potency factors for calculating theoretical noncancer risk estimates, nicotine would play a significant role besides acrolein. Its major role in the risk assessment of smoking has been considered in influencing the smoking dose. The TobReg group thus suggested using all MS chemistry data relative to nicotine to obtain a qualitative measure of the MS composition.

11. STRENGTHS OF USING HAZARD INDICES FOR EVALUATING SMOKE

11.1. Selection or Prioritization of Constituents for Analysis, Regulation, or as Risk Reduction Targets. Quite similar priorities have been identified with the various approaches suggesting that the respective results might be rather robust within the limitations of the applying this concept to smoke. One of the major findings has been the predominant role of acrolein for noncancer effects and the preponderance of the vapor phase relative to particular phase constituents for both cancer and noncancer effects.

11.2. Comparative Evaluation of Types of MS or Smokeless Tobacco. Except by weight-of-the-evidence approaches, there is no other formal and straightforward way to evaluate MS chemistry data. However, such evaluations seem to be necessary because otherwise there is not much use of data sets that show variability between brands, between years, or by the presence of ingredients, in particular if this variability includes both increases and decreases for the various MS constituents analyzed.

11.3. Regulatory Acceptance of the Hazard Index Concept. The HI concept has been used for many years as an approach of evaluating complex exposures. It is still part of the more recent guidance documents on risk assessment of complex or cumulative exposures.[23,25,26] As such, it is an approach that has regulatory precedence, at least in other areas of risk assessment. Even for the assessment of cigarette smoke, it has been used by consulting groups to the governments of Massachusetts and New Zealand;[30,33] it has also been the basis for the recommendation of a number of MS constituents for mandated lowering by the TobReg group;[17,28] and most recently, a group of scientists from European government agencies have used potency values as the basis for selecting MS constituents for regulatory purposes.[19]

11.4. Supplementation of Available Routine Toxicological Tests of Smoke. The most practical argument against using the HI-based approach for noncancer assessments is the dominance of the TNHI contribution by acrolein. For noncancer risk, there are a number of biological assays available that could be used, e.g., for ingredient assessment, such as the *in vitro* cytotoxicity or the subchronic inhalation toxicity assays,[85] some of which also suggest a prominent role for acrolein.[86] However, inhalation cancer studies are no routine part of ingredient assessments. Therefore, some laboratories have extended the classic master list of MS constituents for routine analysis by additional constituents with known or suspected carcinogenicity to humans.[49] With this extended set of MS constituent yield data and the respective cancer potency values, an assessment for a TCHI is possible that may be based on the contribution by 30 to 40 MS constituents. This is certainly not worse than some biological assays, which are also often driven by a relatively small number of most active constituents, such as aldehydes.[86] In addition, the constituents that weigh into the TCHI would mostly not contribute in a significant manner to the activity in *in vitro* genotoxicity assays with MS, such as the bacterial mutagenicity assay. This to some degree is related to their presence in the MS vapor phase, which is not routinely tested in this assay. Moreover, the cancer potency values are based on rodent bioassay studies or even epidemiological studies and should thus be superior by default to results obtained in a bacterial mutagenicity assay. Thus, the determination of the TCHI, in any case, may be a good use of the MS chemistry data sets generated and a reasonable supplementation to the weight-of-evidence analysis of the data generated in a tiered testing battery.

11.5. Application to Comparative Objectives. The main strength of using the HI-based theoretical assessment of MS composition is if used in a comparative manner. The advantage is that a number of assumptions needed to calculate lifetime cancer risks are not required for a comparative assessment. At the same time, the disadvantage of only a limited number of constituents that can enter the assessment is not as severe as long the comparative nature of the assessment is clear. Quite often, on the background of the intrinsic toxicity of MS, a comparative assessment is the only objective to generate MS chemistry data, such as during the evaluation of ingredients, manufacturing techniques, or cigarette design parameters. This is particularly true for the demonstration of substantial equivalence, which has recently been requested for the approval of new brands to the US market.[87] This most current guidance document requests MS chemistry data but does not specify how substantial equivalence would be determined on the basis of these data.

12. CONCLUSIONS

There is a clear demand for a risk analysis-based assessment of the composition of smoke and smokeless tobacco as stereotypes of complex mixture exposures. Various attempts have been made of using the HI approach, both in a comparative mode and for the calculation of theoretical ILCRs, and, more recently, the MoE approach. There is also some regulatory precedence in applying this approach to smoke. Two principal objectives in dealing with smoke have been recognized, (1) the prioritization of constituents for analysis, regulation, and as targets for risk reduction, and (2) the evaluation of products, experimental research cigarettes, e.g., for the evaluation of ingredients, prototypes, e.g., PREPs, or standards, such as the GothaTek standard for smokeless tobacco. All of these applications suffer from inherent weaknesses and limitations, not much different from those of applications to other mixture exposures. All previous applications to smoke have been performed within the boundaries of currently available quantitative composition data and potency values. The suggested application to the exposure from smokeless tobacco has so far not added information beyond what can be obtained by analytical determination of NNK. There is not much advantage of applying the HI concept to smoke for noncancer effects, as this index would be dominated by acrolein. The

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application of the HI concept to cancer effects can be based on 30 or more MS constituents and offers a relevant additional source of information, which is not otherwise covered in current tiered assessment approaches. In particular, information is additionally made available on the impact of carcinogenic constituents, which are predominantly found in the MS vapor phase, with a mostly genotoxic mode of action. Inclusion of this activity in the toxicological assessment of smoke can be considered as an added value supplementing the weight-of-evidence analysis within a tiered approach. This application does not bear more limitations than many other toxicological assays that are currently employed in the toxicological assessments of cigarette types. As is the case for every other assay and interpretation model, the HI concept needs to be applied with its limitations and weaknesses in mind. Its best application is for comparative purposes, which is the objective of many toxicological assessments of cigarette types. In a future application, the utility of the previously suggested larger selections of smoke constituents for regulation may be assessed using the HI approach after having generated the respective current quantitative yield data. It should always be kept in mind that the HI concept is a theoretical concept and does not provide actual risk information.

AUTHOR INFORMATION

Corresponding Author

*Phone: +49-2205-894 0384. Fax: +49-2205-894 0385. E-mail: HansJ.Haussmann@T-Online.de.

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ABBREVIATIONS

ADI, allowable daily intake; AU, arbitrary units; CalEPA, California Environmental Health Agency; COPD, chronic obstructive pulmonary disease; CPDB, Cancer Potency Data Base; CVD, cardiovascular disease; HCI, Health Canada intense (machine-smoking conditions); HI, hazard index; HQ, hazard quotient; ILCR, incremental lifetime cancer risk; ISO, International Organization for Standardization; MoE, margin of exposure; MS, mainstream cigarette smoke; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*′-nitrosonornicotine; OSHA, US Occupational Safety and Health Administration; PEL, permissible exposure level; PREP, potentially reduced exposure product; REI, relative exposure index; REL, chronic reference exposure level; RfC, chronic reference concentration; T25, dose for 25% tumor incidence in laboratory animal bioassays; TD_{50}, dose for 50% tumor incidence in laboratory animal bioassays; TCHI, theoretical cancer hazard index; TCHQ, theoretical cancer hazard quotient; TNHI, theoretical noncancer hazard index; TNHQ, theoretical noncancer hazard quotient; TobReg, WHO Study Group on Tobacco Regulation; TSNA, tobacco-specific *N*-nitrosamine; UCL, upper confidence limit; USEPA, United States Environmental Protection Agency; WHO, World Health Organization

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