

## Quantitative Puff-by-Puff-Resolved Characterization of Selected Toxic Compounds in Cigarette Mainstream Smoke

Thomas Adam,<sup>†,‡</sup> Stefan Mitschke,<sup>†,‡</sup> Thorsten Streibel,<sup>†,‡</sup> Richard R. Baker,<sup>§</sup> and Ralf Zimmermann<sup>\*,†,‡,||</sup>

*Institute of Physics, Analytical Chemistry, University of Augsburg, 86159 Augsburg, Germany, Institute of Ecological Chemistry, GSF-National Research Centre for Environment and Health, 85764 Neuherberg, Germany, British American Tobacco, R&D Centre, Southampton SO15 8TL, U.K., and BfA-Bavarian Institute of Applied Environmental Research and Technology GmbH, Environmental Chemistry, D-86167 Augsburg, Germany*

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Soft single photon ionization (SPI)–time-of-flight mass spectrometry (TOFMS) has been applied for the quantitative puff-by-puff-resolved characterization of selected toxic species in cigarette mainstream smoke, namely, nitric oxide (NO), acetaldehyde, butadiene, acetone, isoprene, benzene, toluene, ethyl benzene, and xylene. The 2R4F research cigarette was investigated for whole smoke (unfiltered) and gas phase (filtered). It has been demonstrated that the existing smoking regime for total smoke analysis (smoke from a complete cigarette) features deficiencies when applied to puff-resolved measurements. This is especially the case for analysis in which a glass fiber filter is used to separate the smoke particulate and gas phases because material is desorbed from the filter and influences succeeding puffs. Regarding whole smoke measurements, succeeding puffs are affected by contamination and memory effects of the smoking machine itself, but to a lower extent. Quantitative puff-resolved smoke profiles show that the puff yields of the various constituents can differ tremendously. Most species' concentrations increase gradually with puff number, which is mainly due to the reduction in length of the cigarette. However, high yields in the first puff are observed for butadiene and isoprene, suggesting different formation mechanisms for these compounds. First results are presented in which these high yields in the first puff are mainly associated with the gaseous fraction of the smoke.

### Introduction

Cigarette smoke is a very complex and highly dynamic matrix with nearly 4800 constituents identified (1). However, estimations of the number of unidentified species have been as high as 100 000 (2). Whole cigarette smoke is an aerosol, containing gaseous and suspended particulate material, and many substances are partitioned between these two phases. The gas phase is comprised of approximately 400 to 500 individual compounds (3), many of them at trace levels, and the greater number of smoke constituents appears in the particulate fraction. The particulate phase is comprised primarily of the many organic compounds that condense together with some water to form liquid droplets when combustion zone gases cool while traveling along the cigarette rod (1). Soot particles only play a minor role in the formation of cigarette smoke.

The composition and partition between the phases can change continuously and are strongly influenced by time as well as temperature and dilution of smoke (1). Investigations of the

effects of cigarette smoke constituents have generally concentrated on the particulate phase of smoke (3). However, knowledge about the phase affiliation of smoke components is important because gas phase and particulate matter have very different deposition characteristics in the respiratory tract, be it oral, pharyngeal, bronchial, or alveolar. For most smokers, the smoking process occurs in two steps. The first stage is a "mouth" phase, whereby smoke is drawn into the mouth without any evidence of inhalation. The second stage begins with a pause of variable duration, often associated with removing the cigarette from the mouth, which is followed by inhalation of smoke. Thus, higher yields in gaseous constituents may result in predominant uptake during the first ('mouth') phase, whereas particulate-related species are mainly absorbed in the second ('inhalation') phase (4). In this regard, animal inhalation tests have indicated that upper respiratory tract changes are specifically associated with the cigarettes' vapor phase (3). Particulate matter can penetrate deep into the respiratory tract, and the regional deposition is influenced by several parameters, for example, the diameter of the inhaled particulate matter (5 and references therein).

High volatile compounds, for example, CO<sub>2</sub>, reside almost exclusively in the gas phase, whereas very low volatile substances (e.g., large polynuclear aromatic hydrocarbons (PAHs)) tend to reside almost exclusively in the particulate

\* Corresponding author. E-mail: ralf.zimmermann@gsf.de.

† University of Augsburg.

‡ GSF-National Research Centre for Environment and Health.

§ British American Tobacco. Author's current address: The Langdales, Lime Walk, Dibden Purlieu, Southampton SO45 4RA, U.K.

|| BfA-Bavarian Institute of Applied Environmental Research and Technology.

phase. In contrast, semivolatile components can be present in significant amounts in both phases (6), and the phase affiliation can differ substantially. In addition, tobacco smoke composition is a highly dynamic matrix. Therefore, to gain analytical information of smoke components relevant for human smokers, it is important to use the conditions in smoke when it is 1 or 2 s old, rather than smoke that has aged over a few minutes (7). Consequently, the ideal way to analyze tobacco smoke would be to analyze (i) simultaneously as (ii) many relevant compounds as possible, (iii) in both phases, (iv) on a real time basis. Inherent in this ideal situation is the use of an unambiguous method for separating the particulate and gas phases in smoke. However, from the scientific point of view, the ideal analysis procedure is difficult to achieve. There is also no completely unambiguous way of separating the two phases; the separation depends on the technique used: Cambridge filter pad, electrostatic precipitation, jet impaction, the use of solid absorbents, and cold traps (8). The objective of the work in this paper was to develop a single instrumental setup which best meets these requirements to analyze at trace levels several volatile and semivolatile compounds which are believed to cause health effects for human smokers. The main goal of the method described is a fast and comprehensive analysis which interferes as little as possible with the complex smoke matrix. Since fast gas-phase measurements require modifications to the setup due to the implementation of particulate matter precipitation techniques, the gas phase and the whole smoke phase were investigated in succession. The approach of analyzing whole smoke and vapor phase allows us to indirectly draw conclusions about the burden of organic matter in the particulate phase.

Quantification of toxic compounds in whole cigarette smoke and its gas phase was carried out on a puff-by-puff basis. To make the results comparable to total amounts per cigarette available in the literature, we tried to meet similar conditions as are commonly used in tobacco science. This included using a Cambridge filter pad at room temperature to separate the gas and particulate phases of smoke, described more fully in Experimental Procedures below, including some of its drawbacks. The analytical method applied is laser-based single-photon-ionization (SPI)–time-of-flight mass spectrometry (TOFMS) coupled to a smoking machine. The experimental setup used was based on the one described by Mitschke et al. (9). Therein, it was demonstrated that laser-based photoionization methods can be applied to comprehensively monitor volatile and semivolatile substances in the cigarette's mainstream smoke (MS). Mainstream smoke is the smoke which emerges from the mouth end of the cigarette during a puff and is usually inhaled by the smoker (1). In addition, the work (9) covered detailed information on previous studies dealing with analytical on-line techniques applied to cigarette smoke, which is therefore not specified in the current paper. The interested reader is referred to, for example, refs 10–20. However, since the paper (9) essentially described a qualitative overview of substances in cigarette smoke being accessible by the combination of two different laser photoionization methods, the resonance-enhanced multiphoton ionization (REMPI) and the single photon ionization (SPI), the work presented here focuses on a quantitative evaluation by SPI–TOFMS only of a few constituents of special interest due to their harmful potential for the human smoker.

Several lists of tobacco smoke components thought to be relevant to smoking-related diseases have been published in the past. The most comprehensive ones are derived from Hoffmann and co-workers (21–23). Moreover, at least three quantitative rankings of cigarette smoke components which are believed to

cause health effects have been published. The first one was done by Vorhees et al. (24) in 1997 and was supported by the Massachusetts Department of Public Health Tobacco Control Program. The second ranking by Fowles and Bates was part of a year 2000 report to the New Zealand Ministry of Health (25) and was summarized in ref (26). The third paper that addresses ranking, by Rodgman and Green (27), ranked the smoke constituents in various ways according to various toxicological criteria. All the reports contain evaluations of smoke constituents for carcinogenic and noncarcinogenic health effects. However, there are disagreements among scientists concerning the relevance, accuracy, and procedure of establishing the rankings, mainly due to the extrapolation of pure-compound knowledge to the biological properties of a complex and dynamic mixture such as smoke containing them (27). To fully prioritize the relative risks of the various smoke components, it is necessary to have a robust disease model and a means of verifying that the sum of the individual contributions of the components is equal to the activity of the whole smoke. The reader is referred to the literature for more information (27).

The target compounds in the framework of this study were chosen according to their accessibility by SPI–TOFMS, possible relevance for the human smoker, and expected yield in tobacco smoke whereby the above-mentioned publications and reports acted as a guideline. The substances studied are nitric oxide, acetaldehyde, butadiene, acetone, isoprene, benzene, toluene, and the C2-benzene derivatives ethyl benzene and xylene. Table 1 illustrates the chemical structures of the target molecules, some important physical properties (vapor pressure and boiling point), as well as toxicological data, such as International Agency for Research on Cancer (IARC) categories and Occupational Safety and Health Administration (OSHA) permissible 8-hour time-weighted average concentration (TWA<sub>8</sub>). Moreover noncarcinogenic health effects and the compounds' position in the three mentioned cancer potential rankings of smoke constituents are illustrated. In addition, yields per plain, nonfilter cigarettes measured under ISO conditions are given, although these numbers cannot necessarily be transferred to filter cigarettes commercially available nowadays.

## Experimental Procedures

A sketch of the SPI–TOFMS coupled to the smoking machine is illustrated in Figure 1. The experimental setup used is described in detail in ref 9 and references therein; as a consequence, only a brief description is given here. The laser-based single-photon ionization technique uses vacuum ultraviolet (VUV) photons for ionization, which are generated via a 2-fold frequency tripling of fundamental Nd:YAG laser pulses (1064 → 355 → 118 nm; repetition rate 10 Hz). The wavelength of 118 nm is equivalent to 10.49 eV, which means that all organic species featuring an ionization potential lower than 10.49 eV are ionized. Subsequently, it can be used to detect a wide range of organic species such as aliphatic hydrocarbons and aromatic and carbonyl compounds (28–30). In contrast, most background gases such as N<sub>2</sub>, O<sub>2</sub>, CO<sub>2</sub>, and H<sub>2</sub>O are not ionized and, therefore, do not overload the detector and spectra. A great benefit compared to conventional ionization techniques, such as electron impact ionization, is the fact that almost no fragmentation occurs. Consequently, complex matrixes containing hundreds of substances can be resolved and investigated. The combination of soft SPI with time-of-flight mass spectrometry enables a comprehensive on-line monitoring of many types of industrial processes, which has been demonstrated for several applications, such as steel recycling (31), coffee roasting (32), and waste incineration (33, 34). In combination with a pyrolysis furnace, it was recently applied to clarify formation mechanisms of tobacco smoke constituents (35) and to characterize different types of tobacco (36).

Table 1. Illustration of the Chemical Structures of the Target Molecules as Well as Some Physical and Toxicological Data

Substance	Nitric oxide	Acetaldehyde	1,3-Butadiene	Acetone	Isoprene	Benzene	Toluene	Ethyl benzene	Xylene
Chemical structure	NO								
CAS No.	10102-43-9	75-07-0	106-99-0	67-64-1	78-79-5	71-43-2	108-88-3	100-41-4	1330-20-7
Molecular weight [g/mol]	30	44	54	58	68	78	92	106	106
Vapour pressure @ 20°C [kPa]	-	101	245	24	53.2	10	2.9	0.9	0.9
Boiling point [°C]	-151.8	-123	-4	56	34	80	111	136	138
Amount per cigarette [µg][1, 62]	100 – 600 (NOx)	400 - 1400	25 - 40	100 - 650	200 - 400	< 70	< 200	n. e.	n. e.
IARC class [63] *	3	2B	2A	3	2B	1	3	3	3
OSHA TWA8 [mg/m³][27, 63]	30	360	2.21	2400	n. e.	3.19	754	434	434
Ranking by Fowles and Bates [25,26]	n. e.	5	1	n. e.	n. e.	6	n. e.	n. e.	n. e.
Ranking by Vorhees[24]	n. e.	2	1	n. e.	n. e.	9	n. e.	n. e.	n. e.
Ranking by Rodgman & Green [27]	n. e.	5	1	n. e.	n. e.	16	n. e.	n. e.	n. e.
further health hazards **	a, b, c	d, e	f, g	d, e	f, g	f, g	f, g	h	h

\* IARC categories: 1, carcinogenic to humans; 2A, probably carcinogenic to humans; 2B, possibly carcinogenic to humans; 3, not classifiable as to its carcinogenicity to humans. n. e.: none established. \*\*: a, Lung inflammation (64); b, nitrosation agent for tobacco alkaloids for formation of tobacco-specific nitrosamines (1, 54, 59); c, precursor of NO<sub>2</sub> which is believed to form small organic radicals by addition to smoke constituents (1, 54, 59); d, respiratory health effects (25); e, eye irritation (25); f, reproductive effects (25); g, developmental effects (25); h, low acute and chronic toxicity (65).

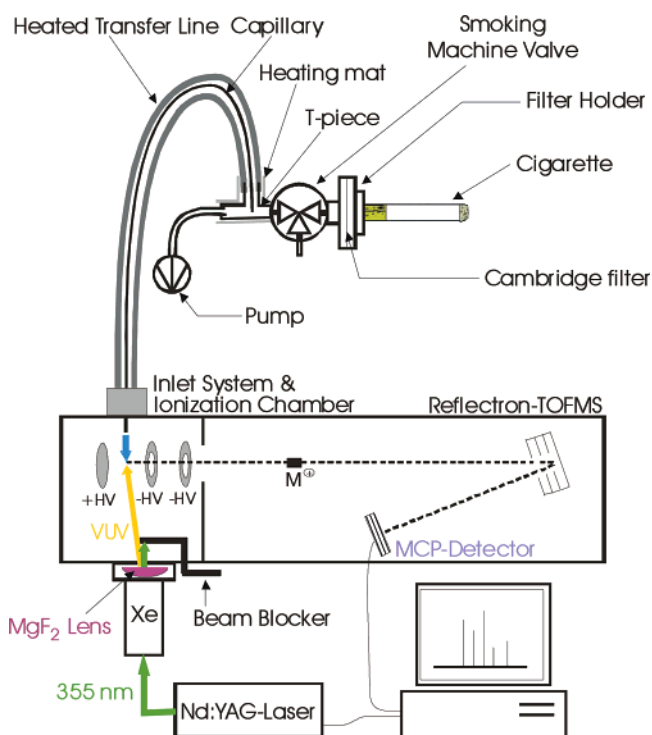


Figure 1. Experimental setup of the SPI-TOFMS coupled to the smoking machine.

The SPI-TOFMS was connected to a custom-made smoking machine based on a Borgwaldt single-port smoking machine. Modifications of the smoking machine were necessary to decrease contamination and memory effects of the commercially available smoking machine. A detailed description of the modified smoking machine can be found in ref 9. The connection element between the smoking-machine and analytical system was a heated transfer

line consisting of a deactivated silica capillary (length 1.5 m; inner diameter 0.32 mm;  $T = 220\text{ }^{\circ}\text{C}$ ; residence time  $< 1\text{ s}$ ). As a result, besides gaseous components, most volatile and semivolatile organic substances deposited on particles are evaporated by passing through the transfer line and, subsequently, are accessible to the photoionization process. Formation of artifacts could not be observed (9). Gas phase measurements without the particulate fraction were carried out by incorporating a filter in the cigarette holder. In tobacco science, separation is mostly done by using a quartz fiber filter pad stabilized by an organic binder for collecting the particulate phase of cigarette smoke (8). This so-called Cambridge filter, since it was originally manufactured by the Cambridge Filter Corporation, Syracuse, New York, was first described in 1959 by Wartman et al. (37). The filter pad traps particles larger than 0.1  $\mu\text{m}$  present in the cigarette smoke aerosol with 99.9% efficiency, while gas phase smoke components pass through the filter. Trapped material is usually referred to as total particulate matter (TPM) (38). It is, however, important remember that this is an arbitrary separation used for experimental convenience. There is nothing absolute about the separation of the vapor and particulate phases of the smoke aerosol by the Cambridge pad. The materials collected on the pad are affected by a host of factors, including moisture content, temperature, flow rate, and specific chemical interaction between aerosol constituents and the fiber glass. The pad can also collect some vapor phase constituents. Different separation techniques, such as electrostatic precipitation, and jet impaction traps give different values for the vapor/aerosol composition (8).

The smoking procedure was carried out according to the International Organization for Standardization (ISO) puff/smoulder conditions (35 mL puff volume, 2 s puff duration, one puff every 60 s, smoked to a butt length of filter plus 8 mm). Cigarettes were lit with a Borgwaldt (Borgwaldt GmbH, Germany) electric lighter. The cigarettes were stored for several days under controlled conditions of 60% relative air humidity and 22  $^{\circ}\text{C}$  (38). Regarding sample cigarettes, the study focused on the University of Kentucky 2R4F research cigarette. This research cigarette, available from the University of Kentucky, Kentucky Tobacco Research & Develop-



ment Center (KTRDC), replaces the former 1R4F and is thoroughly characterized in ref 39.

In principle, 10 mass spectra per second can be recorded since a 10 Hz laser was used. For the purpose of this work, three successive single laser shot mass spectra were averaged (time resolution 3.3 Hz) to improve signal stability. The signal intensities of the compounds of interest were summed up for each single cigarette puff to enable puff-by-puff-resolved quantification. Calibration was done by applying commercial standard gases (Linde AG, Germany). In total, three replicates of gas phase and whole smoke of every cigarette type were measured, and the corresponding mean and standard deviation were calculated.

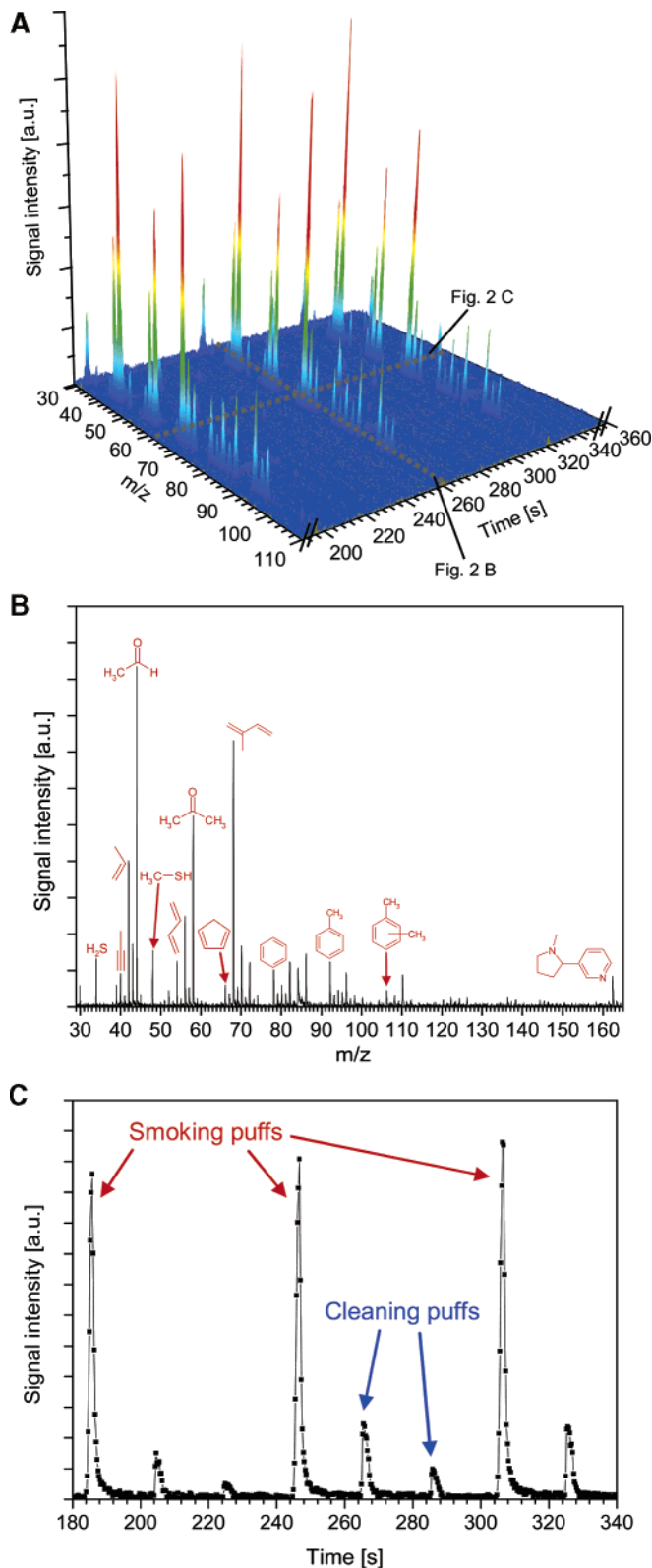
Resolution of the TOFMS (1800  $m/z$ , 33) is such that separation between isobaric compounds is difficult. This is hardly a problem for most substances in the framework of this study, since isobaric species of the selected target compounds are either not at all (ionization potential) or only weakly (low cross section for SPI) accessible by SPI, or their expected concentrations are lower than the standard deviations of the target compounds. Therefore, some quantitative information might be influenced by small amounts of isobaric species. In this context, the main focus was on a fast and robust method that is simple to operate and, at the same time, delivering comprehensive quantitative information rather than a highly accurate but time-consuming technique being limited to a low number of observable compounds. However, since a separation of ethyl benzene and xylene is not possible, their yield is stated as the sum of both.

## Results and Discussion

Figure 2A exhibits a three-dimensional illustration of the time-resolved analysis of the smoking process of a complete cigarette. For an easier illustration, only puff four to puff six are shown. A single mass spectrum featuring all the species in tobacco smoke which are accessible by this method is illustrated in Figure 2B. In there, several important smoke constituents are assigned to the corresponding mass peaks, for example, hydrogen sulfide (34  $m/z$ ), acetaldehyde (44  $m/z$ ), butadiene (54  $m/z$ ), acetone (58  $m/z$ ), isoprene (68  $m/z$ ), benzene (78  $m/z$ ), and nicotine (162  $m/z$ ). In addition, Figure 2C demonstrates the possible on-line capabilities of the setup used by plotting the time profile of three successive puffs (fourth to sixth puff) of the tobacco smoke compound acetaldehyde. These time-resolved signals were added leading to the sum signals of the individual puffs being the base for further discussion.

Smoking of the 2R4F cigarette resulted in puff numbers between eight and nine puffs. This is mainly due to slightly varying lighting behaviors of the cigarette and operator. The mean puff number out of three measurements was 8.7 for the whole smoke analysis and 8.3 for the vapor phase measurements. Consequently, in the following illustrations, the standard deviation for the ninth puff of the gas phase is nonexistent.

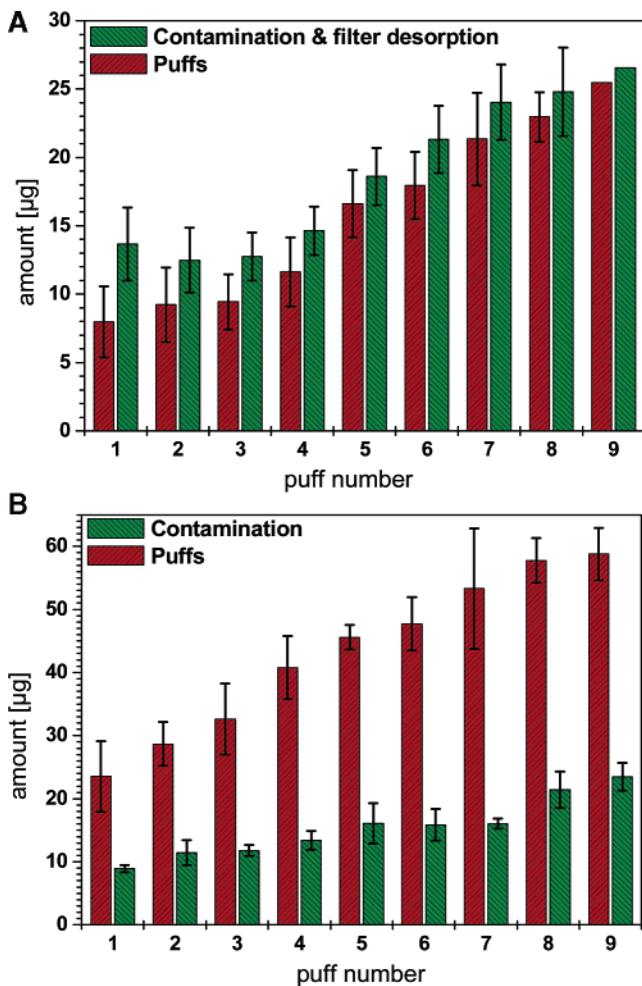
Mitschke et al. (9) have already described contamination problems and memory effects caused by the smoking machine. These effects are even greater for vapor phase measurements due to additional desorption from the filter. The trapped volatile organic material evaporates into the smoke stream of the following puffs. This organic matter is composed of either species in the particulate phase, or gas-phase constituents that condense when in contact with the filter material and pre-deposited particulate matter, respectively. Strictly speaking, the former fraction should not contribute to the gas phase. Contamination of the smoking machine is dependent on the type of machine used and, in this case, is reduced by modifying the filter holder and assembling the sampling device straight behind the filter of the cigarette. However, properties of the Cambridge filter are not modified further since this would cause the



**Figure 2.** (A) Comprehensive on-line monitoring of the cigarette smoking process; (B) SPI mass spectrum of whole smoke with several compounds assigned to mass peaks; (C) time-resolved illustration of the cigarette smoking process by means of acetaldehyde (44  $m/z$ ).

methodology to deviate from the ISO smoking machine sampling conditions.

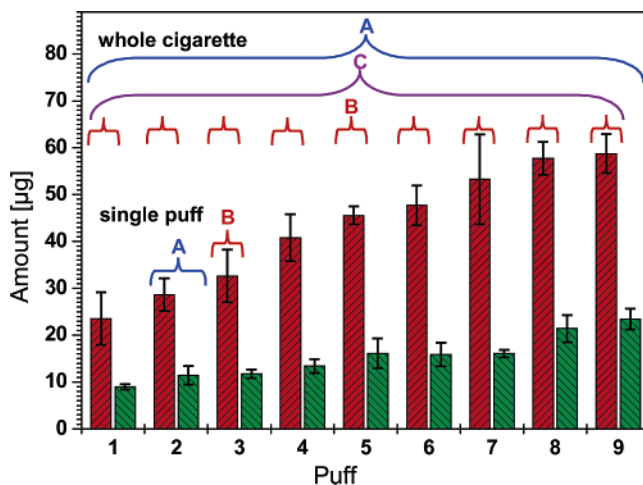
Figure 3, panels A (gas phase) and B (whole smoke) demonstrate the contamination and desorption effect for acetaldehyde. Between the cigarette puffs, several cleaning puffs with no cigarette present were taken until the signals declined to zero.



**Figure 3.** (A) Successive smoking and cleaning puffs of the gas phase of the 2R4F cigarette, shown for acetaldehyde (44 *m/z*); (B) successive smoking and cleaning puffs of whole smoke of the 2R4F cigarette, shown for acetaldehyde (44 *m/z*).

In general, only two cleaning puffs were necessary for this purpose. A further approach would be to replace the filter after every puff by a new one. However, this procedure was not possible for the setup used. The signal intensities were added together and compared to the smoking puffs. For whole smoke, contamination of the smoking machine for each puff amounted to about one-third of the corresponding smoking puff intensity. Contamination of the gas phase measurements with a Cambridge filter pad present resulted in even higher amounts than for the smoking puff, especially for the earlier puffs (Figure 3A). Concerning total yields of gas phase constituents from entire cigarettes, which have been determined routinely for many substances for a long time, this desorption effect also occurs. In this case, it is not as crucial as for single puff characterization because deposited material is evaporated by later puffs. However, when analysis is immediately stopped after the final puff, some material (here, 23 and 26  $\mu\text{g}$  acetaldehyde for whole smoke and vapor phase, respectively) is held back and does not contribute to the measured total amount per cigarette. These considerations are illustrated in Figure 4 by showing three different approaches for the collection of total smoke delivery (A–C) and two approaches for the single puff yield (A and B). Regarding total smoke, approach A was used.

Hence, the definition of “single puff” is problematic, since it is not clear if the subsequent contamination levels should be added to the yield of the previous puff (approach A). If this is not the case (approach B), thorough cleaning or filter changing



**Figure 4.** Illustration of the different approaches for the definition of single puff and whole cigarette.

**Table 2. Total Yields of Smoke Constituents of the 2R4F Research Cigarette of Three Different Studies (ISO Smoking Machine Conditions)**

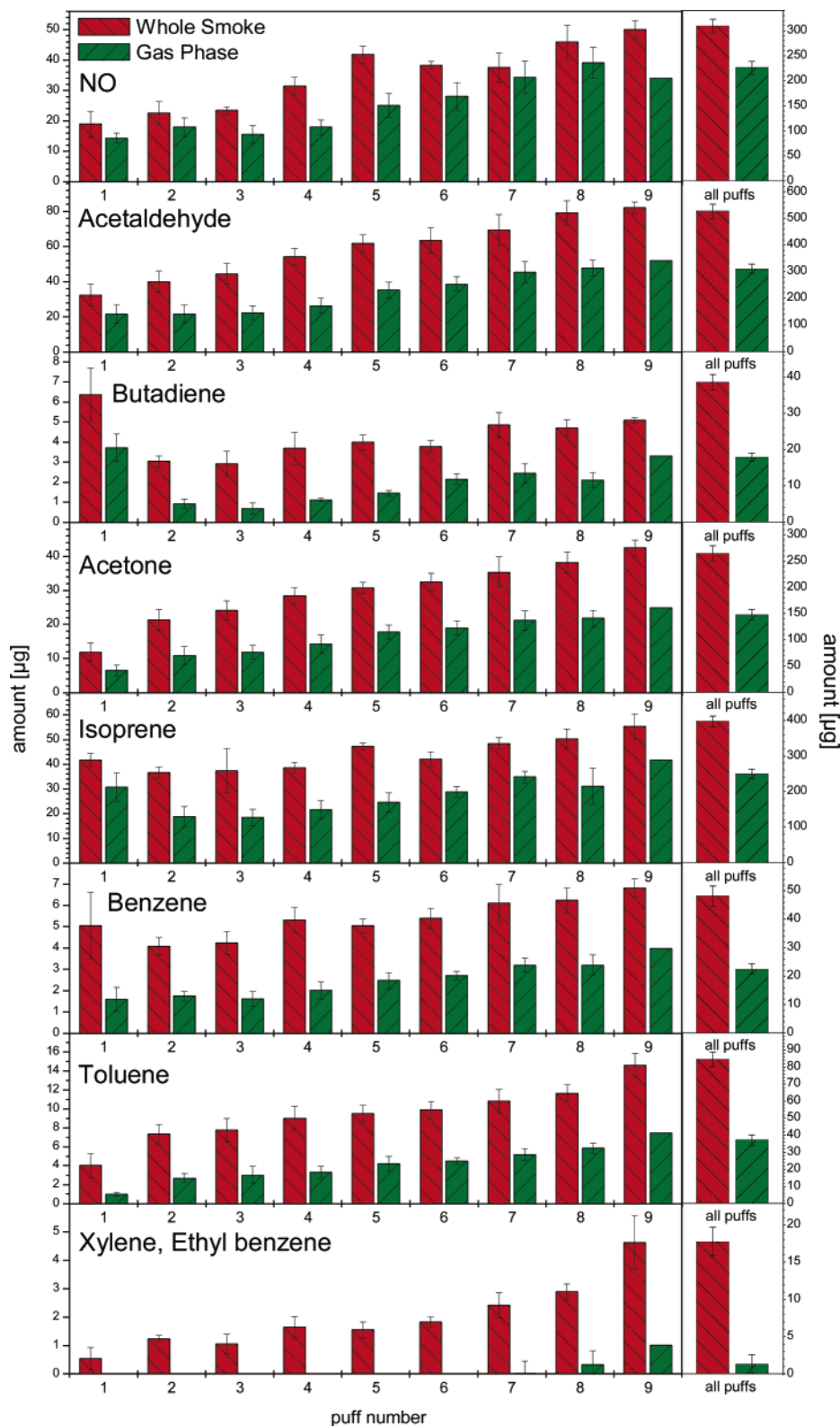
compound	this work	Chen et al. (39)	Wagner et al. (40)
Whole Smoke			
NO [ $\mu\text{g}/\text{cig}$ ]	309.6 $\pm$ 13.9	223.41	–
Acetaldehyde [ $\mu\text{g}/\text{cig}$ ]	527.1 $\pm$ 26.7	560.48;	562
		583.74 $\pm$ 13.18 <sup>a</sup>	
Butadiene [ $\mu\text{g}/\text{cig}$ ]	38.5 $\pm$ 2.2	29.94	37.1
Acetone [ $\mu\text{g}/\text{cig}$ ]	265.1 $\pm$ 15.1	264.74;	248
		261.62 $\pm$ 7.35 <sup>a</sup>	
Isoprene [ $\mu\text{g}/\text{cig}$ ]	397.2 $\pm$ 15.3	297.68	391
Benzene [ $\mu\text{g}/\text{cig}$ ]	48.2 $\pm$ 3.6	43.39	51.8
Toluene [ $\mu\text{g}/\text{cig}$ ]	84.5 $\pm$ 4.3	64.91	88.0
“Gas Phase”			
Acetaldehyde [ $\mu\text{g}/\text{cig}$ ]	310.7 $\pm$ 17.6	396.78 $\pm$ 71.02	–
Acetone [ $\mu\text{g}/\text{cig}$ ]	148.1 $\pm$ 9.6	205.52 $\pm$ 8.35	–

<sup>a</sup> Chen’s publication contained two values for acetaldehyde and acetone because two different analytical techniques were used.

after every puff will be required. Otherwise, this contamination level is added automatically to the subsequent smoking puff, which thereby affects the result of the subsequent puff. Consequently, for the following evaluations, the levels in the cleaning puffs have been added to the corresponding (preceding) puff yields (approach A), to account for the total single puff yield, for the following two reasons. First, it makes the results comparable to published data dealing with total amounts of various smoke compounds. Second, the levels of components in both the smoking puffs and the cleaning puffs are relevant to the human smoker. However, some particulate matter held back by the filter might evaporate into the smoke stream of the following puffs and may be wrongly considered to be in the gas phase. This source of error is also relevant for conventional total yield measurements of species in which a Cambridge filter is used.

To evaluate the quantitative results achieved by SPI–TOFMS and the definitions described above, the total delivery was cross-checked with total yields of the 2R4F research cigarette of two other studies available in the literature, namely, Chen et al. (39) and Wagner et al. (40). The comparison of the compounds’ yields can be found in Table 2. We observe that the achieved total yields are in good agreement with the values in the two referred papers which encouraged to carry out single puff quantification.

Figure 5 and Table 3 illustrate the puff-by-puff behavior and total yields of whole smoke and gas phase of the target



**Figure 5.** Puff-by-puff behavior and total yields of whole smoke (i.e., no Cambridge filter pad present) and "gas phase" (i.e., Cambridge filter pad present) of the target compounds of the 2R4F cigarette.

compounds and are the basis for further discussion. In contrast Table 4 lists the corresponding yields without cleaning puffs for comparison only.

Most substances (NO, acetaldehyde, acetone, benzene, toluene, and the C2-benzenes) feature an increase in concentration with increasing puff number. This trend can be observed in both whole smoke and in the vapor phase. It can be explained by the gradual reduction in cigarette length which causes the

following effects: filtration by the tobacco rod for products in the particulate phase is decreased, condensation of gas-phase constituents onto the tobacco is reduced, and air dilution as well as outward gaseous diffusion through cigarette paper and filter is lowered (41, 42). Also, more mass is burnt due to condensed/filtered material from earlier puffs.

From the results in Table 3 and Figure 5, it appears that more NO occurs in whole smoke than in the gas phase. Total yields



**Table 3. Puff-by-Puff and Total Yields of Whole Smoke and Gas Phase (Smoking Puffs + Cleaning Puffs) of the 2R4F Cigarette**

yield [ $\mu\text{g}$ ]	1 <sup>st</sup> puff	2 <sup>nd</sup> puff	3 <sup>rd</sup> puff	4 <sup>th</sup> puff	5 <sup>th</sup> puff	6 <sup>th</sup> puff	7 <sup>th</sup> puff	8 <sup>th</sup> puff	9 <sup>th</sup> puff	$\Sigma$
Whole Smoke (i.e., No Cambridge Filter Pad Present)										
Nitric oxide	18.9 $\pm$ 4.3	22.5 $\pm$ 3.8	23.4 $\pm$ 1.2	31.4 $\pm$ 2.8	41.7 $\pm$ 2.9	38.3 $\pm$ 1.2	37.6 $\pm$ 4.9	45.9 $\pm$ 5.7	50.0 $\pm$ 2.9	309.6 $\pm$ 13.9
Acetaldehyde	32.4 $\pm$ 6.0	40.1 $\pm$ 6.0	44.4 $\pm$ 6.1	54.2 $\pm$ 4.6	61.7 $\pm$ 5.1	63.6 $\pm$ 7.4	69.3 $\pm$ 9.0	79.2 $\pm$ 6.8	82.2 $\pm$ 3.2	527.1 $\pm$ 26.7
1,3-Butadiene	6.4 $\pm$ 1.3	3.0 $\pm$ 0.3	2.9 $\pm$ 0.6	3.7 $\pm$ 0.8	4.0 $\pm$ 0.4	3.8 $\pm$ 0.3	4.9 $\pm$ 0.6	4.7 $\pm$ 0.4	5.1 $\pm$ 0.1	38.5 $\pm$ 2.2
Acetone	11.9 $\pm$ 2.7	21.3 $\pm$ 3.0	24.1 $\pm$ 2.9	28.4 $\pm$ 2.5	30.7 $\pm$ 1.6	32.5 $\pm$ 2.5	35.3 $\pm$ 4.4	38.3 $\pm$ 2.9	42.5 $\pm$ 2.3	265.1 $\pm$ 15.1
Isoprene	41.6 $\pm$ 2.7	36.6 $\pm$ 2.3	37.4 $\pm$ 8.8	38.6 $\pm$ 2.3	47.2 $\pm$ 1.5	41.9 $\pm$ 3.1	48.3 $\pm$ 2.3	50.2 $\pm$ 4.0	55.3 $\pm$ 5.0	397.2 $\pm$ 15.3
Benzene	5.0 $\pm$ 1.6	4.1 $\pm$ 0.4	4.2 $\pm$ 0.5	5.3 $\pm$ 0.6	5.0 $\pm$ 0.3	5.4 $\pm$ 0.5	6.1 $\pm$ 0.9	6.2 $\pm$ 0.6	6.8 $\pm$ 0.4	48.2 $\pm$ 3.6
Toluene	4.0 $\pm$ 1.3	7.3 $\pm$ 1.0	7.7 $\pm$ 1.2	9.0 $\pm$ 1.3	9.5 $\pm$ 0.9	9.9 $\pm$ 0.9	10.8 $\pm$ 1.2	11.6 $\pm$ 0.9	14.6 $\pm$ 1.2	84.5 $\pm$ 4.3
C2-benzenes	0.5 $\pm$ 0.4	1.2 $\pm$ 0.1	1.1 $\pm$ 0.3	1.6 $\pm$ 0.4	1.6 $\pm$ 0.3	1.8 $\pm$ 0.2	2.4 $\pm$ 0.4	2.9 $\pm$ 0.3	4.6 $\pm$ 0.9	17.8 $\pm$ 1.9
"Gas Phase" (i.e., Cambridge Filter Pad Present)										
Nitric oxide	14.3 $\pm$ 1.6	17.9 $\pm$ 3.0	15.6 $\pm$ 3.0	17.9 $\pm$ 2.4	25.1 $\pm$ 3.9	28.1 $\pm$ 4.5	34.3 $\pm$ 5.4	39.1 $\pm$ 5.1	34.0	226.2 $\pm$ 12.6
Acetaldehyde	21.6 $\pm$ 5.2	21.7 $\pm$ 4.9	22.2 $\pm$ 3.9	26.3 $\pm$ 4.3	35.2 $\pm$ 4.5	38.6 $\pm$ 4.2	45.4 $\pm$ 6.1	47.8 $\pm$ 4.7	52.0	310.7 $\pm$ 17.6
1,3-Butadiene	3.7 $\pm$ 0.7	0.9 $\pm$ 0.2	0.7 $\pm$ 0.3	1.1 $\pm$ 0.1	1.5 $\pm$ 0.1	2.1 $\pm$ 0.3	2.4 $\pm$ 0.5	2.1 $\pm$ 0.4	3.3	17.9 $\pm$ 1.1
Acetone	6.5 $\pm$ 1.6	10.9 $\pm$ 2.6	11.9 $\pm$ 2.0	14.3 $\pm$ 2.7	17.8 $\pm$ 2.1	19.0 $\pm$ 2.1	21.2 $\pm$ 2.8	21.8 $\pm$ 2.4	24.9	148.1 $\pm$ 9.6
Isoprene	30.7 $\pm$ 5.7	18.8 $\pm$ 4.0	18.4 $\pm$ 3.3	21.6 $\pm$ 3.8	24.5 $\pm$ 3.9	28.8 $\pm$ 2.2	34.8 $\pm$ 2.3	31.1 $\pm$ 7.3	41.5	250.2 $\pm$ 13.6
Benzene	1.6 $\pm$ 0.6	1.7 $\pm$ 0.2	1.6 $\pm$ 0.3	2.0 $\pm$ 0.4	2.5 $\pm$ 0.4	2.7 $\pm$ 0.2	3.2 $\pm$ 0.3	3.2 $\pm$ 0.5	4.0	22.5 $\pm$ 1.7
Toluene	1.0 $\pm$ 0.2	2.7 $\pm$ 0.5	3.0 $\pm$ 0.9	3.3 $\pm$ 0.6	4.2 $\pm$ 0.8	4.5 $\pm$ 0.4	5.2 $\pm$ 0.6	5.8 $\pm$ 0.6	7.4	37.1 $\pm$ 3.1
C2-benzenes	0	0	0	0	0	0	0	0.3 $\pm$ 0.5	1.0	1.3 $\pm$ 1.3

**Table 4. Puff-by-Puff and Total Yields of Whole Smoke and Gas Phase (Smoking Puffs Only) of the 2R4F Cigarette**

yield [ $\mu\text{g}$ ]	1 <sup>st</sup> puff	2 <sup>nd</sup> puff	3 <sup>rd</sup> puff	4 <sup>th</sup> puff	5 <sup>th</sup> puff	6 <sup>th</sup> puff	7 <sup>th</sup> puff	8 <sup>th</sup> puff	9 <sup>th</sup> puff	$\Sigma$
Whole Smoke (i.e., No Cambridge Filter Pad Present)										
Nitric oxide	12.2 $\pm$ 2.9	15.6 $\pm$ 2.8	16.4 $\pm$ 0.7	22.6 $\pm$ 2.8	30.5 $\pm$ 2.1	28.6 $\pm$ 1.0	28.7 $\pm$ 4.4	33.5 $\pm$ 3.2	35.8 $\pm$ 1.1	224.5 $\pm$ 10.0
Acetaldehyde	23.5 $\pm$ 5.6	28.7 $\pm$ 3.5	32.6 $\pm$ 5.7	40.8 $\pm$ 5.0	45.6 $\pm$ 1.9	47.7 $\pm$ 4.2	53.3 $\pm$ 9.6	57.7 $\pm$ 3.5	58.8 $\pm$ 4.1	388.7 $\pm$ 21.0
1,3-Butadiene	5.1 $\pm$ 1.2	2.5 $\pm$ 0.2	2.4 $\pm$ 0.5	2.9 $\pm$ 0.6	3.1 $\pm$ 0.1	3.1 $\pm$ 0.2	4.0 $\pm$ 0.7	3.7 $\pm$ 0.5	3.8 $\pm$ 0.3	30.6 $\pm$ 1.9
Acetone	8.7 $\pm$ 2.3	15.8 $\pm$ 1.8	18.3 $\pm$ 2.8	22.0 $\pm$ 2.7	23.4 $\pm$ 1.7	25.0 $\pm$ 1.4	27.8 $\pm$ 4.7	28.6 $\pm$ 1.8	30.6 $\pm$ 2.6	200.4 $\pm$ 12.2
Isoprene	31.1 $\pm$ 3.8	27.5 $\pm$ 2.1	28.7 $\pm$ 7.2	30.3 $\pm$ 2.4	36.8 $\pm$ 2.5	33.1 $\pm$ 2.0	38.1 $\pm$ 3.0	38.1 $\pm$ 1.7	41.8 $\pm$ 5.7	305.3 $\pm$ 13.4
Benzene	4.1 $\pm$ 1.4	3.4 $\pm$ 0.3	3.5 $\pm$ 0.6	4.3 $\pm$ 0.6	4.1 $\pm$ 0.3	4.3 $\pm$ 0.3	5.1 $\pm$ 1.0	5.0 $\pm$ 0.4	5.2 $\pm$ 0.5	38.9 $\pm$ 3.1
Toluene	3.1 $\pm$ 0.8	5.8 $\pm$ 0.7	6.1 $\pm$ 1.3	7.2 $\pm$ 1.3	7.5 $\pm$ 0.8	7.9 $\pm$ 0.6	8.6 $\pm$ 1.3	9.1 $\pm$ 0.7	10.7 $\pm$ 1.3	65.9 $\pm$ 3.6
C2-benzenes	0.5 $\pm$ 0.2	1.2 $\pm$ 0.1	1.1 $\pm$ 0.3	1.4 $\pm$ 0.3	1.5 $\pm$ 0.3	1.7 $\pm$ 0.2	2.4 $\pm$ 0.3	2.5 $\pm$ 0.4	3.6 $\pm$ 0.7	15.8 $\pm$ 1.6
"Gas Phase" (i.e., Cambridge Filter Pad Present)										
Nitric oxide	5.8 $\pm$ 1.1	8.4 $\pm$ 2.6	7.5 $\pm$ 1.9	8.7 $\pm$ 1.7	13.8 $\pm$ 2.4	14.6 $\pm$ 3.1	18.3 $\pm$ 3.8	21.9 $\pm$ 4.6	19.3	118.3 $\pm$ 8.8
Acetaldehyde	8.0 $\pm$ 2.6	9.2 $\pm$ 2.7	9.4 $\pm$ 2.0	11.6 $\pm$ 2.5	16.6 $\pm$ 2.5	18.0 $\pm$ 2.5	21.4 $\pm$ 3.4	23.0 $\pm$ 1.8	25.5	142.6 $\pm$ 8.9
1,3-Butadiene	1.5 $\pm$ 0.4	0.5 $\pm$ 0.2	0.4 $\pm$ 0.1	0.6 $\pm$ 0.1	1.0 $\pm$ 0.1	1.1 $\pm$ 0.2	1.3 $\pm$ 0.2	1.2 $\pm$ 0.1	1.7	9.5 $\pm$ 0.6
Acetone	1.8 $\pm$ 0.7	4.3 $\pm$ 1.5	4.9 $\pm$ 1.0	6.0 $\pm$ 1.5	8.0 $\pm$ 1.0	8.4 $\pm$ 1.1	9.3 $\pm$ 1.4	9.7 $\pm$ 0.9	11.3	63.6 $\pm$ 4.5
Isoprene	12.2 $\pm$ 3.8	8.7 $\pm$ 2.6	8.1 $\pm$ 1.5	10.2 $\pm$ 2.4	12.0 $\pm$ 1.9	13.8 $\pm$ 1.8	17.2 $\pm$ 1.2	15.5 $\pm$ 3.0	21.4	119.1 $\pm$ 7.4
Benzene	0.5 $\pm$ 0.2	0.9 $\pm$ 0.3	0.8 $\pm$ 0.1	0.9 $\pm$ 0.3	1.3 $\pm$ 0.2	1.1 $\pm$ 0.1	1.4 $\pm$ 0.2	1.4 $\pm$ 0.1	1.7	9.9 $\pm$ 0.8
Toluene	0.3 $\pm$ 0	1.0 $\pm$ 0.7	1.0 $\pm$ 0.3	1.1 $\pm$ 0.3	1.4 $\pm$ 0.1	1.5 $\pm$ 0.3	1.7 $\pm$ 0.2	1.8 $\pm$ 0.3	2.2	12.1 $\pm$ 1.1
C2-benzenes	0	0	0	0	0	0	0	0	0	0

in NO for the 2R4F cigarette are  $310.0 \pm 13.9 \mu\text{g}$  (WS) and  $226.2 \pm 12.6 \mu\text{g}$  (GP). Puff-resolved values increase from  $18.9 \pm 4.3$  to  $50.0 \pm 2.9 \mu\text{g}$  for whole smoke (WS) and from  $14.3 \pm 1.6$  to  $39.1 \pm 5.1/34.0 \mu\text{g}$  for the gas phase (8th/9th puff; GP). Of course, NO is a small molecule and will be entirely in the gas phase of cigarette smoke. The observed results for NO must reflect a reaction that removes NO, which is enhanced by the presence of the Cambridge pad. Although such an effect of the Cambridge pad has not been reported previously for NO, Williams (43) has observed recoveries of only 87–91% when NO was added to whole smoke using a nondispersive infrared analytical technique together with a Cambridge filter pad. He postulated that NO was being lost by its reaction with other smoke constituents such as amines and alcohols to form nitrosamines and methyl nitrite, respectively. Nitric oxide is known to oxidize within a few seconds in smoke to generate nitrogen dioxide, and the nitrogen dioxide subsequently reacts within a further few seconds with smoke constituents such as methyl alcohol, isoprene, butadiene, and acrolein (44, 45). The rate of oxidation of nitric oxide to nitrogen dioxide in mainstream smoke has been determined in several studies (43, 45–47). The rate constant in smoke is almost a magnitude higher than for the pure gas phase oxidation, possibly due to catalysis of the reaction by free radicals present in smoke. In the present study, the transit time of the smoke to the analyzer will be greater in the presence of the Cambridge pad because of the tortuous path of the smoke through the filter pad. This allows more time for reaction of NO and will result in the measurement of lower quantities of NO when the Cambridge filter pad is present.

The occurrence of these reactions of NO indicates that, at least for reactive smoke constituents such as NO, the Cambridge pad is not an ideal filter for separating all the gas and particulate phase constituents of cigarette smoke, as discussed above. It is experimentally convenient to use a Cambridge pad in many smoke analyses, but it does not give an absolute separation of the two phases. In the present study, the more authentic NO values in each puff of the smoking regime will be those that were determined in the absence of the Cambridge filter pad. For comparison, in the following, only the amount of the ninth puff of the gas phase is stated. The reason for the missing error margin was discussed earlier. Values for all puffs from the first to the last including standard deviation can be taken from Table 3.

Acetaldehyde exhibited the steepest increase from the first puff ( $32.4 \pm 6.0 \mu\text{g}$  (WS)/ $21.6 \pm 5.2 \mu\text{g}$  (GP)) to the last puff ( $82.2 \pm 3.2 \mu\text{g}$  (WS)/ $52.0 \mu\text{g}$  (GP)) yielding totally  $527.1 \pm 26.7 \mu\text{g}$  (WS)/ $310.7 \pm 17.6 \mu\text{g}$  (GP).

Acetone, similar to acetaldehyde, features a rather steady increase from the first to the last puff for both whole smoke and vapor phase. Puff yields start with  $11.9 \pm 2.7 \mu\text{g}$  (WS)/ $6.5 \pm 1.6 \mu\text{g}$  (GP) and reach up to  $42.5 \pm 2.3 \mu\text{g}$  (WS)/ $24.9 \mu\text{g}$  (GP) which finally sums up to  $265.1 \pm 15.1 \mu\text{g}$  (WS)/ $148.1 \pm 9.6 \mu\text{g}$  (GP).

Regarding the aromatic species benzene and toluene, whole smoke and vapor phase exhibit a similar increase of signal intensity with increasing puff number. Total yield of benzene is  $48.2 \pm 3.6 \mu\text{g}$  (WS)/ $22.5 \pm 1.7 \mu\text{g}$  (GP) and of toluene  $84.5 \pm 4.3 \mu\text{g}$  (WS)/ $37.1 \pm 3.1 \mu\text{g}$  (GP). The substances occur in whole smoke and gas phase and range for benzene from  $5.0 \pm$

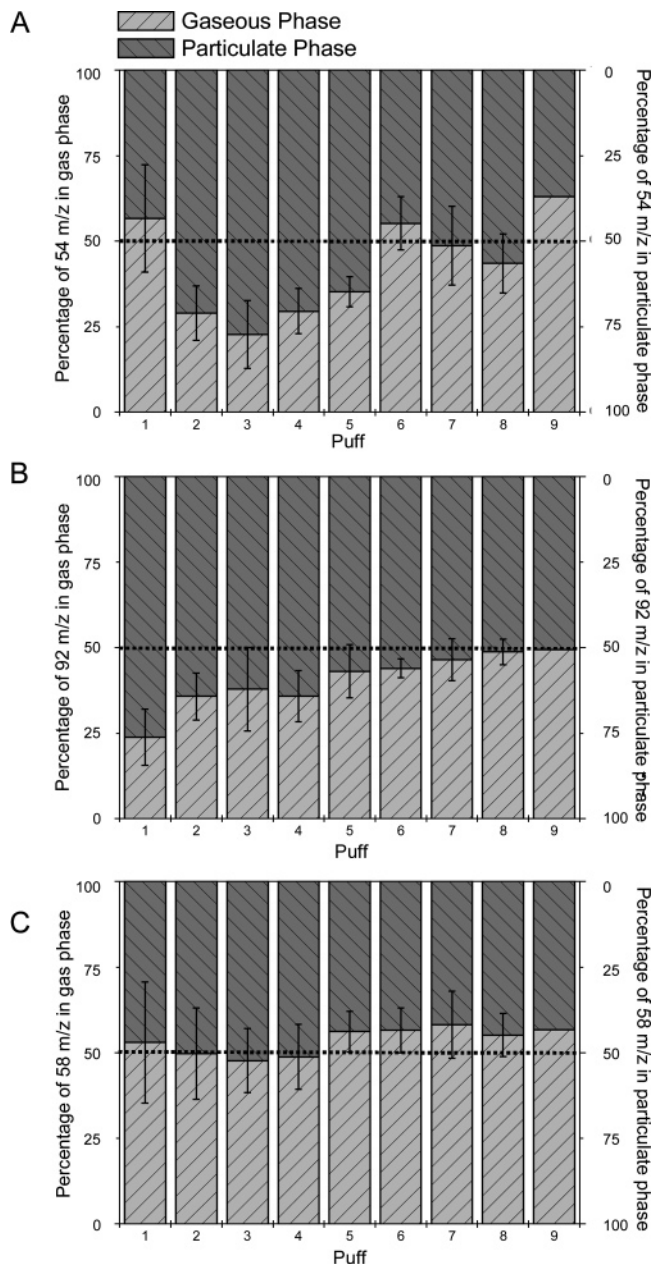
1.6  $\mu\text{g}$  (WS)/1.6  $\pm$  0.6  $\mu\text{g}$  (GP) in the first puff to 6.8  $\pm$  0.4  $\mu\text{g}$  (WS)/4.0  $\mu\text{g}$  (GP) in the last puff and for toluene from 4.0  $\pm$  1.3  $\mu\text{g}$  (WS)/1.0  $\pm$  0.2  $\mu\text{g}$  (GP) to 14.6  $\pm$  1.2  $\mu\text{g}$  (WS)/7.4  $\mu\text{g}$  (GP).

In contrast, the C2-benzenes ethyl benzene and xylene are almost entirely retained by the Cambridge filter, that is, are almost entirely in the particulate phase of smoke. Only the late puffs gave weak signals in the gas phase. For whole smoke, values increased from below 1  $\mu\text{g}$  to about 5  $\mu\text{g}$ . Thereby, the total amount measured added up to 18.8  $\pm$  2.0  $\mu\text{g}$ .

Interestingly, butadiene and isoprene exhibit a high yield in the first puff (butadiene, 6.4  $\pm$  1.3  $\mu\text{g}$  (WS)/3.7  $\pm$  0.7  $\mu\text{g}$  (GP); isoprene, 41.6  $\pm$  2.7  $\mu\text{g}$  (WS)/30.7  $\pm$  5.7  $\mu\text{g}$  (GP)), followed by smaller amounts in the second puff (butadiene, 3.0  $\pm$  0.3  $\mu\text{g}$  (WS)/0.9  $\pm$  0.2  $\mu\text{g}$  (GP); isoprene, 36.7  $\pm$  2.3  $\mu\text{g}$  (WS)/18.8  $\pm$  4.0  $\mu\text{g}$  (GP)) and then the usual increase until the last puff (butadiene, 5.1  $\pm$  0.1  $\mu\text{g}$  (WS)/3.3  $\mu\text{g}$  (GP); isoprene, 55.3  $\pm$  5.0  $\mu\text{g}$  (WS)/41.5  $\mu\text{g}$  (GP)). The reason for this increased first puff behavior, which is more pronounced for butadiene, may be related to the initial lighting of the cigarette before the first puff is taken. During lighting, the tobacco is heated from ambient temperature, resulting in a much higher temperature increase and heating rate than is the case for the subsequent puffs, since the inter-puff smoulder temperature is around 600–700  $^{\circ}\text{C}$ . Thus, tobacco closest to the burning zone is preheated for all puffs except the first one. Similar higher levels in the first puff have also been reported for formaldehyde and ethylene (10, 16, 18, 19) as well as benzo[*a*]pyrene (48, 49). This trend attracts special interest when assuming that human smokers might inhale differently during the first puff in order to light the cigarette properly. The total amounts of these species are 38.5  $\pm$  2.2  $\mu\text{g}$  (WS)/17.9  $\pm$  1.1  $\mu\text{g}$  (GP) and 397.2  $\pm$  15.3  $\mu\text{g}$  (WS)/250.2  $\pm$  13.6  $\mu\text{g}$  (GP) for butadiene and isoprene, respectively.

When subtracting the gas phase (i.e., Cambridge filter pad present) from the whole smoke (i.e., Cambridge filter pad not present), conclusions can be drawn about the ratio of compounds in the gas phase and the particulate phase for the experimental setup applied. In so doing, three slightly different behaviors can be observed, as shown in Figure 6.

Pankow et al. (50) have recently predicted the fraction of a compound in the gas phase of smoke at equilibrium as a function of vapor pressure and particulate matter concentration. The results in Figure 6 differ from those of Pankow et al. The calculations of Pankow et al. were based on their measurements, which were made under conditions very different from those of the present study. First, they used a more intense machine regime of a 45 mL puff of 2 s duration taken every 30 s. This intense smoking regime is known to produce much larger quantities of water in both the gas and particulate phases of smoke than the ISO smoking machine regime of a 35 mL puff of 2 s duration taken every minute, as used in this study (51). The trapping efficiency of the Cambridge pad for smoke components is known to be a function of the moisture on the pad (52). Second, Pankow et al. collected their smoke into a Teflon bag and analyzed it 15 min after collection, whereas fresh smoke (<1 s old) was analyzed in the present study. It is therefore not surprising that the results of the present study (Figure 6) differ from those of Pankow et al. The fresh smoke as it hits the Cambridge pad in the present study, which is less than 1 s old, will not be at equilibrium. Fresh mainstream smoke is a continually changing, complex aerosol. The number concentration and diameter of the smoke particles change substantially in the first few seconds after the mainstream smoke



**Figure 6.** Puff-by-puff-resolved ratio of 1,3-butadiene (A), toluene (B), and acetone (C) in the vapor phase and the particulate phase of the 2R4F cigarette.

exits the cigarette (1); the depletion of volatile constituents from the particles can take fractions of a second (53); and it has been extensively demonstrated by Pryor et al. that the concentrations of many smoke constituents, for example, butadiene, NO, and NO<sub>2</sub>, are interrelated and change substantially within minutes (45, 54–59). The fresh smoke analyzed on a puff-by-puff basis in the present study will be in a similar condition to the smoke that enters the smoker's lungs in a real smoking situation. It will not be aged and certainly not at equilibrium.

Substances such as acetone (Figure 6C) and, to a lesser extent, acetaldehyde occur in both phases in equal amounts. Within the accuracy of measurement, no difference in phase affiliation can be observed between successive puffs. In contrast, toluene (Figure 6B) and benzene feature higher yields in the particulate phase in the first puff, and for the later puffs, the ratio gradually shifts toward being balanced between both phases. The reasons for this behavior are similar to the ones described earlier for the increasing yields with puff number. Reduction of cigarette



length as the cigarette is smoked results in a smaller contact surface and residence time in the shorter tobacco rod and hence less condensation into aerosol particles. Less cooling of smoke in a shorter cigarette may also contribute to this. However, a decrease in dilution should support condensation but, apparently, plays a minor role for these substances. The same holds for the reduced filtration effect of the unburnt tobacco between the cigarette filter and burning zone. Again, the compounds which featured the highest yield in the first puff (butadiene and isoprene) differ (Figure 6A), since the fraction in the gas phase is higher for the first puff. In the second puff, the ratio is shifted toward the particulate phase, followed by a rise of the gas phase fraction for the subsequent puffs. Consequently, it seems as if the high yield in the first puff is predominantly caused by gaseous butadiene and isoprene. From the second puff onward, the typical behavior can be observed again.

In general, when we look only at the smoking puffs without cleaning puffs (Table 4), the puff-by-puff partition looks similar for all compounds but is shifted toward the particulate phase (not shown here) because the cleaning puffs influence the vapor phase results more strongly.

### Conclusion

SPI–TOFMS coupled to a smoking machine has proven to be well-suited for quick and comprehensive puff-resolved quantification of several cigarette smoke constituents. Results obtained gave insight into the varying burden and phase affiliation of compounds from early to late puffs. In addition, influences of the commonly applied separation and sampling techniques could be determined, which points to deficiencies of some existing analytical methodologies. Although these effects on the complex smoke matrix cannot entirely be resolved, the setup would certainly enable us to compare different types of cigarettes and any new developments on a puff-by-puff basis. Coupling of the SPI–TOFMS to an appropriate particle analyzer device may help to unravel the complex smoke formation by incorporating and investigating simultaneous changes of several particle properties, such as particle nature, number, size distribution, and surface area. In the framework of this study, quantification was carried out for nine selected chemicals, but in principle, a much greater number of substances are attainable.

In terms of the photoionization method, the latest improvements are the development of electron-beam-pumped rare-gas excimer VUV-lamp systems for SPI, which replace costly laser instruments (60, 61). Enhancement and commercialization of this technique would make it accessible for a wider range of applications.

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