Absorption efficiency of particulate and gaseous aerosol constituents on aqueous surfaces

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Introduction and Objectives

In vitro aerosol exposures are commonly conducted at the air-liquid interface, that is, test aerosols in their native form are brought into contact with cellular models of the respiratory tract epithelia that are not covered by cell culture medium, but usually only by a mucus layer which is produced by the cells themselves.
Different aerosol constituents are thereby absorbed by the biological test system with different efficiencies. These are functions of physicochemical properties of the constituents such as their vapor pressure or solubility in the (aqueous) mucus, and specific properties of the exposure system such as the used principle of aerosol delivery.
For a selection of constituents of 3R4F reference cigarette smoke, we determined absorption efficiencies by exposing samples of phosphate buffered saline (PBS) in the Vitrocell® 24/48 aerosol exposure system, followed by quantification of the absorbed smoke constituents and comparison to their presence in the native smoke. PBS thereby served as a model for the aqueous surface of cell cultures.
The reported, compound specific absorption efficiencies show that the relative contribution of individual smoke constituents to the overall composition of the applied and the delivered smoke may differ by orders of magnitude. A grouping according to chemical structure indicates that, using a larger set of compounds, a predictive model of absorption efficiencies can potentially be developed.

Methods

Smoke generation: 3R4F reference cigarettes (University of Kentucky) were smoked on a rotary smoking machine according to the Health Canada smoking regime (55 mL puffs, 2 seconds aspiration, 30 seconds between puffs (Health Canada Test Method T-115;1999))
Smoke trapping for determining smoke composition: A total of 22 puffs (two cigarettes, 11 puffs per cigarette) were passed through the Vitrocell system and bubbled through three serially connected glass impingers, each containing 5 mL N,N-dimethylformamide (DMF) at -50 ± 5°C. The trapping was repeated 12 times with the smoke being diluted to 22% or 45% with air of 65 ± 5% relative humidity and a temperature of 37°C. DMF retrieved from the three impingers was pooled and subjected to chemical analysis.
Smoke exposures in the Vitrocell aerosol exposure system for determining smoke absorption: 100 µL PBS were pipetted into 24-well format cell culture inserts and exposed in the Vitrocell system to 110 puffs of 3R4F smoke (ten cigarettes, 11 puffs per cigarette). The system was operated at 37°C, the smoke was diluted to 22% (65 ± 5% relative humidity, 37°C). Exposed PBS samples were collected and subjected to chemical anaylsis. 4 independent repetitions were performed.
Analytical procedures: In order to adress 22 cigarette smoke-relevant compounds covering a broad spectrum of physicochemical properties, 5 analytical methods were developed. Method 1 was developed in-house, methods 2 - 5 by Analytisch Biologisches Forschungslabor (ABF), Munich, Germany (Table 1).
Calculation of absorption efficiencies: The masses per puff detected in PBS and DMF samples were calculated and normalized by the used smoke concentration. Values obtained from PBS samples were further corrected for the internal smoke sampling in the Vitrocell system (volume flow rate of 2 mL/min through the exposure chambers). The average value obtained from PBS samples was then expressed in % of the average value obtained from DMF samples.
As smoke trapping in DMF was performed downstream the Vitrocell system, these absorption efficiencies are not affected by smoke losses inside the system and only describe the internal smoke sampling (sampling with a flow speed of 5 mm/s from a stream of 0.95 m/s at a 90° angle) and the absorption under the aerosol delivery principle applied in the Vitrocell system (stagnation flow conditions over the cell culture).

Table 1: Description of the five analytical methods used. Method 1 was developed in-house, methods 2 - 5 by Analytisch Biologisches Forschungslabr (ABF) , a certified bioanalytical contract research laboratory in Munich, Germany

	Method 1	Method 2	Method 3	Method 4	Method 5
Method description	HS-GC-HRMS Electron ionization Positive mode	LCMS-MS Mobile phase buffers: A) 0.1 % ammonium acetate in water (pH 5.0) B) methanol Gradient A:B over 13 minutes 95:15, 55:45, 15:95, 95:15 Electrospray ionization Positive mode	Liquid-liquid extraction (hexane) followed by LC-MS Mobile phase buffer (isocratic): 0.1 % ammonium acetate in water and acetonitrile Chemical ionization Positive mode	LCMS-MS Mobile phase buffers (gradient) A) 0.1 % ammonium acetate in water (pH 6.3) B) Acetonitrile + 0.1% formic acid Gradient A:B over 17 minutes 90:10, 40:60, 30:70, 90:10 Electrospray ionization Positive mode	Liquid-liquid extraction (cyclohexane) followed by GC-MS Electron ionization Positive mode
Targeted compounds	Diacetyl 2,3-pentanedione Benzene Isobutyraldehyde Isovaleraldehyde Thiophene Toluene	Nicotine Nornicotine Anabasine Anatabine 3-Ethenylpyridine (3-EP)	Solanesol	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) N-Nitrosonornicotine (NNN) N-Nitrosoanabasine (NAB) N-Nitrosoanatabine (NAT)	Naphthalene Phenanthrene Fluorene Pyrene Benzo[a]pyrene

Results

Table 2: The targeted smoke constituents, sorted by their absorption efficiencies. Selected structural and physicochemical properties are listed

Smoke constituent	CAS #	Absorption efficiency	Structural and physicochemical properties								
			Amines	Nitrosamines	Terpenes	Aldehydes	Ketones	Partly or fully aromatic	Aromatic heterocycles	Solubility in water[a] (mg/L at 25°C)	Vapor pressure[a] (kPa, at 25°C)
Benzo[a]pyrene	50-32-8	0%[d]						x		1.62E-03	7.32E-10
Naphthalene	91-20-3	0%[d]						x		31.00	1.13E-02
Toluene	108-88-3	0.04%						x		5.26E+02	3.79
Benzene	71-43-2	0.05%						x		1.79E+03	12.64
Solanesol	13190-97-1	0.05%			x					2.3E-03[e]	9.21E-22[e]
Pyrene	129-00-0	0.24%						x		1.35E-01	6.00E-07
Thiophene	110-02-1	0.33%						x	S	3.02E+03	10.62
Phenanthrene	85-01-8	0.88%						x		1.15	1.61E-05
Fluorene	86-73-7	2.8%						x		1.69	8.00E-05
Isovaleraldehyde	590-86-3	5.7%				x				1.40E+03	6.67
Isobutyraldehyde	78-84-2	6.5%				x				8.90E+04	23.06
Nornicotine	5746-86-1	11%	x					x	N	1E+06[e]	1.23E-03
N-Nitrosoanabasine	37620-20-5	12%		x				x	N	6.9E+03[e]	1.21E-05[e]
NNN	80508-23-2	14%		x				x	N	1E+04[e]	2.52E-05[e]
N-Nitrosoanatabine	887407-16-1	15%		x				x	N	3.6E+03[e]	2.83E-05[e]
NNK	64091-91-4	16%		x			x	x	N	1.03E+05	9.06E-06
Nicotine	54-11-5	20%	x					x	N	1.00E+06	5.07E-03
3-EP	1121-55-7	21%						x	N	1.00E+05	2.79
2,3-pentanedione	600-14-6	29%				x	x			6.67E+04[b]	2.66[c]
Diacetyl	431-03-8	37%				x	x			2.00E+05	7.50
Anatabine	2743-90-0	42%	x					x	N	1.00E+03	7.46E-03[e]
Anabasine	13078-04-1	53%	x					x	N	1.00E+03	6.62E-03[e]

a) data retrieved from PubChem, unless otherwise stated
b) value at 20°C
c) value at 15°C
d) absorption efficiencies of 0% result from the lower limit of detection and do not indicate the absolute absence of the compound in PBS samples
e) data retreived from: Scydinder.cas.org ; Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2017 ACD/Labs)

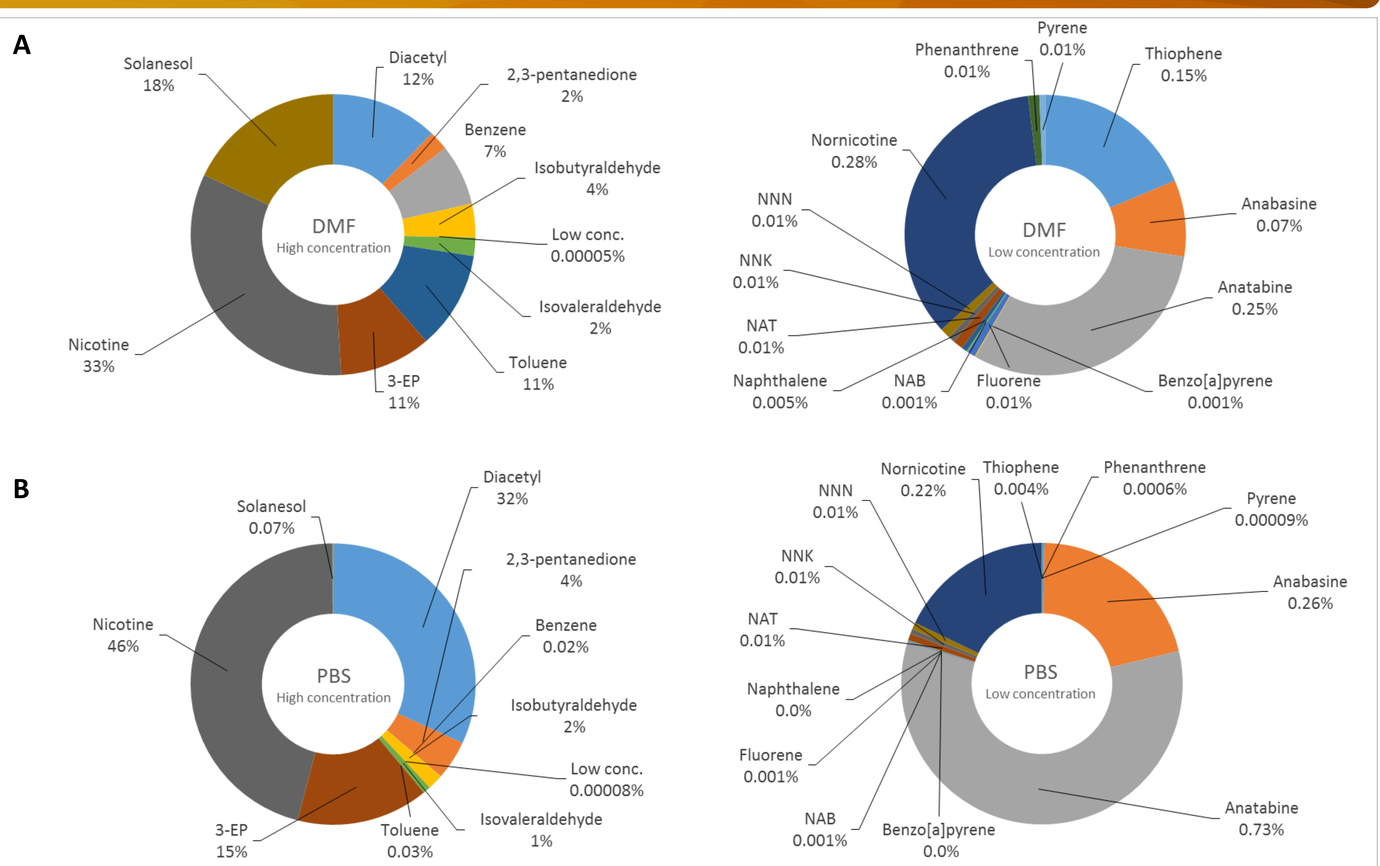

Figure 1: Relative contribution of 22 cigarette smoke-relevant compounds to A) the smoke generated from a 3R4F reference cigarettes and trapped in DMF downstream the Vitrocell system, B) the smoke fraction that is absorbed into PBS samples exposed in the Vitrocell aerosol exposure system. All values are expressed as % of the total mass of the 22 compounds in the according samples. Diagrams on the left show compounds present in high amounts, diagrams on the right side enhance the compounds present in low amounts.

Conclusions

- Analytical Methods for the quantification of various 3R4F smoke constituents trapped in DMF or transferred to PBS during exposures have been developed
- Absorption efficiencies of the 22 smoke constituents targeted in this study vary by more than 3 orders of magnitude, resulting in relevant differences between the composition of the smoke entering the exposure chambers and the smoke fraction being transferred into PBS, a model for the liquid lining covering cell cultures
- As a conclusion, *in vitro* exposures are poorly described by the chemical composition of the applied aerosol. The delivered doses need to be measured directly or predicted based on absorption efficiencies as measured in this work
- The results can be considered valid for exposure systems other than the Vitrocell 24/48, if aerosol delivery relies on stagnation flow conditions in the exposure chambers.
- Sorting the targeted smoke constituents according to their absorption efficiencies allows to a certain extent grouping them according to compound classes/structural properties (but not according to the physicochemical properties listed here)
- This indicates that models for predicting efficiencies of absorption at liquid surfaces can potentially be developed empirically, a larger data set for this purpose will be generated in follow up studies

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