Current Medicinal Chemistry, 2016, *23*, 2112-2131

REVIEW ARTICLE

A Compendium of Volatile Organic Compounds (VOCs) Released By Human Cell Lines

Wojciech Filipiak[1,2], Pawel Mochalski[1], Anna Filipiak[1], Clemens Ager[1], Raquel Cumeras[3], Cristina E. Davis[3], Agapios Agapiou[4], Karl Unterkofler[1] and Jakob Troppmair[5,*]

[1]*Breath Research Institute of the University of Innsbruck, Dornbirn, Austria*
[2]*Department of Anesthesiology and Critical Care Medicine, Innsbruck Medical University, Innsbruck, Austria*
[3]*Department of Mechanical and Aerospace Engineering, University of California, Davis, CA, USA*
[4]*Department of Chemistry, University of Cyprus, Nicosia, Cyprus*
[5]*Daniel Swarovski Research Laboratory, Department of Visceral-, Transplant- and Thoracic Surgery, Innsbruck Medical University, Innsbruck, Austria*

Jakob Troppmair

ARTICLE HISTORY

Received: February 10, 2016
Revised: May 02, 2016
Accepted: May 10, 2016

DOI: http://dx.doi.org/10.2174/0929867323666160316125 05

Abstract: Volatile organic compounds (VOCs) offer unique insights into ongoing biochemical processes in healthy and diseased humans. Yet, their diagnostic use is hampered by the limited understanding of their biochemical or cellular origin and their frequently unclear link to the underlying diseases. Major advancements are expected from the analyses of human primary cells, cell lines and cultures of microorganisms. In this review, a database of 125 reliably identified VOCs previously reported for human healthy and diseased cells was assembled and their potential origin is discussed. The majority of them have also been observed in studies with other human matrices (breath, urine, saliva, feces, blood, skin emanations). Moreover, continuing improvements of qualitative and quantitative analyses, based on the recommendations of the ISO-11843 guidelines, are suggested for the necessary standardization of analytical procedures and better comparability of results. The data provided contribute to arriving at a more complete human volatilome and suggest potential volatile biomarkers for future validation.

Dedication*:This review is dedicated to the memory of Prof. Dr. Anton Amann, who sadly passed away on January 6, 2015. He was motivator and motor for the field of breath research.*

Keywords: Biomarker, Breath analysis, Cancer, GC-MS, Human cell lines, Standardization, Volatile organic compounds (VOCs), Volatilome.

1. INTRODUCTION

The complexity of biological processes only now begins to be matched by similarly complex methodological advances for their analysis. Most recently volatile organic compounds (VOCs) have received considerable interest to obtain insights into physiological and pathophysiological processes, and to exploit the knowledge of their absence/ presence or changes in their concentration profiles or VOC composition in various body matrices for disease detection and therapeutic monitoring [1, 2]. A major advantage of VOCs in this regard is the fact that they are readily and noninvasively obtain able and may be sampled as often as desired with acceptable discomfort. However, their main disadvantage in comparison to *e.g.* nucleic acid- or protein-based markers is the current shortage of information on the total number of VOCs produced by human cells and an insight how their normal composition in breath is qualitatively or quantitatively altered by stress, age, time of day, gender, activity, disease status or the transport to the site of their detection. Moreover, in most cases the information about the metabolic pathways leading to their production or degradation is

* Address correspondence to this author at the Daniel Swarovski Research Laboratory, Department of Visceral-, Transplant- and Thoracic Surgery, Innsbruck Medical University, Innsbruck, Austria; Tel: +4351250427819; Fax: +4351250424625; E-mail: jakob.troppmair@i-med.ac.at

1875-533X/16 $58.00+.00

missing. Due to these reasons it is crucial to investigate the production of volatile metabolites on the cellular level, performing *in vitro* experiments with cancer and non-transformed human cell lines or *ex vivo* experiments with human tumor and healthy tissues surgically excised from respective patients. The main challenges towards establishing breath analysis as a diagnostic or monitoring tool are:

- Establishing of standardized procedures and overcoming the technical issues for sampling and analyses of VOCs found in different emanations of the human body
- Achieving unambiguous identification of VOCs
- Linking VOCs to a particular cellular source and the underlying biochemical processes, which lead to their generation
- Identifying the underlying causes for differences in the VOC fingerprints between different cell types or different phenotypes of the same cell population
- Understanding the inter/intra- person variability
- Establishing the genotype-phenotype relationship for VOC production

The main goal of this review was to create a database of volatile organic compounds found in the analyses of human cell lines. The database contains only reliably identified species with confirmation of *e.g*. chromatographic parameters in addition to the mass spectra library match. Additionally, potential biochemical processes underlying VOCs production as well as technical issues affecting analysis of those volatiles in cell culture studies are discussed. This review is current as of January 2016.

2. THE COMPLEXITY OF THE HUMAN VOLATILOME

Attempts have been made to identify VOCs produced by primary cells, established cell lines as well as by healthy and diseased humans. The study of small samples of tumor and normal tissue obtained during surgery may provide an additional information [3 - 5], circumventing the problems associated with the ideal experimental conditions for *in vitro* studies with cell lines (type of medium, nutrients availability, oxygen supply, *etc*.) or problems with *in vivo* study on humans (breath analysis) such as dietary regimes, physical activity, smoking, *etc*. While the analysis of cell culture head space or human breath is most frequently performed, also other body fluids are targeted including breath, urine, saliva, feces, blood, and sweat [6].

A compendium of 1849 VOCs contributing to the volatilome of healthy humans has been recently published [6]. Out of these species 874 compounds were detected in breath, 279 in urine, 504 in skin emanations, 353 in saliva, 130 in blood, and 381 in feces. Amongst the breath VOCs, 15.7% are also listed for urine, 11.9% for skin emanations [7 - 13], 8.0% for blood [14, 15], 16.2% for saliva [16 - 18] and 22.8% for feces [2]. Hence, numerous compounds appearing in exhaled breath are present in other matrices (urine, skin, blood, saliva and feces) but these matrices also have unique compounds. The overlap between feces and breath may be striking given the difference in the origin, location and composition of these sources. Breath volatiles mainly would be derived from living cells of the body and eventually also from the presence of infectious agents, while feces will contain a small fraction of cells derived from the gut lining and a substantial fraction of bacteria. A particular focus of VOC research has been on their possible use as biomarkers for various diseases [19 - 22], most prominently cancer [4, 21 - 33], but also liver [34 - 37], or renal diseases [38 - 41]. However, the problem with selection of breath biomarkers for cancer is very complex and advanced quantitative statistical tests need to be applied as those compounds are typically present not only in cancer patients but also in healthy controls, hence the clinical relevance could not be demonstrated for any of the so far reported candidate VOCs. For some promising cancer-related metabolites their application as biomarker may be limited as they originate also from other, often exogenous, sources. One of the most prominent examples for this is ethanol (CAS: 64-17-5), which is produced by lung tumour tissues, released into the headspace of A-549 non-small cell lung cancer cells and exhaled by lung cancer patients [4] is present in food,

beverages and is produced by intestinal bacteria such as *Escherichia coli*.

Frequently underestimated sources of volatile compounds in breath gas are *inter alia* (i) nutrition and medication uptake [42 - 45], (ii) physiology (hemodynamics, distribution in body compartments) [46, 47] and (iii) metabolism of intestinal bacteria [48, 49]. Detection of VOCs in general, but also their further identification, may be additionally complicated by the fact that their concentration levels are affected by metabolic processes, which may undergo large fluctuations, *e.g*. in response to physical activity [50 - 52], or smoking [42]. Also the individual VOC fingerprint of a certain pathological state may be shaped by the immune response [53]. Published work also suggests that VOCs can exert immunomodulatory effects. *Toluene* (CAS: 108-88-3) inhibits the secretion of interferon-gamma (IFN-gamma), interleukin-4 (IL-4) and IL-13 but increases the production rate of the tumor-necrosis-factor-alpha (TNF-alpha) from human peripheral blood mononuclear cells (PBMC) [54]. Similarly, the study of Sarma *et al*. [55] confirms that aromatic hydrocarbons such as *benzene* (CAS: 71-43-2), *toluene* (CAS: 108-88-3), *o-xylene* (CAS. 95-47-6) and *ethylbenzene* (CAS: 100-41-4) but also chlorinated hydrocarbons such as *dichloromethane* (CAS: 75-09-2) and trichloroethylene (CAS: 79-01-6) induce the apoptosis of human promyelocytic leukemia HL-60 cells.

3. COMPOUNDS APPEARING IN THE HEADSPACE OF CULTURED HUMAN CELLS

Bearing in mind that human beings are hosts for bacterial, fungal, or other cells of non-human origin, which outnumber the body's cells by far [56], the identification of those VOCs, which are really human cell-derived becomes a formidable challenge. Metabolites produced by microbiota will be present not only in breath, but also in all the other above mentioned sources. Thus, it is essential to study clean populations of cells and microorganisms, to identify the cellular origin of a given volatile compound. A major challenge is that not all human or microbial cells will grow in culture and thus are amenable to analyses of VOC metabolism. In particular human primary cells have limited life spans and any attempt for immortalization may alter cellular metabolism. Due to the complexity of studying VOC production at the organismic level the use of human primary and established cell lines, both of normal and transformed origin, will be an important part in correctly assessing the full range of human-derived VOCs and in assigning the cellular origin of particular VOCs. This work will be paralleled by the analysis of microorganisms associated with healthy and diseased human individuals. Cell culture studies should be also useful in identifying compounds, which are associated with a specific phenotype, *e.g*. oncogenic transformation [53, 57 - 69], or infection [63, 70]. Moreover, primary cells from humans and genetically altered animals may be analyzed to establish genotype/phenotype relationships for particular VOCs.

The work on cell lines has already started to yield invaluable insights into the biochemistry of volatile compounds observed in human organism [53, 57 - 62, 64 - 68]. In the literature survey, altogether 125 volatile compounds were found to fulfill the criterion of reliable identification by spectral library match and retention time confirmation (Table **1**). For this purpose, the present study is focused only on CAS-identified VOCs which have been either released or consumed by specific cell lines. Among them are 23 alcohols, 32 hydrocarbons, 25 aldehydes, 17 ketones, 11 esters, 7 carboxylic acids, 2 ethers, 1 aromatic amine, 1 nitrile and 6 sulfur-containing compounds. Amongst VOCs forming this set 84 were observed also in exhaled breath, 58 in saliva, 48 in skin emanations, 31 in blood, 50 in urine and 79 in feces [6]. 18 compounds are not present in this recently published compilation [6].

Disease states may go along with both quantitative and qualitative differences in particular metabolites. Metabolic analyses of tumors have shown that tumor progression frequently results in a metabolic rewiring of the cell (*e.g*. Warburg effect), which is driven by alterations in cancer genes or the metabolic enzymes themselves [72]. The existence of VOCs more readily produced or consumed by transformed than normal cells is supported by work with cell lines [58, 59, 61, 62, 64-67]. VOC profiles

Table 1. List of 125 VOCs which have been found to be released (↑) or taken up (↓) by different cell lines. The table takes into consideration the results of studies published untill the end of 2015. All these compounds have been identified by spectral library match and retention time by GC-MS, except acetaldehyde which was determined exclusively with SIFT-MS. Information on the presence of a VOC of interest in feces, urine, breath, skin emanations, blood and saliva is based on Ref. [6].

Class	Compound	CAS	Body Fluids						Normal Cells			Cancer Cells		
			F	U	Br	Sk	Bl	Sa	Cell type	Profile	Reference	Cell type	Profile	Reference
Alcohols	Ethanol	64-17-5	F	U	Br		Bl	Sa				A549	↑	[58]
												A549	↑	[85]
	1-Propanol	71-23-8	F	U	Br		Bl	Sa				A549	↑	[84]
												Lu7466	↑	[84]
												Lu7387	↑	[84]
												A549	↑	[85]
												Lu7466	↑	[85]
	2-Propanol	67-63-0	F	U	Br		Bl	Sa				A549	↑	[84]
												A549	↑	[85]
	2-Methyl-1-propanol	78-83-1	F	U	Br	Bl	Sa		hFBÂ	↑	[58]			
	2-Methyl-2-propanol	75-65-0			Br		Bl		HBEpC	↑	[58]	Lu7466	↓	[85]
	2-Methyl-1-butanol	137-32-6			U							VGP	↑	[60]
												Mm	↑	[60]
	3-Methyl-1-butanol	123-51-3	F	U			Bl	Sa	hFBÂ	↑	[58]	RGP	↑	[60]
												Mm	↑	[60]
												SW1116	↑	[68]
	1,4-Butanediol	110-63-4										GES-1	↑	[67]
	Cyclohexanol	108-93-0		U								Lu7387	↑	[84]
	1-Hexanol	111-27-3	F	U		Sk		Sa				Mm	↑	[60]
	2-Ethyl-1-hexanol	104-76-7	F	U	Br		Bl	Sa	hFBÂ	↑	[58]	NCI-H2087	↑	[65]
									HDF	↑	[162]			
	1-Heptanol	111-70-6	F									SW1116	↑	[68]
	1-Octanol	111-87-5	F	U		Sk			NCM460	↓	[68]			
	1-Nonanol	143-08-8	F									GES-1	↑	[67]
												MGC-803	↑	[67]
	1-Decanol	112-30-1	F			Sk			NCM460	↓	[68]	SW1116	↓	[68]
												SW480	↓	[68]
	2-Undecanol	1653-30-1							NCM460	↑	[68]	SW1116	↑	[68]
												SW480	↑	[68]
	Phenol	108-95-2	F	U	Br	Sk		Sa	TBE	↑	[63]			
	Benzyl alcohol	100-51-6	F	U	Br	Sk		Sa				RGP	↑	[60]
												VGP	↑	[60]
												Mm	↑	[60]
	2-Phenylethanol	60-12-8	F			Sk		Sa	FOM	↑	[60]	RGP	↑	[60]
												Mm	↑	[60]
	Carveol	99-48-9		U	Br				TBE	↑	[63]			
	4-Butoxy-1-butanol	4161-24-4										GES-1	↑	[67]
												MGC-803	↑	[67]
	4-Isopropoxybutanol	31600-69-8										GES-1	↑	[67]
												MGC-803	↑	[67]
	1-Tetradecanol	112-72-1				Sk						HeLa	↓	[83]
Aldehydes	Acetaldehyde	75-07-0	F	U	Br	Sk	Bl	Sa	NL-20	↑	[66]	CALU-1	↑	[66]
									HBEpC	↓	[58]	CALU-1	↓	[59]
									35FL121 Tel+	↓	[66]	NCI-H2087	↓	[65]
									HMSC	↓	[66]	HL-60	↑	[114]
	Acrolein	107-02-8	F	U	Br	Sk		Sa				CALU-1	↓	[59]
												MCF-7	↑	[115]
												MCF-7/Adr	↑	[115]
	n-Propanal	123-38-6	F	U	Br	Sk		Sa				A549	↓	[84]
												Lu7387	↓	[84]
	Hydroxyacetaldehyde	141-46-8										HeLa	↓	[83]
	(E)-2-Butenal	123-73-9	F		Br			Sa	hFB	↓	[58]			

(Table 1) contd.....

Class	Compound	CAS	Body Fluids						Normal Cells			Cancer Cells		
			F	U	Br	Sk	Bl	Sa	Cell type	Profile	Reference	Cell type	Profile	Reference
Aldehydes									HBEpC	↓	[58]			
	Methacrolein	78-85-3	F	U	Br			Sa	HBEpC	↓	[58]	CALU-1	↓	[59]
									HUVEC	↓	[62]	NCI-H1666	↓	[64]
												A549	↓	[58]
												HepG2	↓	[61]
												A549	↓	[84]
												Lu7466	↓	[84]
												Lu7387	↓	[84]
	n-Butanal	123-72-8	F		Br	Sk						A549	↓	[84]
												Lu7466	↓	[84]
												Lu7387	↓	[84]
												A549	↓	[85]
	2-Methylpropanal	78-84-2	F	U	Br	Sk			hFB	↓	[58]	CALU-1	↓	[59]
									HBEpC	↓	[58]	NCI-H2087	↓	[65]
									HUVEC	↓	[62]	A549	↓	[58]
												HepG2	↓	[61]
												A549	↓	[85]
	2-Methyl-2-butenal	1115-11-3										CALU-1	↓	[59]
												A549	↓	[58]
												A549	↓	[84]
												Lu7466	↓	[84]
												Lu7387	↓	[84]
	2-Ethylacrolein	922-63-4										A549	↓	[58]
												HepG2	↓	[61]
	n-Pentanal	110-62-3	F	U	Br			Sa				A549	↓	[84]
												Lu7466	↓	[84]
												Lu7387	↓	[84]
	2-Methylbutanal	96-17-3	F	U	Br	Sk		Sa	hFB	↓	[58]	NCI-H2087	↓	[65]
									HUVEC	↓	[62]			
	3-Methylbutanal	590-86-3	F	U	Br			Sa	hFB	↓	[58]	CALU-1	↓	[59]
									HBEpC	↓	[58]	NCI-H2087	↓	[65]
									HUVEC	↓	[62]	NCI-H1666	↓	[64]
												A549	↓	[58]
												HepG2	↓	[61]
												A549	↓	[84]
												A549	↓	[85]
	n-Hexanal	66-25-1	F	U	Br	Sk	Bl	Sa	HBEpC	↓	[58]	CALU-1	↓	[59]
									HUVEC	↓	[62]	NCI-H1666	↓	[64]
												HepG2	↓	[61]
												HL-60	↑	[114]
	n-Heptanal	111-71-7	F	U	Br	Sk	Bl	Sa				Lu7466	↓	[84]
												Lu7387	↓	[84]
	2-Octenal, (E)	2548-87-0	F									HeLa	↓	[83]
	n-Octanal	124-13-0	F	U	Br	Sk	Bl	Sa	HBEpC	↓	[58]			
									HUVEC	↓	[62]			
	2-Ethylhexanal	12/5/2007			Br							HeLa	↓	[83]
	2,4-Nonadienal, (E,E)	5910-87-2										HeLa	↓	[83]
	2-Nonenal, (E)	18829-56-6				Sk		Sa				HeLa	↓	[83]
	n-Nonanal	124-19-6	F	U	Br	Sk		Sa	HUVEC	↓	[62]	HeLa	↓	[83]
									FOM	↓	[60]			
	Benzeneacetaldehyde, α-ethylidene	4411-89-6	F									HeLa	↓	[83]
	2,4-Decadienal, (E,E)	25152-84-5										HeLa	↓	[83]
	Benzaldehyde	100-52-7	F	U	Br	Sk	Bl	Sa	hFB	↓	[58]	HepG2	↓	[61]
									HUVEC	↓	[62]	RGP	↓	[60]
									HDF	↑	[162]	VGP	↓	[60]
									NCM460	↓	[68]	Mm	↓	[60]
												SW1116	↓	[68]
												HeLa	↓	[83]
												SW480	↓	[68]
	Tetradecanal	124-25-4	F			Sk		Sa				HeLa	↓	[83]

(Table 1) contd.....

Class	Compound	CAS	Body Fluids						Normal Cells			Cancer Cells		
			F	U	Br	Sk	Bl	Sa	Cell type	Profile	Reference	Cell type	Profile	Reference
Acids	Acetic acid	64-19-7	F	U	Br	Sk		Sa	TBE	↑	[63]	HeLa	↓	[83]
												A549	↑	[85]
												Lu7466	↑	[85]
	2-Methylpropionic acid	79-31-2	F		Br			Sa	FOM	↑	[60]			
	n-Butyric acid	107-92-6	F	U	Br	Sk		Sa	FOM	↑	[60]			
	2-Methylbutyric acid	116-53-0	F	U					FOM	↑	[60]			
	3-Methylbutyric acid	503-74-2	F		Br	Sk			FOM	↑	[60]	VGP	↑	[60]
	n-Octanoic acid	124-07-2	F		Br	Sk						Mm	↑	[60]
	n-Dodecanoic acid	143-07-7				Sk		Sa				HeLa	↓	[83]
Esters	Methyl acetate	79-20-9	F		Br				HBEpC	↑	[58]			
	Methyl acrylate	96-33-3										A549	↑	[85]
	Ethyl acetate	141-78-6	F	U	Br		Bl	Sa	HUVEC	↑	[62]	A549	↑	[84]
												A549	↑	[85]
	n-Propyl acetate	109-60-4	F		Br			Sa	HBEpC	↑	[58]	HepG2	↑	[61]
	n-Butyl acetate	123-86-4	F		Br				hFB	↓	[58]	CALU-1	↓	[59]
									HBEpC	↓	[58]	NCI-H2087	↓	[65]
									HUVEC	↓	[62]	NCI-H1666	↓	[64]
												A549	↓	[58]
												HepG2	↓	[61]
												A549	↓	[84]
												Lu7387	↓	[84]
	Ethyl propionate	105-37-3	F		Br				HUVEC	↑	[62]			
	n-Propyl formate	110-74-7			Br							GES-1	↑	[67]
	n-Propyl propionate	106-36-5	F		Br							HepG2	↓	[61]
	Ethyl butanoate	105-54-4	F		Br				HUVEC	↑	[62]			
	Methyl decanoate	110-42-9				Sk			NCM460	↓	[68]	SW1116	↓	[68]
												SW480	↓	[68]
	2-Octyl benzoate	6938-52-9										HeLa	↑	[83]
Ethers	Methyl tert-butyl ether	1634-04-4			Br			Bl	hFB	↑	[58]	A549	↑	[58]
												CALU-1	↓	[59]
												NCI-H1666	↓	[64]
	Ethyl tert-butyl ether	637-92-3			Br				HBEpC	↑	[58]	A549	↑	[58]
												CALU-1	↓	[59]
												NCI-H1666	↓	[64]
Hydrocarbons	Isoprene	78-79-5			Br	Sk	Bl					HepG2	↓	[61]
	n-Pentane	109-66-0	F		Br		Bl					A549	↓	[84]
												A549	↓	[85]
	Methylcyclopentane	96-37-7			Br		Bl	Sa				A549	↓	[84]
	Cyclohexane	110-82-7	F		Br			Sa				A549	↓	[84]
												A549	↓	[85]
	2-Methyl-1-pentene	763-29-1			Br							A549	↑	[58]
												A549	↓	[84]
	2-Methylpentane	107-83-5	F		Br		Bl	Sa				NCI-H2087	↑	[65]
	3-Methylpentane	96-14-0	F		Br		Bl	Sa				A549	↓	[84]
												A549	↓	[85]
	2-Heptene	14686-13-6			Br			Sa				HepG2	↑	[61]
	n-Heptane	142-82-5			Br	Sk	Bl	Sa				A549	↓	[84]
												A549	↓	[85]
	3-Methylhexane	589-34-4			Br							A549	↓	[84, 85]
	3-Methylheptane	589-81-1	F		Br			Sa	hFB	↑	[58]			
									HBEpC	↑	[58]			
	4-Methylheptane	589-53-7			Br				hFB	↑	[58]			
									HBEpC	↑	[58]			
	n-Octane	111-65-9	F		Br	Sk		Sa	hFB	↑	[58]	A549	↑	[58]
												A549	↓	[84]
	2,3,3-Trimethylpentane	560-21-4			Br				hFB	↑	[58]	CALU-1	↑	[59]
									HBEpC	↑	[58]			
	2,3,4-Trimethylpentane	565-75-3			Br				hFB	↑	[58]			

(Table 1) contd.....

Class	Compound	CAS	Body Fluids						Normal Cells			Cancer Cells		
			F	U	Br	Sk	Bl	Sa	Cell type	Profile	Reference	Cell type	Profile	Reference
Hydrocarbons	2,4-Dimethylhexane	589-43-5			Br	Sk			hFB	↑	[58]			
	n-Nonane	111-84-2			Br	Sk		Sa				A549	↓	[84]
	2,3,5-Trimethylhexane	1069-53-0			Br				hFB	↑	[58]	CALU-1	↑	[59]
	4-Methyloctane	2216-34-4	F		Br							CALU-1	↑	[59]
	2,4-Dimethylheptane	2213-23-2	F	U	Br							CALU-1	↑	[59]
	2,4-Dimethyl-1-heptene	19549-87-2			Br	Sk			hFB	↑	[58]	A549	↑	[58]
									HBEpC	↑	[58]			
	1,3,5-Undecatriene, (3E,5Z)-	51447-08-6										HeLa	↓	[83]
	2,9-Dimethyl-5-decyne	19550-56-2										HeLa	↓	[83]
	2,6,11-Trimethyl-dodecane	31295-56-4	F									GES-1	↑	[67]
	3-Hexadecene, (Z)-	34303-81-6										HeLa	↓	[83]
	Benzene	71-43-2	F	U	Br	Sk	Bl	Sa	hFB	↑	[58]	A549	↓	[84]
												A549	↓	[85]
	Toluene	108-88-3	F	U	Br	Sk	Bl	Sa	HUVEC	↑	[62]			
	o-Xylene	95-47-6	F	U	Br		Bl	Sa				A549	↓	[84]
	p-Xylene	106-42-3	F	U	Br	Sk	Bl	Sa				A549	↓	[84]
	Styrene	100-42-5	F	U	Br	Sk	Bl	Sa				A549	↑	[84]
	Ethylbenzene	100-41-4	F	U	Br	Sk	Bl					A549	↓	[84]
	Cumene	98-82-8	F		Br							A549	↓	[84]
Ketones	Acetone	67-64-1	F	U	Br	Sk	Bl	Sa	HBEpC	↑	[58]	A549	↑	[58]
									TBE	↓	[63]	VGP	↑	[60]
												A549	↓	[84]
	2-Butanone	78-93-3	F	U	Br	Sk	Bl	Sa				MGC-803	↑	[67]
												CALU-1	↓	[59]
												A549	↑	[85]
	Hydroxyacetone	116-09-6	F		Br							HeLa	↓	[83]
	3-Hydroxy-2-butanone	513-86-0	F		Br			Sa	FOM	↑	[60]	VGP	↑	[60]
	2-Pentanone	107-87-9	F	U	Br	Sk		Sa	hFB	↑	[58]	A549	↑	[58]
									HBEpC	↑	[58]	HepG2	↑	[61]
	3-Pentanone	96-22-0	F	U		Sk	Bl					A549	↓	[84]
	3-Penten-2-one	3102-33-8							hFB	↓	[58]			
	2-Hexanone	591-78-6	F	U	Br	Sk			hFB	↑	[58]			
	2-Heptanone	110-43-0	F	U	Br			Sa				HepG2	↑	[61]
	3-Heptanone	106-35-4	F	U	Br				HUVEC	↑	[62]	HepG2	↑	[61]
	Acetophenone	98-86-2	F	U	Br	Sk		Sa				Lu7387	↓	[84]
	2-Octanone	111-13-7							HUVEC	↑	[62]			
	3-Octanone	106-68-3	F	U	Br			Sa				HepG2	↑	[61]
												MGC-803	↑	[67]
	2-Nonanone	821-55-6	F	U	Br	Sk		Sa	NCM460	↑	[68]	HepG2	↑	[61]
									HUVEC	↑	[62]	SW1116	↑	[68]
												SW480	↑	[68]
	2-Undecanone	112-12-9	F			Sk		Sa				HeLa	↑	[83]
	2-Tridecanone	593-08-8				Sk		Sa				HeLa	↑	[83]
	2-Pentadecanone	2345-28-0				Sk		Sa				SW480	↑	[68]
												HeLa	↑	[83]
VSCs	Dimethyl sulfide	75-18-3	F		Br	Sk	Bl		TBE	↑	[63]	HepG2	↑	[61]
	Ethyl methyl sulfide	624-89-5		U	Br							HepG2	↑	[61]
	3-Methylthiophene	616-44-4	F		Br							HepG2	↑	[61]
	Isobutyl methyl sulfide	5008-69-5										HepG2	↑	[61]
	2-Methyl-5-(methylthio)-furan	13678-59-6		U					HUVEC	↑	[62]	HepG2	↑	[61]
	Methanesulfonic anhydride	1/3/7143										HeLa	↓	[83]
VNCs	Pyrrole	109-97-7	F	U	Br			Sa				A549	↓	[58]
	Acetonitrile	75-05-8	F		Br		Bl	Sa				CALU-1	↓	[59]

VSCs: Volatile Sulfur-containing Compounds, VNCs: Volatile Nitrogen-containing Compounds. Cell line abbre*via*tions: NSCLC, non-small-cell lung carcinoma, GES-1: SV40 transformed human fetal gastric epithelial, cell line MGC-803: human gastric cancer; SW1116: large intestine, carcinoma, adenocarcinoma; NCI-H2087: non-small-cell lung carcinoma, NSCLC; NCM460: NCM460, a normal human colon mucosal epithelial cell line; SW480: Colorectal Adenocarcinoma; CALU-1: lung cancer; HMSC: human mesenchymal stem cells; 35FL121 Tel+: Telomerase positive lung fibroblast cells; HL-60: human promyelocytic leukemia; HepG2: hepatocyte carcinoma; A549: lung adenocarcinoma; NCI-H1666: bronchioloalveolar and lung adenocarcinoma; FOM: Neonatal foreskin melanocytes; TBE: Human primary tracheobronchial epithelial cells; VGP: Vertical growth phase primary melanoma; RGP: radial growth phase primary melanoma; HBEpC: human bronchial epithelial primary cells; MCF-7: human breast adenocarcinoma cells; MCF-7/Adr: human breast adenocarcinoma cells a doxorubicin-resistant subline; Mm: metastatic melanoma; HUVEC: human umbilical vein endothelial cells; NL-20: normal lung epithelial cells; hFB: human fibroblasts; HDF: human dermal fibroblasts; HeLa: human epithelial cervical carcinoma cells; Lu7466: lung adenocarcinoma; Lu7387: lung adenocarcinoma.

released from cell lines were also compared with those of paired samples of isolated tumors/healthy lung tissues and exhaled breath from lung cancer patients and healthy controls, respectively. This work demonstrated significantly higher concentrations in all three types of cancer samples studied (cells, tissues, patients' breath) for *ethanol* (CAS 64-17-5) and *n-octane* (111-65-9) [4]. Additionally, 2-*methylpentane* (CAS 107-83-5) and *n-hexane* (CAS 110-54-3), released by NCI-H2087 lung cancer cells (adenocarcinoma) were observed at significantly higher levels in exhaled breath of lung cancer patients. Importantly, these compounds are not related to smoking [42] and have also been traced in other body emanations (Table **1**).

Furthermore, *2-methylpentane* was also found by Poli *et al.,* [5] at higher levels in breath of lung cancer patients. All seven compounds present in all sources tested have been found in the headspace of bacteria [73 - 79], although so far mostly pathogenic strains have been analyzed. These findings suggest that complementary research on human-produced VOCs from different sources with tissue and culture studies will help in the validation of the true human origin of candidate compounds as well as their potential disease association. However, cancer cell lines may also differ from real cancer. For instance*, straight-chained saturated aldehydes (e.g. n-hexanal* (CAS 66-25-1) and *n-octanal* (CAS 922-63-4)) exhibit higher concentrations in breath gas of lung cancer patients as compared to healthy volunteers [27, 28, 80, 81] or blood [82] of lung cancer patients as compared to healthy volunteers, whereas cancer cells have been shown to metabolize these compounds [59, 64, 65, 83 - 85]. The contrary profiles for several aldehydes in the breath gas samples and the headspace of transformed cell lines may be related to differences in the expression of aldehyde dehydrogenase (ALDH) isoforms (see section 4.2 below) or hypoxic conditions in the cultures under *in vitro* conditions, not observed *in vivo* (see section 4.3 below).

4. BIOCHEMICAL PROCESSES UNDERLYING VOC PRODUCTION

Even though more than 100 different volatile biomarkers for lung cancer have been proposed during the past 10 years [21, 22, 32], the biochemical background of most of these compounds has not been elucidated. Biochemical pathways leading to the production of various classes of potentially cancer-related VOCs (hydrocarbons, alcohols, aldehydes, ketones, esters, nitriles, and aromatic compounds) have been reviewed recently [21, 22, 86]. Alkanes, alcohols and aldehydes are produced *via* various processes, including the reduction of hydroperoxide by cytochrome P450, as a secondary product of lipid peroxidation. Aldehydes are generated also from amino acid and carbohydrate catabolism. Other sources are related to smoking [87], dietary intake or exposure to indoor-air pollutants [88]. Ketone production has been linked to stress conditions, *e.g.* cancer, where increased fatty acid oxidation results in the formation of ketone bodies, which in turn can be secreted *via* breath, urine and skin. Moreover, increased protein metabolism during cachexia or under ketogenic diet results in the increase of ketone bodies levels [89]. *Acetone* (CAS: 67-64-1) concentrations can also vary in response to food consumption [90]. Esters are present in many natural fats and oils, and can be hydrolyzed by esterases (*e.g*. lipases) to yield alcohols and acids. Nitriles and aromatic VOCs are usually considered to be contaminants from exogenous sources, mainly cigarette smoke [42, 91], but also outdoor-air / environmental pollutant [92, 93] such as automobile emission [94]. Acetonitrile (CAS: 75-05-8) is predominant in the exhaled breath of smokers and practically absent in the breath of non-smokers; it is also readily detected in the urine of smokers [95, 96]. The combined knowledge of the cellular source and underlying biochemical process will provide a rationale base for the use of VOCs in the detection of metabolic alterations, which are caused or associated with diseases. *Isoprene* (CAS: 78-79-5), which is thought to be produced in the liver [97 - 99] and in muscles [46, 100] has not yet been observed in cell culture experiments. In the headspace of HepG2-cells isoprene levels decrease (about 5-fold) when compared to headspace of medium only [61], and may be caused by the cytochrome P450 oxidation of this hydrocarbon to mono- and di-epoxides by liver microsomes [101 - 103].

4.1. Oxidative Stress and CYP-Oxidase Enzymes

The production of some hydrocarbons has been linked to the presence of reactive oxygen species, the oxygen free radicals that may leak from site of their production in mitochondria into a cytoplasm. In particular, the peroxidation of polyunsaturated fatty acids (PUFA) such as *linoleic acid* (CAS: 60-33-3) [104] present in the cell membrane, may result in the production of straight chained alkanes (*e.g*. *ethane*, CAS: 74-84-0 and *pentane*, CAS: 109-66-0). It has been also suggested that free radical peroxidation of PUFA leads to generation of methylated hydrocarbons [105], which has been disputed by others [106, 107]. Further oxidation of those alkanes by cytochrome P450 (CYP) oxidase enzymes – activated in transformed cells [108] and induced by aromatic hydrocarbons from tobacco smoke [109] – leads to formation of alcohols, ketones and aldehydes that may be detected in diverse body fluids and breath gas [21]. Nevertheless, alcohols are mainly derived from the uptake of food and beverages and may enter the blood stream through simple diffusion. Additionally, low molecular alcohols, mainly *ethanol* (CAS: 64-17-5), can be produced *via* pyruvate metabolism by intestinal bacteria, *e.g*. *Escherichia coli* [49].

4.2. Aldehyde Dehydrogenase

Aldehyde dehydrogenase (ALDH) enzymes (19 isoforms) are responsible mainly for oxidation of cytotoxic aldehydes to carboxylic acids [110]. Additionally they are involved in *(i)* ester hydrolysis, *(ii)* scavenging the hydroxyl radical, *(iii)* potentially serving as antioxidants by NAD(P)H production, and *(iv)* contributing to the regulation of retinoic acid production [111 - 113]. Elevated metabolic activity of ALDH in the transformed cells will lead to the strong degradation of aldehydes. This was confirmed in the *in vitro* studies with human transformed cells, where uptake of aldehydes was observed for lung carcinoma cells NCI-H1666, NCI-H2087, CALU-1, A549, Lu7466, Lu7387 [58, 59, 64, 65, 84, 85] and liver carcinoma cells HepG2 [61]. Intriguingly, for NL20, HL60, MCF-7 transformed cell lines the release of low-molecular aldehydes was observed [66, 114, 115] suggesting down-regulation of ALDH activity.

Indeed, there is evidence that only particular isoform(s) are active in specific tumor types [111], for instance increased expression of the ALDH1 isoform could result from smoking and substantially contribute to malignant transformation of lung cells [116]. The ALDH1 isoform was also positively correlated to stage and grade of lung tumors in the clinical study based on 303 human specimens from independent cohorts of lung cancer patients [117].

4.3. Culture Conditions and Oxygen Supply

A fundamental concern in all *in vitro* experiments is how well they recapitulate the *in vivo* situation. Cell culture experiments are also not able to reproduce changes, which may affect VOCs after their production to the site where they are released. While analyzing cells in the standard tissue culture setting (which usually provides optimized growth conditions) may provide a first approximation, the task for the future is to imitate the real conditions present in the body. The effect of changing growth conditions on VOC production has been noted in the analysis of tumor cells growing in 2D versus 3D cultures, the latter being more similar to *in vivo* conditions of the tumor growth [118]. Availability of nutrients and oxygen in tissue is typically optimized in the *in vitro* experiments and may thus differ considerably from the situation in the tissue. In the human body, the growth of a tumor will result in hypoxic conditions due to insufficient diffusion of oxygen [119, 120]. An altered respiration process (anaerobic fermentation in cytosol) leads to different intermediate products (conversion of pyruvate into lactate), which may cause qualitative and quantitative changes in the profile of volatile metabolites. Other processes induced under hypoxic conditions, such as degradation of cellular components during autophagocytosis [121] may further complicate the metabolic fate of VOCs. It was recently suggested that hypoxia may lead to increased oxidative stress and enhanced lipid peroxidation hence to appearance of aliphatic hydrocarbons in the breath of cancer patients, respectively in the headspace of cancer cell lines, whereas hyperoxic conditions prompt to generation of oxidized products, such as alcohols [105]. Also intermediates or end products will accumulate in

tissue culture but may be further metabolized or excreted in the *in vivo* situation.

4.4. Cell Death

Another important issue concerning *in vitro* studies with cells is to discern whether volatile metabolites detected in a headspace originate from metabolism of living cells or from decaying dead cells. This task was addressed by Pyo *et al*. [122] in their experiments with human non-small cell lung cancer A549 cells. Depending on the dose, the cisplatin treatment of A549 cells caused apoptosis (manifested by cell shrinkage, DNA fragmentation or chromatin condensation), or necrosis (characterized cytosolic swelling and loss of plasma membrane integrity) at higher concentrations. A strong correlation (R^2=0.99) between the percentage of apoptotic cells and the quantities of *nonanal* (CAS: 124-19-6), *1,3-di-tert-butylbenzene* (CAS: 1014-60-4) and *2,6-di-tertbutyl-1,4-benzoquinone* (CAS: 719-22-2) was observed. Additionally, *n-decane* (CAS: 124-18-5) was assigned to the necrotic phase of these cells. The release of alkane and aldehyde from apoptotic and necrotic cells suggests that lipid peroxidation mediates the cytotoxic effects of cisplatin in addition to the well-known formation of DNA adducts leading to cell cycle arrest.

4.5. Immune Activation

Distinct profiles of VOCs released from different types of human B-cells under *in vitro* conditions were reported by Aksenov *et al*. [53]. They have observed that a specific allele in the Human Leukocyte Antigen (HLA) complex (class I antigen) impacts on downstream signal transduction and metabolic pathways in the human B-lymphoblastoic cell line C1R. Those results suggest possible detection of unique VOCs produced by human B-cells circulating in an organism during infection, cancer or other affected medical state. However, VOCs discussed in these works were identified only by mass spectra match (not confirmed with standards) [53, 57].

5. ANALYTICAL CHALLENGES

Amongst the analytical techniques used for determination of volatile metabolites, gas chromatography-mass spectrometry (GC-MS) is considered to be the gold standard [14, 24, 26, 28, 30, 33, 42, 78, 123, 124]. Other analytical techniques embrace proton transfer reaction-mass spectrometry (PTR-MS) [125], selected ion flow tube-mass spectrometry (SIFT-MS) [71, 126, 127], ion mobility spectrometry (IMS) [128 - 130], laser spectrometry [131 - 133], and sensors or sensor arrays [21, 100, 134 - 138]. The major merit of the GC-MS techniques lies in advanced identification mechanism and ability of analyzing hundreds of species simultaneously, whereas SIFT-MS and PTR-MS methods offer near real-time analyses. In turn, IMS, laser spectroscopy and especially sensor arrays have a potential for miniaturization, although they provide limited identification and very complex mathematical algorithms (such as neural network and machine learning) are involved in the interpretation of acquired signals [139 - 141].

Although GC-MS is the most versatile tool amongst the analytical techniques, there is an issue of comparability between results gathered in different laboratories. The main reason for this, is the lack of standardization in methodology *e.g*. for sample collection, preparation, GC-MS analysis and the missing validation of the analytical method used. Both, identification and quantitative VOCs analyses are questionable in some cases and important parameters as detection limits are either inadequately determined or entirely missing. The quality of some of the published results from *in vitro* studies suffers largely due to questionable identification of analytes which is often the case in direct mass spectrometric techniques (inadequate VOC identification based on single mass-to-charge ratio “m/z” is discussed elsewhere [142 - 144]) or sensors (no attempt of VOC identification at all) but, unfortunately, appears also in GC-MS-based studies.

5.1. Sampling Procedure

Contrary to breath or other body fluids, the collection of the headspace gas from cell lines or bacteria cultures does not require transport containers (bags or bulbs) and the sample can be directly entrapped on an adsorption device such as solid phase microextraction (SPME) fiber, sorption tube or needle trap. Despite the time-consuming

handling, these techniques ensure well controlled sampling, high reproducibility, and stability. The most commonly used technique for adsorptive preconcentration is SPME, where a thin layer of adsorbent coats a tiny fiber [145]. The benefits of SPME are: low costs (desorption directly in GC injector), relatively small as compared to tubes uptake of water from humid gases thus no influence on chromatographic separation, good sensitivity, and automation of adsorption as well as desorption processes. Since the appropriate quantification is important to reveal a relation between VOC profiles and health status in general, the sample preparation technique must ensure quantitative preconcentration for sensitive analysis. In this regard, SPME is considered to be a "semi-quantitative" technique, as the masses of adsorbed analytes depends on their partition coefficient and reaches maximum when equilibrium between their concentration in a "sample" gaseous phase and a "fiber" solid/liquid phase is reached. Nevertheless, when parameters for adsorption, storage and desorption are well controlled, both sensitivity and reproducibility are very high and SPME technique can be successfully used in *in vitro* studies focused on the cellular metabolism of VOCs. While most research on volatile metabolites released from human cell lines focuses on very volatile organic compounds (vVOCs), in some particular cases also the heavier (semi-volatile) fraction, respectively unstable reactive VOCs, is targeted. For this purpose the detection limits and quantitation with SPME-GC-MS may need improvement. It is typically achieved by increasing SPME extraction efficiency through a *derivatization* process that converts polar analytes into their less polar analogues. Such conversion of selected low volatile compounds into more volatile derivatives increases their partition coefficients (SPME fiber/gas rarely SPME fiber/water) and may also improve their GC separations without a need of GC column exchange [146], but most importantly it improves the stability of reactive species, such as aldehydes, on SPME fiber [80, 147, 148]. Nevertheless, to ensure the lowest detection limits and the broadest spectrum of collected VOCs, so called "exhaustive" adsorption techniques can be used, whereby the gaseous sample is drawn through a tube filled with one or more sorbents in a quantity considerable larger than in SPME. Thus in contrast to SPME, all sample components present at trace concentration levels are quantitatively adsorbed on sorption tube, if adsorbent selection and adsorption parameters optimization were done correctly. The sorptive properties of adsorbents commonly used for preconcentration of the gaseous samples have been investigated in numerous research articles [149 - 152] review papers [153] and the guidelines for the selection of appropriate adsorbents for VOC sampling are available on manufacturer websites.

Presence of pollutants or loss of volatiles can considerably distort the original chemical fingerprint produced by cells under study conditions. Thus, the isolation of the head-space atmosphere from the external environment can considerably improve the reliability of analyses. Another important issue is the selection of inert and contaminant-free materials (tubing, cultivation flasks *etc*.) for experimental setups. Glass or some inert polymers such as Teflon, or polyetheretherketone (PEEK) seem to be the materials of choice in this context. While the gas phase from a closed system can be easily adsorbed on a SPME fiber (so called static headspace), the application of sorption tubes or needle trap devices normally requires a carrier gas to drag the sample (so called dynamic headspace), which must be additionally purified with filters or catalyst to reduce the risk of sample contamination and improve the background [58, 59, 61, 62, 64, 65].

Regardless of preconcentration technique, it is recommended to investigate the adsorption parameters (saturation/breakthrough, sample volume, temperature, *etc*.) and desorption parameters (temperature, duration, split ratio, *etc*.) before using a method for sample analysis. Especially important is to minimize/eliminate water uptake, for example by elevating slightly the adsorption temperature ("warm trap"), that decreases sample's relative humidity [154].

5.2. Reliable VOC Identification

Unfortunately, numerous GC-MS studies report compounds which were only identified on the basis of the spectral library match. Apart from fundamental mistakes in VOC identification, such as multiple detection of a single analyte on a sample

chromatogram or attribution of more than one compound to a single peak (assuming baseline resolution), researchers seem to forget that although a mass spectrum is characteristic for a certain substance, it is not unique. Compounds – especially structural isomers containing the same functional group – can have very similar spectra. Consequently, only comparing the mass spectrum of an unknown sample component to the reference spectra in a commercial MS library (NIST, Wiley and others) may be misleading. This is particularly important in case of unresolved peaks, where the spectrum of a certain analyte is "contaminated" by the spectrum of a neighboring, unseparated sample components or by the impurities originating from a background. Therefore, in agreement to existing guidelines [155] the identification of an unknown analyte has to be supported with independent data, *i.e*. the chromatographic parameters (retention time or retention index) have to be analyzed for reference standards under identical experimental conditions. Furthermore, compound names provided are often not supported by unique numeric identifiers such as, *e.g*. *Chemical Abstracts Service registry number* (CAS-number, see https://scifinder .cas.org), which could assist the unambiguous identification but also the comparison of different studies. This issue plays a particular role in case of complex volatiles such as, *e.g*. *5-Isopropenyl-2-methyl-7-oxabicyclo [4.1.0]heptan-2 -ol* detectable in breath gas [33]. Therefore, it is recommended supporting a CAS number respective to the reported analytes for the purpose of comparability of the published data from diverse research groups.

5.3. Reliability of the Analytical Method

Once the optimal conditions for sample collection, adsorptive preconcentration, storage, thermal desorption and off-line GC-MS measurement are investigated, the analytical method needs to be validated to prove that it is acceptable for its intended purpose. The repeated measurements (at least 3 times) of reference standards at increasing concentrations spiked with sample matrix (calibration) can be used to determine the dynamic range of response, which demonstrates the optimum range of linearity, precision (repeatability), and accuracy (closeness to a "true" value) for quantitative analysis. The last one depends also on the selectivity that refers to the extent to which a method as a whole can determine the target analyte without interferences [156]. In the case of adsorptive preconcentration techniques an important parameter to evaluate is the robustness of an analytical method, which demonstrates if the measured analyte's concentration remains unaffected as a consequence of a small change in a certain parameter (for instance slightly elevated temperature of adsorption that affects sample relative humidity).

Particular attention should be paid to correct determination of the detection limit of an applied analytical method. According to the IUPAC "the limit of detection (LOD) is *expressed as the concentration or the quantity derived from the smallest measure that can be detected with reasonable certainty for a given analytical procedure*" [157]. This definition is, unfortunately, not precise and leaves quite some room for interpretation. In many analytical techniques, including chromatography and mass spectrometry, it relays on the signal to noise ratio (S/N) determined for repeated blank measurements, which is then multiplied with a factor to achieve a desired confidence level, for instance k=3 for 99% of confidence level [157]. Consequently, unrealistic low LODs can sometimes be found in the published literature, which under realistic circumstance would fall far short of the required performance.

Instead, it is recommended to determine the LOD according to the standards outlined in the ISO-11843 guidelines [158], which evaluate the detection capability of an analytical method by actually calculating the risk of both false positive and false negative errors. It is an important difference that the limits here are a function of the acceptable error rates – meaning the same analytical method can have different limits depending on the acceptable errors of different tasks. The critical values determined (*detection limit, quantification limit, and detection decision*) derive from the calibration data performed under the same analytical conditions as for real sample instead of blank [159]. Importantly, the other sub-standards from the set ISO-11843 provide procedures for more complex scenarios and additionally for linear calibration cases (ISO 11843-2). They also address

cases of non-linear calibration (ISO 11843-5) that may occur when SPME is used for high concentration of target VOCs, Poisson distributed measurements (ISO 11843-6) particularly important for direct mass spectrometric techniques or situations, where no calibration data are used, if the preparation of standards close to detection limit is impossible (ISO 11843-3).

CONCLUSION

Compiling the human volatilome is a formidable task, which is still at a very early stage. A first comprehensive overview of candidate compounds found in various emanations of the human body comprising 1765 candidates with an associated CAS-number, has been recently published [6]. Almost all substances encountered in the analyses of normal and transformed human cells *in vitro* (Table **1**) have been observed in exhaled breath. At present, there are no *single* tumor-specific volatile compounds for which clinical relevance could be proved. Alternatively, panels of compounds may be preferentially associated with the transformed phenotype. The relation between volatile profiles of cancer cell lines and the breath profiles of cancer patients is still far from being understood. If required, growth conditions can be changed drastically to meticulously investigate the particular metabolic pathway leading to VOCs production. Otherwise, the conditions of *in vitro* experiments with cell cultures, such as oxygen supply or nutrients availability, need to be carefully adjusted to mimic as much as possible the *in vivo* situation. We also propose the implementation of the ISO-11843 guidelines for the standardization of analytical procedures to ensure the comparability of results gathered in different research laboratories. The use of cell lines but also of cultures of microorganisms making up the human microbiota will be essential to unambiguously assign VOCs to their cellular source and the underlying biochemical processes leading to their generation and metabolism. This combined knowledge will provide the basis for a rationale use of VOCs as biomarkers for disease detection or treatment monitoring.

The library of endogenously produced compounds, released by human cells *in vitro*, is an important starting point for future work and discussions. It will, in particular, be interesting to look at all these compounds in experiments with *real-time* analysis of exhaled breath under different conditions and with different exhalation kinetics. Following the trend of miniaturization, candidate biomarkers validated in patients studies may be used to develop selective nanosensors as a point of care devices for screening purposes [160, 161].

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

PM and KU gratefully acknowledge the support by the Austrian Science Fund (FWF) under Grant No. P24736-B23. This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 644031.

REFERENCES

[1] *Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring*; Amann, A.; Smith, D., Eds.; World Scientific: New Jersey, **2005**.

[2] Amann, A.; Smith, D. *Volatile Biomarkers: Non-invasive Diagnosis in Physiology and Medicine*; Elsevier: Amsterdam, **2013**.

[3] Buszewski, B.; Rudnicka, J.; Ligor, T.; Walczak, M.; Jezierski, T.; Amann, A. Analytical and unconventional methods of cancer detection using odor. *Trac-Trend Anal. Chem.,* **2012**, *38*, 1-12. [http://dx.doi.org/10.1016/j.trac.2012.03.019]

[4] Filipiak, W.; Filipiak, A.; Sponring, A.; Schmid, T.; Zelger, B.; Ager, C.; Klodzinska, E.; Denz, H.; Pizzini, A.; Lucciarini, P.; Jamnig, H.; Troppmair, J.; Amann, A. Comparative analyses of volatile organic compounds (VOCs) from patients, tumors and transformed cell lines for the validation of lung cancer-derived breath markers. *J. Breath Res.,* **2014**, *8*(2), 027111. [http://dx.doi.org/10.1088/1752-7155/8/2/027111] [PMID: 24862102]

[5] Poli, D.; Carbognani, P.; Corradi, M.; Goldoni, M.; Acampa, O.; Balbi, B.; Bianchi, L.; Rusca, M.; Mutti, A. Exhaled volatile organic compounds in patients with non-small cell lung cancer: cross sectional and nested short-term follow-up study. *Respir. Res.,* **2005**, *6*, 71. [http://dx.doi.org/10.1186/1465-9921-6-71] [PMID: 16018807]

[6] de Lacy Costello, B.; Amann, A.; Al-Kateb, H.; Flynn, C.; Filipiak, W.; Khalid, T.; Osborne, D.; Ratcliffe, N.M. A review of the volatiles from the healthy human body. *J. Breath Res.,* **2014**, *8*(1), 014001. [http://dx.doi.org/10.1088/1752-7155/8/1/014001] [PMID: 24421258]

[7] Bernier, U.R.; Kline, D.L.; Barnard, D.R.; Schreck, C.E.; Yost, R.A. Analysis of human skin emanations by gas chromatography/mass spectrometry. 2. Identification of volatile compounds that are candidate attractants for the

yellow fever mosquito (Aedes aegypti). *Anal. Chem.,* **2000**, *72*(4), 747-756.
[http://dx.doi.org/10.1021/ac990963k] [PMID: 10701259]

[8] Gallagher, M.; Wysocki, C.J.; Leyden, J.J.; Spielman, A.I.; Sun, X.; Preti, G. Analyses of volatile organic compounds from human skin. *Br. J. Dermatol.,* **2008**, *159*(4), 780-791.
[http://dx.doi.org/10.1111/j.1365-2133.2008.08748.x] [PMID: 18637798]

[9] Schmidt, F.M.; Vaittinen, O.; Metsälä, M.; Lehto, M.; Forsblom, C.; Groop, P.H.; Halonen, L. Ammonia in breath and emitted from skin. *J. Breath Res.,* **2013**, *7*(1), 017109.
[http://dx.doi.org/10.1088/1752-7155/7/1/017109] [PMID: 23445955]

[10] Haze, S.; Gozu, Y.; Nakamura, S.; Kohno, Y.; Sawano, K.; Ohta, H.; Yamazaki, K. 2-Nonenal newly found in human body odor tends to increase with aging. *J. Invest. Dermatol.,* **2001**, *116*(4), 520-524.
[http://dx.doi.org/10.1046/j.0022-202x.2001.01287.x] [PMID: 11286617]

[11] Mochalski, P.; King, J.; Unterkofler, K.; Hinterhuber, H.; Amann, A. Emission rates of selected volatile organic compounds from skin of healthy volunteers. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.,* **2014**, *959*, 62-70.
[http://dx.doi.org/10.1016/j.jchromb.2014.04.006] [PMID: 24768920]

[12] Mochalski, P.; Unterkofler, K.; Hinterhuber, H.; Amann, A. Monitoring of selected skin-borne volatile markers of entrapped humans by selective reagent ionization time of flight mass spectrometry in NO+ mode. *Anal. Chem.,* **2014**, *86*(8), 3915-3923.
[http://dx.doi.org/10.1021/ac404242q] [PMID: 24611620]

[13] Curran, A.M.; Prada, P.A.; Furton, K.G. The differentiation of the volatile organic signatures of individuals through SPME-GC/MS of characteristic human scent compounds. *J. Forensic Sci.,* **2010**, *55*(1), 50-57.
[http://dx.doi.org/10.1111/j.1556-4029.2009.01236.x] [PMID: 20002268]

[14] Mochalski, P.; King, J.; Klieber, M.; Unterkofler, K.; Hinterhuber, H.; Baumann, M.; Amann, A. Blood and breath levels of selected volatile organic compounds in healthy volunteers. *Analyst (Lond.),* **2013**, *138*(7), 2134-2145.
[http://dx.doi.org/10.1039/c3an36756h] [PMID: 23435188]

[15] Miekisch, W.; Schubert, J.K.; Vagts, D.A.; Geiger, K. Analysis of volatile disease markers in blood. *Clin. Chem.,* **2001**, *47*(6), 1053-1060.
[PMID: 11375291]

[16] Larsson, B.T. Gas Chromatography of Organic Volatiles in Human Breath and Saliva. *Acta Chem. Scand.,* **1965**, *19*(1), 159-164.
[http://dx.doi.org/10.3891/acta.chem.scand.19-0159] [PMID: 14280839]

[17] Soini, H.A.; Klouckova, I.; Wiesler, D.; Oberzaucher, E.; Grammer, K.; Dixon, S.J.; Xu, Y.; Brereton, R.G.; Penn, D.J.; Novotny, M.V. Analysis of volatile organic compounds in human saliva by a static sorptive extraction method and gas chromatography-mass spectrometry. *J. Chem. Ecol.,* **2010**, *36*(9), 1035-1042.
[http://dx.doi.org/10.1007/s10886-010-9846-7] [PMID: 20809147]

[18] AL-Kateb, H.; Costello, B.D.; Ratcliffe, N. An investigation of volatile organic compounds from the saliva of healthy individuals using headspace-trap/GC-MS. *J. Breath Res.,* **2013**, *7*(3), 036004.

[19] Broza, Y.Y.; Mochalski, P.; Ruzsanyi, V.; Amann, A.; Haick, H. Hybrid volatolomics and disease detection. *Angew. Chem. Int. Ed. Engl.,* **2015**, *54*(38), 11036-11048.
[http://dx.doi.org/10.1002/anie.201500153] [PMID: 26235374]

[20] Amann, A.; Ligor, M.; Ligor, T.; Bajtarevic, A.; Ager, C.; Pienz, M.; Denz, H.; Fiegl, M.; Hilbe, W.; Weiss, W.; Lukas, P.; Jamnig, H.; Hackl, M.; Haidenberger, A.; Sponring, A.; Filipiak, W.; Miekisch, W.; Schubert, J.; Troppmair, J.; Buszewski, B. Analysis of exhaled breath for screening of lung cancer patients. *MEMO,* **2010**, *3*, 106-112.
[http://dx.doi.org/10.1007/s12254-010-0219-2]

[21] Haick, H.; Broza, Y.Y.; Mochalski, P.; Ruzsanyi, V.; Amann, A. Assessment, origin, and implementation of breath volatile cancer markers. *Chem. Soc. Rev.,* **2014**, *43*(5), 1423-1449.
[http://dx.doi.org/10.1039/C3CS60329F] [PMID: 24305596]

[22] Hakim, M.; Broza, Y.Y.; Barash, O.; Peled, N.; Phillips, M.; Amann, A.; Haick, H. Volatile organic compounds of lung cancer and possible biochemical pathways. *Chem. Rev.,* **2012**, *112*(11), 5949-5966.
[http://dx.doi.org/10.1021/cr300174a] [PMID: 22991938]

[23] Mazzone, P. Progress in the development of a diagnostic test for lung cancer through the analysis of breath volatiles. *J. Breath Res.,* **2008**, *2*(3), 037014.
[http://dx.doi.org/10.1088/1752-7155/2/3/037014] [PMID: 21386175]

[24] Ligor, M.; Ligor, T.; Bajtarevic, A.; Ager, C.; Pienz, M.; Klieber, M.; Denz, H.; Fiegl, M.; Hilbe, W.; Weiss, W.; Lukas, P.; Jamnig, H.; Hackl, M.; Buszewski, B.; Miekisch, W.; Schubert, J.; Amann, A. Determination of volatile organic compounds in exhaled breath of patients with lung cancer using solid phase microextraction and gas chromatography mass spectrometry. *Clin. Chem. Lab. Med.,* **2009**, *47*(5), 550-560.
[http://dx.doi.org/10.1515/CCLM.2009.133] [PMID: 19397483]

[25] Amann, A.; Corradi, M.; Mazzone, P.; Mutti, A. Lung cancer biomarkers in exhaled breath. *Expert Rev. Mol. Diagn.,* **2011**, *11*(2), 207-217.
[http://dx.doi.org/10.1586/erm.10.112] [PMID: 21405971]

[26] Bajtarevic, A.; Ager, C.; Pienz, M.; Klieber, M.; Schwarz, K.; Ligor, M.; Ligor, T.; Filipiak, W.; Denz, H.; Fiegl, M.; Hilbe, W.; Weiss, W.; Lukas, P.; Jamnig, H.; Hackl, M.; Haidenberger, A.; Buszewski, B.; Miekisch, W.; Schubert, J.; Amann, A. Noninvasive detection of lung cancer by analysis of exhaled breath. *BMC Cancer,* **2009**, *9*, 348.
[http://dx.doi.org/10.1186/1471-2407-9-348] [PMID: 19788722]

[27] Fuchs, P.; Loeseken, C.; Schubert, J.K.; Miekisch, W. Breath gas aldehydes as biomarkers of lung cancer. *Int. J. Cancer,* **2010**, *126*(11), 2663-2670.
[PMID: 19839051]

[28] Kischkel, S.; Miekisch, W.; Sawacki, A.; Straker, E.M.; Trefz, P.; Amann, A.; Schubert, J.K. Breath biomarkers for lung cancer detection and assessment of smoking related effects--confounding variables, influence of normalization and statistical algorithms. *Clin. Chim. Acta,* **2010**, *411*(21-22), 1637-1644.
[http://dx.doi.org/10.1016/j.cca.2010.06.005] [PMID: 20542019]

[29] Mazzone, P.J.; Wang, X.F.; Xu, Y.; Mekhail, T.; Beukemann, M.C.; Na, J.; Kemling, J.W.; Suslick, K.S.; Sasidhar, M. Exhaled breath analysis with a colorimetric sensor array for the identification and characterization of lung cancer. *J. Thorac. Oncol.,* **2012**, *7*(1), 137-142.
[http://dx.doi.org/10.1097/JTO.0b013e318233d80f] [PMID: 22071780]

[30] Kischkel, S.; Miekisch, W.; Fuchs, P.; Schubert, J.K. Breath analysis during one-lung ventilation in cancer patients. *Eur. Respir. J.,* **2012**, *40*(3), 706-713.
[http://dx.doi.org/10.1183/09031936.00125411] [PMID:

22267752]

[31] Leja, M.; Amal, H.; Funka, K.; Lasina, I.; Skapars, R.; Ancans, G.; Liepniece-Karele, I.; Haick, H. Volatile Organic Compound in the Breath to Differentiate between Gastric Cancer and Benign Conditions. *Helicobacter,* **2013**, *18*, 119-119.

[32] Amann, A.; Mochalski, P.; Ruzsanyi, V.; Broza, Y.Y.; Haick, H. Assessment of the exhalation kinetics of volatile cancer biomarkers based on their physicochemical properties. *J. Breath Res.,* **2014**, *8*(1), 016003. [http://dx.doi.org/10.1088/1752-7155/8/1/016003] [PMID: 24566039]

[33] Phillips, M.; Altorki, N.; Austin, J.H.; Cameron, R.B.; Cataneo, R.N.; Kloss, R.; Maxfield, R.A.; Munawar, M.I.; Pass, H.I.; Rashid, A.; Rom, W.N.; Schmitt, P.; Wai, J. Detection of lung cancer using weighted digital analysis of breath biomarkers. *Clin. Chim. Acta,* **2008**, *393*(2), 76-84. [http://dx.doi.org/10.1016/j.cca.2008.02.021] [PMID: 18420034]

[34] Chen, S.; Zieve, L.; Mahadevan, V. Mercaptans and dimethyl sulfide in the breath of patients with cirrhosis of the liver. Effect of feeding methionine. *J. Lab. Clin. Med.,* **1970**, *75*(4), 628-635. [PMID: 5444348]

[35] Chen, S.; Mahadevan, V.; Zieve, L. Volatile fatty acids in the breath of patients with cirrhosis of the liver. *J. Lab. Clin. Med.,* **1970**, *75*(4), 622-627. [PMID: 5444347]

[36] Kazui, M.; Andreoni, K.A.; Norris, E.J.; Klein, A.S.; Burdick, J.F.; Beattie, C.; Sehnert, S.S.; Bell, W.R.; Bulkley, G.B.; Risby, T.H. Breath ethane: a specific indicator of free-radical-mediated lipid peroxidation following reperfusion of the ischemic liver. *Free Radic. Biol. Med.,* **1992**, *13*(5), 509-515. [http://dx.doi.org/10.1016/0891-5849(92)90145-7] [PMID: 1459476]

[37] Sehnert, S.S.; Jiang, L.; Burdick, J.F.; Risby, T.H. Breath biomarkers for detection of human liver diseases: preliminary study. *Biomarkers,* **2002**, *7*(2), 174-187. [http://dx.doi.org/10.1080/13547500110118184] [PMID: 12101636]

[38] Davies, S.; Spanel, P.; Smith, D. Quantitative analysis of ammonia on the breath of patients in end-stage renal failure. *Kidney Int.,* **1997**, *52*(1), 223-228. [http://dx.doi.org/10.1038/ki.1997.324] [PMID: 9211367]

[39] Simenhoff, M.L.; Burke, J.F.; Saukkonen, J.J.; Ordinario, A.T.; Doty, R. Biochemical profile or uremic breath. *N. Engl. J. Med.,* **1977**, *297*(3), 132-135. [http://dx.doi.org/10.1056/NEJM197707212970303] [PMID: 865584]

[40] Endre, Z.H.; Pickering, J.W.; Storer, M.K.; Hu, W.P.; Moorhead, K.T.; Allardyce, R.; McGregor, D.O.; Scotter, J.M. Breath ammonia and trimethylamine allow real-time monitoring of haemodialysis efficacy. *Physiol. Meas.,* **2011**, *32*(1), 115-130. [http://dx.doi.org/10.1088/0967-3334/32/1/008] [PMID: 21149927]

[41] Mochalski, P.; King, J.; Haas, M.; Unterkofler, K.; Amann, A.; Mayer, G. Blood and breath profiles of volatile organic compounds in patients with end-stage renal disease. *BMC Nephrol.,* **2014**, *15*, 43. [http://dx.doi.org/10.1186/1471-2369-15-43] [PMID: 24607025]

[42] Filipiak, W.; Ruzsanyi, V.; Mochalski, P.; Filipiak, A.; Bajtarevic, A.; Ager, C.; Denz, H.; Hilbe, W.; Jamnig, H.; Hackl, M.; Dzien, A.; Amann, A. Dependence of exhaled breath composition on exogenous factors, smoking habits and exposure to air pollutants. *J. Breath Res.,* **2012**, *6*(3), 036008. [http://dx.doi.org/10.1088/1752-7155/6/3/036008] [PMID: 22932429]

[43] Fischer, S.; Bergmann, A.; Steffens, M.; Trefz, P.; Ziller, M.; Miekisch, W.; Schubert, J.S.; Köhler, H.; Reinhold, P. Impact of food intake on *in vivo* VOC concentrations in exhaled breath assessed in a caprine animal model. *J. Breath Res.,* **2015**, *9*(4), 047113. [http://dx.doi.org/10.1088/1752-7155/9/4/047113] [PMID: 26670078]

[44] Fischer, S.; Trefz, P.; Bergmann, A.; Steffens, M.; Ziller, M.; Miekisch, W.; Schubert, J.S.; Köhler, H.; Reinhold, P. Physiological variability in volatile organic compounds (VOCs) in exhaled breath and released from faeces due to nutrition and somatic growth in a standardized caprine animal model. *J. Breath Res.,* **2015**, *9*(2), 027108. [http://dx.doi.org/10.1088/1752-7155/9/2/027108] [PMID: 25971714]

[45] Taucher, J.; Hansel, A.; Jordan, A.; Lindinger, W. Analysis of compounds in human breath after ingestion of garlic using proton-transfer-reaction mass spectrometry. *J. Agric. Food Chem.,* **1996**, *44*(12), 3778-3782. [http://dx.doi.org/10.1021/jf960640e]

[46] King, J.; Koc, H.; Unterkofler, K.; Mochalski, P.; Kupferthaler, A.; Teschl, G.; Teschl, S.; Hinterhuber, H.; Amann, A. Physiological modeling of isoprene dynamics in exhaled breath. *J. Theor. Biol.,* **2010**, *267*(4), 626-637. [http://dx.doi.org/10.1016/j.jtbi.2010.09.028] [PMID: 20869370]

[47] Unterkofler, K.; King, J.; Mochalski, P.; Jandacka, M.; Koc, H.; Teschl, S.; Amann, A.; Teschl, G. Modeling-based determination of physiological parameters of systemic VOCs by breath gas analysis: a pilot study. *J. Breath Res.,* **2015**, *9*(3), 036002. [http://dx.doi.org/10.1088/1752-7155/9/3/036002] [PMID: 25972041]

[48] Umber, B.J.; Shin, H.W.; Meinardi, S.; Leu, S.Y.; Zaldivar, F.; Cooper, D.M.; Blake, D.R. Gas signatures from Escherichia coli and Escherichia coli-inoculated human whole blood. *Clin. Transl. Med.,* **2013**, *2*, 13. [http://dx.doi.org/10.1186/2001-1326-2-13] [PMID: 23842518]

[49] Chippendale, T.W.; Španěl, P.; Smith, D. Time-resolved selected ion flow tube mass spectrometric quantification of the volatile compounds generated by E. coli JM109 cultured in two different media. *Rapid Commun. Mass Spectrom.,* **2011**, *25*(15), 2163-2172. [http://dx.doi.org/10.1002/rcm.5099] [PMID: 21710596]

[50] King, J.; Kupferthaler, A.; Frauscher, B.; Hackner, H.; Unterkofler, K.; Teschl, G.; Hinterhuber, H.; Amann, A.; Högl, B. Measurement of endogenous acetone and isoprene in exhaled breath during sleep. *Physiol. Meas.,* **2012**, *33*(3), 413-428. [http://dx.doi.org/10.1088/0967-3334/33/3/413] [PMID: 22370046]

[51] King, J.; Kupferthaler, A.; Unterkofler, K.; Koc, H.; Teschl, S.; Teschl, G.; Miekisch, W.; Schubert, J.; Hinterhuber, H.; Amann, A. Isoprene and acetone concentration profiles during exercise on an ergometer. *J. Breath Res.,* **2009**, *3*(2), 027006. [http://dx.doi.org/10.1088/1752-7155/3/2/027006] [PMID: 21383461]

[52] King, J.; Mochalski, P.; Kupferthaler, A.; Unterkofler, K.; Koc, H.; Filipiak, W.; Teschl, S.; Hinterhuber, H.; Amann, A. Dynamic profiles of volatile organic compounds in exhaled breath as determined by a coupled PTR-MS/GC-MS study. *Physiol. Meas.,* **2010**, *31*(9), 1169-1184. [http://dx.doi.org/10.1088/0967-3334/31/9/008] [PMID:

20664160]

[53] Aksenov, A.A.; Gojova, A.; Zhao, W.; Morgan, J.T.; Sankaran, S.; Sandrock, C.E.; Davis, C.E. Characterization of volatile organic compounds in human leukocyte antigen heterologous expression systems: a cell's "chemical odor fingerprint". *ChemBioChem,* **2012**, *13*(7), 1053-1059. [http://dx.doi.org/10.1002/cbic.201200011] [PMID: 22488873]

[54] Wichmann, G.; Mühlenberg, J.; Fischäder, G.; Kulla, C.; Rehwagen, M.; Herbarth, O.; Lehmann, I. An experimental model for the determination of immunomodulating effects by volatile compounds. *Toxicol. In Vitro,* **2005**, *19*(5), 685-693. [http://dx.doi.org/10.1016/j.tiv.2005.03.012] [PMID: 15885978]

[55] Sarma, S.N.; Kim, Y.J.; Ryu, J.C. Gene expression profiles of human promyelocytic leukemia cell lines exposed to volatile organic compounds. *Toxicology,* **2010**, *271*(3), 122-130. [http://dx.doi.org/10.1016/j.tox.2010.03.014] [PMID: 20359517]

[56] Proctor, L.M. The Human Microbiome Project in 2011 and beyond. *Cell Host Microbe,* **2011**, *10*(4), 287-291. [http://dx.doi.org/10.1016/j.chom.2011.10.001] [PMID: 22018227]

[57] Aksenov, A.A.; Sandrock, C.E.; Zhao, W.; Sankaran, S.; Schivo, M.; Harper, R.; Cardona, C.J.; Xing, Z.; Davis, C.E. Cellular scent of influenza virus infection. *ChemBioChem,* **2014**, *15*(7), 1040-1048. [http://dx.doi.org/10.1002/cbic.201300695] [PMID: 24719290]

[58] Filipiak, W.; Sponring, A.; Filipiak, A.; Ager, C.; Schubert, J.; Miekisch, W.; Amann, A.; Troppmair, J. TD-GC-MS Analysis of Volatile Metabolites of Human Lung Cancer and Normal Cells *in vitro*. In: *Cancer epidemiology, biomarkers & prevention,* American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology, 19(1), **2010**, pp. 182-195.

[59] Filipiak, W.; Sponring, A.; Mikoviny, T.; Ager, C.; Schubert, J.; Miekisch, W.; Amann, A.; Troppmair, J. Release of volatile organic compounds (VOCs) from the lung cancer cell line CALU-1 *in vitro*. *Cancer Cell Int.,* **2008**, *8*, 17. [http://dx.doi.org/10.1186/1475-2867-8-17] [PMID: 19025629]

[60] Kwak, J.; Gallagher, M.; Ozdener, M.H.; Wysocki, C.J.; Goldsmith, B.R.; Isamah, A.; Faranda, A.; Fakharzadeh, S.S.; Herlyn, M.; Johnson, A.T.; Preti, G. Volatile biomarkers from human melanoma cells. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.,* **2013**, *931*, 90-96. [http://dx.doi.org/10.1016/j.jchromb.2013.05.007] [PMID: 23770738]

[61] Mochalski, P.; Sponring, A.; King, J.; Unterkofler, K.; Troppmair, J.; Amann, A. Release and uptake of volatile organic compounds by human hepatocellular carcinoma cells (HepG2) *in vitro*. *Cancel Cell Int.,* **2013**, *13*(1), 72.

[62] Mochalski, P.; Theurl, M.; Sponring, A.; Unterkofler, K.; Kirchmair, R.; Amann, A. Analysis of volatile organic compounds liberated and metabolised by human umbilical vein endothelial cells (HUVEC) *in vitro*. *Cell Biochem. Biophys.,* **2015**, *71*(1), 323-329. [http://dx.doi.org/10.1007/s12013-014-0201-4] [PMID: 25123840]

[63] Schivo, M.; Aksenov, A.A.; Linderholm, A.L.; McCartney, M.M.; Simmons, J.; Harper, R.W.; Davis, C.E. Volatile emanations from *in vitro* airway cells infected with human rhinovirus. *J. Breath Res.,* **2014**, *8*(3), 037110. [http://dx.doi.org/10.1088/1752-7155/8/3/037110] [PMID: 25189196]

[64] Sponring, A.; Filipiak, W.; Ager, C.; Schubert, J.; Miekisch, W.; Amann, A.; Troppmair, J. Analysis of volatile organic compounds (VOCs) in the headspace of NCI-H1666 lung cancer cells. *Cancer Biomark,* **2010**, *7*(3), 153-161.

[65] Sponring, A.; Filipiak, W.; Mikoviny, T.; Ager, C.; Schubert, J.; Miekisch, W.; Amann, A.; Troppmair, J. Release of volatile organic compounds from the lung cancer cell line NCI-H2087 *in vitro*. *Anticancer Res.,* **2009**, *29*(1), 419-426. [PMID: 19331181]

[66] Sulé-Suso, J.; Pysanenko, A.; Spanel, P.; Smith, D. Quantification of acetaldehyde and carbon dioxide in the headspace of malignant and non-malignant lung cells *in vitro* by SIFT-MS. *Analyst (Lond.),* **2009**, *134*(12), 2419-2425. [http://dx.doi.org/10.1039/b916158a] [PMID: 19918611]

[67] Zhang, Y.; Gao, G.; Liu, H.; Fu, H.; Fan, J.; Wang, K.; Chen, Y.; Li, B.; Zhang, C.; Zhi, X.; He, L.; Cui, D. Identification of volatile biomarkers of gastric cancer cells and ultrasensitive electrochemical detection based on sensing interface of Au-Ag alloy coated MWCNTs. *Theranostics,* **2014**, *4*(2), 154-162. [http://dx.doi.org/10.7150/thno.7560] [PMID: 24465273]

[68] Zimmermann, D.; Hartmann, M.; Moyer, M.P.; Nolte, J.; Baumbach, J.I. Determination of volatile products of human colon cell line metabolism by GC/MS analysis. *Metabolomics,* **2007**, *3*(1), 13-17. [http://dx.doi.org/10.1007/s11306-006-0038-y]

[69] Peled, N.; Barash, O.; Tisch, U.; Ionescu, R.; Broza, Y.Y.; Ilouze, M.; Mattei, J.; Bunn, P.A., Jr; Hirsch, F.R.; Haick, H. Volatile fingerprints of cancer specific genetic mutations. *Nanomedicine (Lond.),* **2013**, *9*(6), 758-766. [PMID: 23428987]

[70] Filipiak, W.; Beer, R.; Sponring, A.; Filipiak, A.; Ager, C.; Schiefecker, A.; Lanthaler, S.; Helbok, R.; Nagl, M.; Troppmair, J.; Amann, A. Breath analysis for *in vivo* detection of pathogens related to ventilator-associated pneumonia in intensive care patients: a prospective pilot study. *J. Breath Res.,* **2015**, *9*(1), 016004. [http://dx.doi.org/10.1088/1752-7155/9/1/016004] [PMID: 25557917]

[71] Diskin, A.M.; Spanel, P.; Smith, D. Time variation of ammonia, acetone, isoprene and ethanol in breath: a quantitative SIFT-MS study over 30 days. *Physiol. Meas.,* **2003**, *24*(1), 107-119. [http://dx.doi.org/10.1088/0967-3334/24/1/308] [PMID: 12636190]

[72] Kroemer, G.; Pouyssegur, J. Tumor cell metabolism: cancer's Achilles' heel. *Cancer Cell,* **2008**, *13*(6), 472-482. [http://dx.doi.org/10.1016/j.ccr.2008.05.005] [PMID: 18538731]

[73] Zechman, J.M.; Aldinger, S.; Labows, J.N., Jr Characterization of pathogenic bacteria by automated headspace concentration-gas chromatography. *J. Chromatogr. A,* **1986**, *377*, 49-57. [http://dx.doi.org/10.1016/S0378-4347(00)80760-4] [PMID: 3086354]

[74] Preti, G.; Thaler, E.; Hanson, C.W.; Troy, M.; Eades, J.; Gelperin, A. Volatile compounds characteristic of sinus-related bacteria and infected sinus mucus: analysis by solid-phase microextraction and gas chromatography-mass spectrometry. *J. Chromatogr. B.,* **2009**, *877*(22), 2011-2018. [http://dx.doi.org/10.1016/j.jchromb.2009.05.028] [PMID: 19553163]

[75] Allardyce, R.A.; Langford, V.S.; Hill, A.L.; Murdoch, D.R. Detection of volatile metabolites produced by bacterial growth in blood culture media by selected ion flow tube mass spectrometry (SIFT-MS). *J. Microbiol. Methods,* **2006**, *65*(2), 361-365. [http://dx.doi.org/10.1016/j.mimet.2005.09.003] [PMID:

16249043]

[76] Bunge, M.; Araghipour, N.; Mikoviny, T.; Dunkl, J.; Schnitzhofer, R.; Hansel, A.; Schinner, F.; Wisthaler, A.; Margesin, R.; Märk, T.D. On-line monitoring of microbial volatile metabolites by proton transfer reaction-mass spectrometry. *Appl. Environ. Microbiol.,* **2008**, *74*(7), 2179-2186. [http://dx.doi.org/10.1128/AEM.02069-07] [PMID: 18245241]

[77] Scotter, J.M.; Allardyce, R.A.; Langford, V.S.; Hill, A.; Murdoch, D.R. The rapid evaluation of bacterial growth in blood cultures by selected ion flow tube-mass spectrometry (SIFT-MS) and comparison with the BacT/ALERT automated blood culture system. *J. Microbiol. Methods,* **2006**, *65*(3), 628-631. [http://dx.doi.org/10.1016/j.mimet.2005.09.016] [PMID: 16271408]

[78] Filipiak, W.; Sponring, A.; Baur, M.M.; Ager, C.; Filipiak, A.; Wiesenhofer, H.; Nagl, M.; Troppmair, J.; Amann, A. Characterization of volatile metabolites taken up by or released from Streptococcus pneumoniae and Haemophilus influenzae by using GC-MS. *Microbiology,* **2012**, *158*(Pt 12), 3044-3053. [http://dx.doi.org/10.1099/mic.0.062687-0] [PMID: 23059976]

[79] Filipiak, W.; Sponring, A.; Baur, M.M.; Filipiak, A.; Ager, C.; Wiesenhofer, H.; Nagl, M.; Troppmair, J.; Amann, A. Molecular analysis of volatile metabolites released specifically by Staphylococcus aureus and Pseudomonas aeruginosa. *BMC Microbiol.,* **2012**, *12*, 113. [http://dx.doi.org/10.1186/1471-2180-12-113] [PMID: 22716902]

[80] Poli, D.; Goldoni, M.; Corradi, M.; Acampa, O.; Carbognani, P.; Internullo, E.; Casalini, A.; Mutti, A. Determination of aldehydes in exhaled breath of patients with lung cancer by means of on-fiber-derivatisation SPME-GC/MS. *J. Chromatogr. B,* **2010**, *878*(27), 2643-2651. [http://dx.doi.org/10.1016/j.jchromb.2010.01.022] [PMID: 20149763]

[81] Wehinger, A.; Schmid, A.; Mechtcheriakov, S.; Ledochowski, M.; Grabmer, C.; Gastl, G.A.; Amann, A. Lung cancer detection by proton transfer reaction mass-spectrometric analysis of human breath gas. *Int. J. Mass Spectrom.,* **2007**, *265*(1), 49-59. [http://dx.doi.org/10.1016/j.ijms.2007.05.012]

[82] Deng, C.; Zhang, X.; Li, N. Investigation of volatile biomarkers in lung cancer blood using solid-phase microextraction and capillary gas chromatography-mass spectrometry. *J. Chromatogr. B,* **2004**, *808*(2), 269-277. [http://dx.doi.org/10.1016/j.jchromb.2004.05.015] [PMID: 15261821]

[83] Nozoe, T.; Goda, S.; Selyanchyn, R.; Wang, T.; Nakazawa, K.; Hirano, T.; Matsui, H.; Lee, S.W. *in vitro* detection of small molecule metabolites excreted from cancer cells using a Tenax TA thin-film microextraction device. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.,* **2015**, *991*, 99-107. [http://dx.doi.org/10.1016/j.jchromb.2015.04.016] [PMID: 25932789]

[84] Schallschmidt, K.; Becker, R.; Jung, C.; Rolff, J.; Fichtner, I.; Nehls, I. Investigation of cell culture volatilomes using solid phase micro extraction: Options and pitfalls exemplified with adenocarcinoma cell lines. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.,* **2015**, *1006*, 158-166. [http://dx.doi.org/10.1016/j.jchromb.2015.10.004] [PMID: 26551208]

[85] Schallschmidt, K.; Becker, R.; Zwaka, H.; Menzel, R.; Johnen, D.; Fischer-Tenhagen, C.; Rolff, J.; Nehls, I. *in vitro* cultured lung cancer cells are not suitable for animal-based breath biomarker detection. *J. Breath Res.,* **2015**, *9*(2), 027103. [http://dx.doi.org/10.1088/1752-7155/9/2/027103] [PMID: 25667342]

[86] Calenic, B.; Amann, A. Detection of volatile malodorous compounds in breath: current analytical techniques and implications in human disease. *Bioanalysis,* **2014**, *6*(3), 357-376. [http://dx.doi.org/10.4155/bio.13.306] [PMID: 24471956]

[87] Baker, R.R. Smoke generation inside a burning cigarette: Modifying combustion to develop cigarettes that may be less hazardous to health. *Prog. Energ. Combust.,* **2006**, *32*(4), 373-385. [http://dx.doi.org/10.1016/j.pecs.2006.01.001]

[88] Uhde, E.; Salthammer, T. Impact of reaction products from building materials and furnishings on indoor air quality - A review of recent advances in indoor chemistry. *Atmos. Environ.,* **2007**, *41*(15), 3111-3128. [http://dx.doi.org/10.1016/j.atmosenv.2006.05.082]

[89] Musa-Veloso, K.; Likhodii, S.S.; Rarama, E.; Benoit, S.; Liu, Y.M.; Chartrand, D.; Curtis, R.; Carmant, L.; Lortie, A.; Comeau, F.J.; Cunnane, S.C. Breath acetone predicts plasma ketone bodies in children with epilepsy on a ketogenic diet. *Nutrition,* **2006**, *22*(1), 1-8. [http://dx.doi.org/10.1016/j.nut.2005.04.008] [PMID: 16183255]

[90] Spaněl, P.; Dryahina, K.; Rejšková, A.; Chippendale, T.W.; Smith, D. Breath acetone concentration; biological variability and the influence of diet. *Physiol. Meas.,* **2011**, *32*(8), N23-N31. [http://dx.doi.org/10.1088/0967-3334/32/8/N01] [PMID: 21725144]

[91] Buszewski, B.; Ulanowska, A.; Ligor, T.; Denderz, N.; Amann, A. Analysis of exhaled breath from smokers, passive smokers and non-smokers by solid-phase microextraction gas chromatography/mass spectrometry. *Biomed. Chromatogr.,* **2009**, *23*(5), 551-556. [http://dx.doi.org/10.1002/bmc.1141] [PMID: 19039804]

[92] Scheepers, P.T.; Konings, J.; Demirel, G.; Gaga, E.O.; Anzion, R.; Peer, P.G.; Dogeroglu, T.; Ornektekin, S.; van Doorn, W. Determination of exposure to benzene, toluene and xylenes in Turkish primary school children by analysis of breath and by environmental passive sampling. *Sci. Total Environ.,* **2010**, *408*(20), 4863-4870. [http://dx.doi.org/10.1016/j.scitotenv.2010.06.037] [PMID: 20619876]

[93] Brugnone, F.; Perbellini, L.; Romeo, L.; Bianchin, M.; Tonello, A.; Pianalto, G.; Zambon, D.; Zanon, G. Benzene in environmental air and human blood. *Int. Arch. Occup. Environ. Health,* **1998**, *71*(8), 554-559. [http://dx.doi.org/10.1007/s004200050323] [PMID: 9860165]

[94] Holzinger, R.; Jordan, A.; Hansel, A.; Lindinger, W. Automobile emissions of acetonitrile: Assessment of its contribution to the global source. *J. Atmos. Chem.,* **2001**, *38*(2), 187-193. [http://dx.doi.org/10.1023/A:1006435723375]

[95] Abbott, S.M.; Elder, J.B.; Spanel, P.; Smith, D. Quantification of acetonitrile in exhaled breath and urinary headspace using selected ion flow tube mass spectrometry. *Int. J. Mass Spectrom.,* **2003**, *228*(2-3), 655-665. [http://dx.doi.org/10.1016/S1387-3806(03)00212-4]

[96] Pinggera, G.M.; Lirk, P.; Bodogri, F.; Herwig, R.; Steckel-Berger, G.; Bartsch, G.; Rieder, J. Urinary acetonitrile concentrations correlate with recent smoking behaviour. *BJU Int.,* **2005**, *95*(3), 306-309. [http://dx.doi.org/10.1111/j.1464-410X.2005.05288.x]

[PMID: 15679783]

[97] Kushch, I.; Arendacká, B.; Stolc, S.; Mochalski, P.; Filipiak, W.; Schwarz, K.; Schwentner, L.; Schmid, A.; Dzien, A.; Lechleitner, M.; Witkovský, V.; Miekisch, W.; Schubert, J.; Unterkofler, K.; Amann, A. Breath isoprene--aspects of normal physiology related to age, gender and cholesterol profile as determined in a proton transfer reaction mass spectrometry study. *Clin. Chem. Lab. Med.,* **2008**, *46*(7), 1011-1018. [http://dx.doi.org/10.1515/CCLM.2008.181] [PMID: 18605961]

[98] Gelmont, D.; Stein, R.A.; Mead, J.F. Isoprene-the main hydrocarbon in human breath. *Biochem. Biophys. Res. Commun.,* **1981**, *99*(4), 1456-1460. [http://dx.doi.org/10.1016/0006-291X(81)90782-8] [PMID: 7259787]

[99] Foster, W.M.; Jiang, L.; Stetkiewicz, P.T.; Risby, T.H. Breath isoprene: temporal changes in respiratory output after exposure to ozone. *J. Appl. Physiol.,* **1996**, *80*(2), 706-710. [PMID: 8929619]

[100] King, J.; Mochalski, P.; Unterkofler, K.; Teschl, G.; Klieber, M.; Stein, M.; Amann, A.; Baumann, M. Breath isoprene: muscle dystrophy patients support the concept of a pool of isoprene in the periphery of the human body. *Biochem. Biophys. Res. Commun.,* **2012**, *423*(3), 526-530. [http://dx.doi.org/10.1016/j.bbrc.2012.05.159] [PMID: 22683640]

[101] Del Monte, M.; Citti, L.; Gervasi, P.G. Isoprene metabolism by liver microsomal mono-oxygenases. *Xenobiotica,* **1985**, *15*(7), 591-597. [http://dx.doi.org/10.3109/00498258509045888] [PMID: 4049899]

[102] Bogaards, J.J.; Freidig, A.P.; van Bladeren, P.J. Prediction of isoprene diepoxide levels *in vivo* in mouse, rat and man using enzyme kinetic data *in vitro* and physiologically-based pharmacokinetic modelling. *Chem. Biol. Interact.,* **2001**, *138*(3), 247-265. [http://dx.doi.org/10.1016/S0009-2797(01)00276-9] [PMID: 11714482]

[103] Watson, W.P.; Cottrell, L.; Zhang, D.; Golding, B.T. Metabolism and molecular toxicology of isoprene. *Chem. Biol. Interact.,* **2001**, *135-136*, 223-238. [http://dx.doi.org/10.1016/S0009-2797(01)00192-2] [PMID: 11397393]

[104] Mlakar, A.; Spiteller, G. Reinvestigation of lipid peroxidation of linolenic acid. *Biochim. Biophys. Acta,* **1994**, *1214*(2), 209-220. [http://dx.doi.org/10.1016/0005-2760(94)90046-9] [PMID: 7918602]

[105] Kalluri, U.; Naiker, M.; Myers, M.A. Cell culture metabolomics in the diagnosis of lung cancer-the influence of cell culture conditions. *J. Breath Res.,* **2014**, *8*(2), 027109. [http://dx.doi.org/10.1088/1752-7155/8/2/027109] [PMID: 24861817]

[106] Mitsui, T.; Kondo, T. Inadequacy of theoretical basis of breath methylated alkane contour for assessing oxidative stress. *Clin. Chim. Acta,* **2003**, *333*(1), 91-91. [http://dx.doi.org/10.1016/S0009-8981(03)00173-6] [PMID: 12809740]

[107] Kneepkens, C.M.; Lepage, G.; Roy, C.C. The potential of the hydrocarbon breath test as a measure of lipid peroxidation. *Free Radic. Biol. Med.,* **1994**, *17*(2), 127-160. [http://dx.doi.org/10.1016/0891-5849(94)90110-4] [PMID: 7959173]

[108] Watanabe, M. Polymorphic CYP genes and disease predisposition--what have the studies shown so far? *Toxicol. Lett.,* **1998**, *102-103*, 167-171. [http://dx.doi.org/10.1016/S0378-4274(98)00303-8] [PMID: 10022250]

[109] Roos, P.H.; Bolt, H.M. Cytochrome P450 interactions in human cancers: new aspects considering CYP1B1. *Expert Opin. Drug Metab. Toxicol.,* **2005**, *1*(2), 187-202. [http://dx.doi.org/10.1517/17425255.1.2.187] [PMID: 16922636]

[110] Marchitti, S.A.; Brocker, C.; Stagos, D.; Vasiliou, V. Non-P450 aldehyde oxidizing enzymes: the aldehyde dehydrogenase superfamily. *Expert Opin. Drug Metab. Toxicol.,* **2008**, *4*(6), 697-720. [http://dx.doi.org/10.1517/17425255.4.6.697] [PMID: 18611112]

[111] Marcato, P.; Dean, C.A.; Giacomantonio, C.A.; Lee, P.W. Aldehyde dehydrogenase: its role as a cancer stem cell marker comes down to the specific isoform. *Cell Cycle,* **2011**, *10*(9), 1378-1384. [http://dx.doi.org/10.4161/cc.10.9.15486] [PMID: 21552008]

[112] Zhao, D.; McCaffery, P.; Ivins, K.J.; Neve, R.L.; Hogan, P.; Chin, W.W.; Dräger, U.C. Molecular identification of a major retinoic-acid-synthesizing enzyme, a retinaldehyde-specific dehydrogenase. *Eur. J. Biochem.,* **1996**, *240*(1), 15-22. [http://dx.doi.org/10.1111/j.1432-1033.1996.0015h.x] [PMID: 8797830]

[113] Rexer, B.N.; Zheng, W.L.; Ong, D.E. Retinoic acid biosynthesis by normal human breast epithelium is *via* aldehyde dehydrogenase 6, absent in MCF-7 cells. *Cancer Res.,* **2001**, *61*(19), 7065-7070. [PMID: 11585737]

[114] Shin, H.W.; Umber, B.J.; Meinardi, S.; Leu, S.Y.; Zaldivar, F.; Blake, D.R.; Cooper, D.M. Acetaldehyde and hexanaldehyde from cultured white cells. *J. Transl. Med.,* **2009**, *7*, 31. [http://dx.doi.org/10.1186/1479-5876-7-31] [PMID: 19402909]

[115] Kato, S.; Post, G.C.; Bierbaum, V.M.; Koch, T.H. Chemical ionization mass spectrometric determination of acrolein in human breast cancer cells. *Anal. Biochem.,* **2002**, *305*(2), 251-259. [http://dx.doi.org/10.1006/abio.2002.5682] [PMID: 12054454]

[116] Patel, M.; Lu, L.; Zander, D.S.; Sreerama, L.; Coco, D.; Moreb, J.S. ALDH1A1 and ALDH3A1 expression in lung cancers: correlation with histologic type and potential precursors. *Lung Cancer,* **2008**, *59*(3), 340-349. [http://dx.doi.org/10.1016/j.lungcan.2007.08.033] [PMID: 17920722]

[117] Jiang, F.; Qiu, Q.; Khanna, A.; Todd, N.W.; Deepak, J.; Xing, L.; Wang, H.; Liu, Z.; Su, Y.; Stass, S.A.; Katz, R.L. Aldehyde dehydrogenase 1 is a tumor stem cell-associated marker in lung cancer. *Mol. Cancer Res.,* **2009**, *7*(3), 330-338. [http://dx.doi.org/10.1158/1541-7786.MCR-08-0393] [PMID: 19276181]

[118] Rutter, A.V.; Chippendale, T.W.; Yang, Y.; Španěl, P.; Smith, D.; Sulé-Suso, J. Quantification by SIFT-MS of acetaldehyde released by lung cells in a 3D model. *Analyst (Lond.),* **2013**, *138*(1), 91-95. [http://dx.doi.org/10.1039/C2AN36185J] [PMID: 23136643]

[119] Vaupel, P.; Mayer, A.; Höckel, M. Tumor hypoxia and malignant progression. *Methods Enzymol.,* **2004**, *381*, 335-354. [http://dx.doi.org/10.1016/S0076-6879(04)81023-1] [PMID: 15063685]

[120] Vaupel, P. Tumor microenvironmental physiology and its implications for radiation oncology. *Semin. Radiat. Oncol.,* **2004**, *14*(3), 198-206.

[http://dx.doi.org/10.1016/j.semradonc.2004.04.008] [PMID: 15254862]

[121] Frezza, C.; Zheng, L.; Tennant, D.A.; Papkovsky, D.B.; Hedley, B.A.; Kalna, G.; Watson, D.G.; Gottlieb, E. Metabolic profiling of hypoxic cells revealed a catabolic signature required for cell survival. *PLoS One,* **2011**, *6*(9), e24411. [http://dx.doi.org/10.1371/journal.pone.0024411] [PMID: 21912692]

[122] Pyo, J.S.; Ju, H.K.; Park, J.H.; Kwon, S.W. Determination of volatile biomarkers for apoptosis and necrosis by solid-phase microextraction-gas chromatography/mass spectrometry: a pharmacometabolomic approach to cisplatin's cytotoxicity to human lung cancer cell lines. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.,* **2008**, *876*(2), 170-174. [http://dx.doi.org/10.1016/j.jchromb.2008.10.031] [PMID: 19027370]

[123] Phillips, M.; Basa-Dalay, V.; Blais, J.; Bothamley, G.; Chaturvedi, A.; Modi, K.D.; Pandya, M.; Natividad, M.P.; Patel, U.; Ramraje, N.N.; Schmitt, P.; Udwadia, Z.F. Point-of-care breath test for biomarkers of active pulmonary tuberculosis. *Tuberculosis (Edinb.),* **2012**, *92*(4), 314-320. [http://dx.doi.org/10.1016/j.tube.2012.04.002] [PMID: 22647661]

[124] Phillips, M.; Basa-Dalay, V.; Bothamley, G.; Cataneo, R.N.; Lam, P.K.; Natividad, M.P.; Schmitt, P.; Wai, J. Breath biomarkers of active pulmonary tuberculosis. *Tuberculosis (Edinb.),* **2010**, *90*(2), 145-151. [http://dx.doi.org/10.1016/j.tube.2010.01.003] [PMID: 20189456]

[125] Erhart, S.; Amann, A.; Haberlandt, E.; Edlinger, G.; Schmid, A.; Filipiak, W.; Schwarz, K.; Mochalski, P.; Rostasy, K.; Karall, D.; Scholl-Bürgi, S. 3-Heptanone as a potential new marker for valproic acid therapy. *J. Breath Res.,* **2009**, *3*(1), 016004. [http://dx.doi.org/10.1088/1752-7155/3/1/016004] [PMID: 21383452]

[126] Diskin, A.M.; Spanel, P.; Smith, D. Increase of acetone and ammonia in urine headspace and breath during ovulation quantified using selected ion flow tube mass spectrometry. *Physiol. Meas.,* **2003**, *24*(1), 191-199. [http://dx.doi.org/10.1088/0967-3334/24/1/314] [PMID: 12636196]

[127] Pysanenko, A.; Spaněl, P.; Smith, D. A study of sulfur-containing compounds in mouth- and nose-exhaled breath and in the oral cavity using selected ion flow tube mass spectrometry. *J. Breath Res.,* **2008**, *2*(4), 046004. [http://dx.doi.org/10.1088/1752-7155/2/4/046004] [PMID: 21386191]

[128] Baumbach, J.I.; Vautz, W.; Ruzsanyi, V.; Freitag, L. *Metabolites in Human Breath: Ion Mobility Spectrometers as Diagnostic Tools for Lung Diseases,* In: Conference of Breath Analysis: For Clinical Diagnosis and Atherapeutic Monitoring, **2005**, pp. 53-66.

[129] Ruzsanyi, V. Ion mobility spectrometry for pharmacokinetic studies--exemplary application. *J. Breath Res.,* **2013**, *7*(4), 046008. [http://dx.doi.org/10.1088/1752-7155/7/4/046008] [PMID: 24287589]

[130] Ruzsanyi, V.; Baumbach, J.I.; Sielemann, S.; Litterst, P.; Westhoff, M.; Freitag, L. Detection of human metabolites using multi-capillary columns coupled to ion mobility spectrometers. *J. Chromatogr. A,* **2005**, *1084*(1-2), 145-151. [http://dx.doi.org/10.1016/j.chroma.2005.01.055] [PMID: 16114247]

[131] Parameswaran, K.R.; Rosen, D.I.; Allen, M.G.; Ganz, A.M.; Risby, T.H. Off-axis integrated cavity output spectroscopy with a mid-infrared interband cascade laser for real-time breath ethane measurements. *Appl. Opt.,* **2009**, *48*(4), B73-B79. [http://dx.doi.org/10.1364/AO.48.000B73] [PMID: 19183584]

[132] von Basum, G.; Dahnke, H.; Halmer, D.; Hering, P.; Mürtz, M. Online recording of ethane traces in human breath *via* infrared laser spectroscopy. *J. Appl. Physiol.,* **2003**, *95*(6), 2583-2590. [http://dx.doi.org/10.1152/japplphysiol.00542.2003] [PMID: 12897034]

[133] Von Basum, G.; Halmer, D.; Hering, P.; Murtz, M. *Laser Spectroscopic On-Line Monitoring of Exhaled Trace Gases.,* In: Conference of Breath Analysis: For Clinical Diagnosis and Atherapeutic Monitoring, **2005**, pp. 67-74.

[134] Machado, R.F.; Laskowski, D.; Deffenderfer, O.; Burch, T.; Zheng, S.; Mazzone, P.J.; Mekhail, T.; Jennings, C.; Stoller, J.K.; Pyle, J.; Duncan, J.; Dweik, R.A.; Erzurum, S.C. Detection of lung cancer by sensor array analyses of exhaled breath. *Am. J. Respir. Crit. Care Med.,* **2005**, *171*(11), 1286-1291. [http://dx.doi.org/10.1164/rccm.200409-1184OC] [PMID: 15750044]

[135] Hunter, G.W.; Xu, J.C.; Biaggi-Labiosa, A.M.; Laskowski, D.; Dutta, P.K.; Mondal, S.P.; Ward, B.J.; Makel, D.B.; Liu, C.C.; Chang, C.W.; Dweik, R.A. Smart sensor systems for human health breath monitoring applications. *J. Breath Res.,* **2011**, *5*(3), 037111. [http://dx.doi.org/10.1088/1752-7155/5/3/037111] [PMID: 21896970]

[136] Righettoni, M.; Schmid, A.; Amann, A.; Pratsinis, S.E. Correlations between blood glucose and breath components from portable gas sensors and PTR-TOF-MS. *J. Breath Res.,* **2013**, *7*(3), 037110. [http://dx.doi.org/10.1088/1752-7155/7/3/037110] [PMID: 23959908]

[137] Righettoni, M.; Tricoli, A. Toward portable breath acetone analysis for diabetes detection. *J. Breath Res.,* **2011**, *5*(3), 037109. [http://dx.doi.org/10.1088/1752-7155/5/3/037109] [PMID: 21828897]

[138] Xu, Z.Q.; Broza, Y.Y.; Ionsecu, R.; Tisch, U.; Ding, L.; Liu, H.; Song, Q.; Pan, Y.Y.; Xiong, F.X.; Gu, K.S.; Sun, G.P.; Chen, Z.D.; Leja, M.; Haick, H. A nanomaterial-based breath test for distinguishing gastric cancer from benign gastric conditions. *Br. J. Cancer,* **2013**, *108*(4), 941-950. [http://dx.doi.org/10.1038/bjc.2013.44] [PMID: 23462808]

[139] Gardner, J.W.; Craven, M.; Dow, C.; Hines, E.L. The prediction of bacteria type and culture growth phase by an electronic nose with a multi-layer perceptron network. *Meas. Sci. Technol.,* **1998**, *9*(1), 120-127. [http://dx.doi.org/10.1088/0957-0233/9/1/016]

[140] Hockstein, N.G.; Thaler, E.R.; Torigian, D.; Miller, W.T., Jr; Deffenderfer, O.; Hanson, C.W. Diagnosis of pneumonia with an electronic nose: correlation of vapor signature with chest computed tomography scan findings. *Laryngoscope,* **2004**, *114*(10), 1701-1705. [http://dx.doi.org/10.1097/00005537-200410000-00005] [PMID: 15454757]

[141] Broza, Y.Y.; Haick, H. Nanomaterial-based sensors for detection of disease by volatile organic compounds. *Nanomedicine (Lond.),* **2013**, *8*(5), 785-806. [http://dx.doi.org/10.2217/nnm.13.64] [PMID: 23656265]

[142] Buhr, K.; van Ruth, S.; Delahunty, C. Analysis of volatile flavour compounds by Proton Transfer Reaction-Mass Spectrometry: fragmentation patterns and discrimination between isobaric and isomeric compounds. *Int. J. Mass*

Spectrom., **2002**, *221*(1), 1-7. [http://dx.doi.org/10.1016/S1387-3806(02)00896-5]

[143] Schwarz, K.; Filipiak, W.; Amann, A. Determining concentration patterns of volatile compounds in exhaled breath by PTR-MS. *J. Breath Res.,* **2009**, *3*(2), 027002. [http://dx.doi.org/10.1088/1752-7155/3/2/027002] [PMID: 21383457]

[144] Mochalski, P.; Unterkofler, K.; Španěl, P.; Smith, D.; Amann, A. Product ion distributions for the reactions of NO(+) with some physiologically significant aldehydes obtained using a SRI-TOF-MS instrument. *Int. J. Mass Spectrom.,* **2014**, *363*, 23-31. [http://dx.doi.org/10.1016/j.ijms.2014.02.016] [PMID: 25844049]

[145] Bojko, B.; Reyes-Garces, N.; Bessonneau, V.; Gorynski, K.; Mousavi, F.; Silva, E.A.; Pawliszyn, J. Solid-phase microextraction in metabolomics. *Trac-Trend Anal Chem,* **2014**, *61*, 168-180. [http://dx.doi.org/10.1016/j.trac.2014.07.005]

[146] Pan, L.; Pawliszyn, J. Derivatization/solid-phase microextraction: New approach to polar analytes. *Anal. Chem.,* **1997**, *69*(2), 196-205. [http://dx.doi.org/10.1021/ac9606362]

[147] Svensson, S.; Lärstad, M.; Broo, K.; Olin, A.C. Determination of aldehydes in human breath by on-fibre derivatization, solid-phase microextraction and GC-MS. *J. Chromatogr. B,* **2007**, *860*(1), 86-91. [http://dx.doi.org/10.1016/j.jchromb.2007.10.021] [PMID: 17996502]

[148] Deng, C.; Li, N.; Zhang, X. Development of headspace solid-phase microextraction with on-fiber derivatization for determination of hexanal and heptanal in human blood. *J. Chromatogr. B.,* **2004**, *813*(1-2), 47-52. [http://dx.doi.org/10.1016/j.jchromb.2004.09.007] [PMID: 15556514]

[149] Filipiak, W.; Filipiak, A.; Ager, C.; Wiesenhofer, H.; Amann, A. Optimization of sampling parameters for collection and preconcentration of alveolar air by needle traps. *J. Breath Res.,* **2012**, *6*(2), 027107. [http://dx.doi.org/10.1088/1752-7155/6/2/027107] [PMID: 22621839]

[150] Guillot, J.M.; Fernandez, B.; Le Cloirec, P. Advantages and limits of adsorption sampling for physico-chemical measurements of odorous compounds. *Analusis,* **2000**, *28*(3), 180-187. [http://dx.doi.org/10.1051/analusis:2000280180]

[151] Dettmer, K.; Knobloch, T.; Engewald, W. Stability of reactive low boiling hydrocarbons on carbon based adsorbents typically used for adsorptive enrichment and thermal desorption. *Fresenius J. Anal. Chem.,* **2000**, *366*(1), 70-78. [http://dx.doi.org/10.1007/s002160050014]

[152] Buszewski, B.; Ligor, T.; Filipiak, W.; Vasconcelos, M.T.; Pompe, M.; Veber, M. Study of sorptive properties of trap systems for selective enrichment of volatile organic compounds from tobacco smoke samples. *Toxicol. Environ. Chem.,* **2008**, *90*(1), 51-64. [http://dx.doi.org/10.1080/02772240701384418]

[153] Dettmer, K.; Engewald, W. Ambient air analysis of volatile organic compounds using adsorptive enrichment. *Chromatographia,* **2003**, *57*, S339-S347. [http://dx.doi.org/10.1007/BF02492126]

[154] Fastyn, P.; Kornacki, W.; Gierczak, T.; Gawłowski, J.; Niedzielski, J. Adsorption of water vapour from humid air by selected carbon adsorbents. *J. Chromatogr. A,* **2005**, *1078*(1-2), 7-12. [http://dx.doi.org/10.1016/j.chroma.2005.04.096] [PMID: 16007975]

[155] Sumner, L.W.; Amberg, A.; Barrett, D.; Beale, M.H.; Beger, R.; Daykin, C.A.; Fan, T.W.; Fiehn, O.; Goodacre, R.; Griffin, J.L.; Hankemeier, T.; Hardy, N.; Harnly, J.; Higashi, R.; Kopka, J.; Lane, A.N.; Lindon, J.C.; Marriott, P.; Nicholls, A.W.; Reily, M.D.; Thaden, J.J.; viant, M.R. Proposed minimum reporting standards for chemical analysis Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics,* **2007**, *3*(3), 211-221. [http://dx.doi.org/10.1007/s11306-007-0082-2] [PMID: 24039616]

[156] Vessman, J.; Stefan, R.I.; Van Staden, J.F.; Danzer, K.; Lindner, W.; Burns, D.T.; Fajgelj, A.; Muller, H. Selectivity in analytical chemistry - (IUPAC Recommendations 2001). *Pure Appl. Chem.,* **2001**, *73*(8), 1381-1386. [http://dx.doi.org/10.1351/pac200173081381]

[157] Nomenclature, D.C.; Pure, S.R.; Chemistry, A.; Nic, M.; Jirat, J.; Kosata, B. *IUPAC goldbook*; IUPAC, **2006**.

[158] Standard, M.J. *Capability of Detection- Part 2: Methodology in the Linear Calibration Case (ISO 11843-2: 2000, IDT)*; Department of Standards Malaysia, **2002**.

[159] Currie, L.A. Detection and quantification limits: origins and historical overview. *Anal. Chim. Acta,* **1999**, *391*(2), 127-134. [http://dx.doi.org/10.1016/S0003-2670(99)00105-1]

[160] Konvalina, G.; Haick, H. Sensors for breath testing: from nanomaterials to comprehensive disease detection. *Acc. Chem. Res.,* **2014**, *47*(1), 66-76. [http://dx.doi.org/10.1021/ar400070m] [PMID: 23926883]

[161] Vishinkin, R.; Haick, H. Nanoscale Sensor Technologies for Disease Detection *via* Volatolomics. *Small,* **2015**, *11*(46), 6142-6164. [http://dx.doi.org/10.1002/smll.201501904] [PMID: 26448487]

[162] Acevedo, C.A.; Sánchez, E.Y.; Reyes, J.G.; Young, M.E. Volatile organic compounds produced by human skin cells. *Biol. Res.,* **2007**, *40*(3), 347-355. [http://dx.doi.org/10.4067/S0716-97602007000400009] [PMID: 18449462]