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Assessment, origin, and implementation of breath volatile cancer markers

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Abstract

A new non-invasive and potentially inexpensive frontier in the diagnosis of cancer relies on the detection of volatile organic compounds (VOCs) in exhaled breath samples. Breath can be sampled and analyzed in *real-time*, leading to fascinating and cost-effective clinical diagnostic procedures. Nevertheless, breath analysis is a very young field of research and faces challenges, mainly because the biochemical mechanisms behind the cancer-related VOCs are largely unknown. In this review, we present a list of 115 validated cancer-related VOCs published in the literature during the past decade, and classify them with respect to their “fat-to-blood” and “blood-to-air” partition coefficients. These partition coefficients provide an estimation of the relative concentrations of VOCs in alveolar breath, in blood and in the fat compartments of the human body. Additionally, we try to clarify controversial issues concerning possible experimental malpractice in the field, and propose ways to translate the basic science results as well as the mechanistic understanding to tools (sensors) that could serve as point-of-care diagnostics of cancer. We end this review with a conclusion and a future perspective.

1 Introduction

According to the WHO statistics for 2008, cancer is a leading cause of mortality with more than 7.5 million deaths worldwide and more than 12 million new cases every year.2 While the incidence of lung cancer, as reflected by occurrence and mortality, is among the highest in the world, other cancers (*e.g.*, stomach, liver, colon and breast cancer) are also responsible for many cancer deaths each year.2,3 Approximately 30% of the cancer deaths are associated with one or a combination of the following risk factors: high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use, and alcohol use.2 In a few instances, the cause for cancer is hereditary.2 Patterns of cancer incidence and mortality differ strongly from region to region worldwide; more than 50% of cancer incidence and 60% of deaths occur in less-developed countries.2,3

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1.1 Available approaches for cancer diagnosis

Evaluation of cancer prognosis involves disease confirmation and disease staging.4 Depending on the cancer type, a variety of techniques for the diagnosis and staging are applied in the clinical setting. These techniques include blood tests, X-ray,5 mammography, 6 colonoscopy,7 computed tomography (CT),8 magnetic resonance imaging (MRI),9 positron emission tomography (PET),10 and ultrasonography.11 Although one or a combination of these techniques can show, to a limited extent, the presence, location and size of an abnormal mass, the final determination of cancer is made through a biopsy taken from the specific tissue.12 In this approach, the tissue is generally examined under a microscope by a pathologist to determine the shape and/or concentration of the cells which, in turn, could give indications of the stage(s), sub-type(s) and/or genetic mutations of the disease. Nevertheless, a biopsy is neither convenient for the patient nor free of complications.13 Furthermore, there is a possibility to miss small lesions, because the diseased areas may be patchy.14 In some instances, such as in the lower stages of gastric mucosal atrophy,14,15 there are great inter-observer variations in the identification of pre-malignant lesions. In other instances, such as in the lung or liver biopsy, there is a morbidity and even mortality risk following a biopsy process, mainly due to bleeding.11,13,16

Currently, there is a trend towards personalized medicine in cancer care to optimize clinical response and to minimize toxicity.4,17–19 This trend is based on the search for molecular cancer biomarkers that could complement the conventional diagnostic methods and improve their diagnostic yield.17–31 Gene expression profiling of cancer cells is associated with tumor heterogeneity and treatment outcome, thus allowing a global picture of cellular functioning. Protein expression profiling of cancer cells provides important information to the treating physician, as most targeted therapeutic agents are designed to inhibit the activity of proteins.27–31 Although much progress has been made in these fields, some difficulties must still be overcome towards developing effective biomarkers, including tumor heterogeneity, genetic, epigenetic, and micro environmental effects. Moreover, the related technologies require relatively large amounts of tissue, and are often costly, time consuming, and not available in many medical facilities as described earlier.32–38

1.2 Volatile organic compounds for cancer diagnosis

An evolving approach in cancer diagnostics is based on volatile organic compounds (VOCs), which are organic compounds that have a high vapor pressure under ordinary room-temperature conditions. Cancer VOCs originate from the cell or disease location, enter the surrounding environment39 and can be identified (i) from the headspace of cancer cell lines (*i.e.*, the blend of VOCs confined above the cells in a sealed flask);40–51 (ii) through the urine;52 (iii) through the skin;53,54 (iv) through the blood;55,56 and/or (v) through the exhaled breath.15,40–49,51,55,57–81 The current review focuses on the cancer VOCs examined through exhaled breath.

A typical population of breath samples might contain around 3000 different VOCs in total, mostly at low concentrations that range from pptv to ppbv.82 A major part of the VOC spectrum varies amongst different individuals, while only few VOCs share a common health condition in a given population.83,84 This outcome has been supported by extensive

empirical data.4,39,85–88 A number of first-rate reviews on cancer-related VOCs and an outlook on the potential developments in the area of VOC analysis can be found elsewhere.4,39,57,58,80,87–91 Nevertheless, the pathophysiology underlying the alteration of the cancer VOCs has been vague to a large extent. In this review, we shed light on the pathophysiology causing metabolic changes in the VOC levels and compositions in cancer. Towards this end, we have narrowed the wide spectrum of reported cancer-related VOCs (approximately 3000; the significance for most is unknown)92 to some hundred candidates. We have then used specific VOCs and combinations thereof to discuss important issues related to their possible biochemical origin and the underlying pathophysiological causes (Section 2) – a subject that has so far been insufficiently targeted.4 In our discussion, we have tried to clarify controversial issues concerning possible experimental malpractice in the field. Based on this discussion, we propose ways to translate the lab results as well as the mechanistic understanding to tools (sensors) that could serve as point-of-care diagnostics of cancer (Section 3). We end this review with a conclusion and a future perspective (Section 4).

2 Assessing the origin of cancer VOCs

2.1 Emission of VOCs from cancer cells

In normal and abnormal processes in the body, metabolic changes occur all the time. It has been shown, for example, that different liver enzymes affect the construction of cell membranes.93,94 In metabolic illnesses, such abnormal processes can alter the body's chemistry either by changing the VOCs concentration or by producing new VOCs.

A vital risk factor for cancer development is linked to boosted oxidative stress and induction of cytochrome p-450 enzymes (CYP450, a group of oxidase enzymes).95 Oxidative stress in the body is related to the general equilibrium between formation and deactivation of reactive oxygen species (ROS) and free radicals. As part of the cellular process in the mitochondria, the cell manufactures ROS that have an unpaired electron in the outer shell. Other sources of ROS could be from exogenous origins, for example cigarette smoke, pollution and radiation.4,72 Once accumulated in the tissue, ROS can attack different molecules in the body such as polyunsaturated fatty acids (PUFA) and proteins. During oxidative stress, ROS and free radicals are excreted from the mitochondria in the cell, generating volatile alkanes that are emitted in the breath (see Fig. 1).4 In addition, cytochrome p-450 enzymes that catalyze the oxidation of organic chemicals can be upregulated by ROS molecules in the human tissue.96,97 This enzyme family has been shown to be over-expressed in human breast cancer tissue, for example aromatase which synthesizes estrogens.98 Note that most inflammatory conditions are associated with ROS production, and, hence, ROS products might not be specific for cancer.

A complementary pathophysiologic model suggests that during the early stages of cancer development, some of the normal cells proliferate at prompt rates, reach the oxygen diffusion boundary and become hypoxic (less than 0.1% oxygen in the gaseous phase).99 Because of the increased demand for energy and macromolecular biosynthesis these cells prefer the use of glycolysis over oxidative phosphorylation (Warburg effect). This process is associated with high rate of glycolysis and lactic acid formation,100–103 thus allowing cell

survival in the hypoxic micro-environment.104,105 The excessive lactate production causes the tissue to become acidic and eventually causes the breakage of the basement membrane. Moreover, the acidic surroundings defend the tumor from the immune system.106

Tumor growth generally goes along with gene changes and/or protein changes.107,108 As a result, individual alleles can create a unique VOC profile that is further secreted in the body fluids.109 Although most models relate to VOCs that are produced endogenously, exogenous VOCs detected in breath are of great interest as well, mainly because they relate to exposure of an individual to carcinogens. Exogenous VOCs are typically highly reactive, causing peroxidative damage to DNA, proteins, and PUFA. The negative impact of such processes accumulates during the years and is assumed to promote cancer.110 Particularly, very lipophilic chemical compounds are stored in the fat compartments of the body and can be released over a period of weeks and months after exposure.111

2.2 VOC exchange between various body fluids

As indicated in the previous section, it has been hypothesized that the abnormal cancer VOCs are produced by tumor cells, from which they are excreted into the endobronchial cavity, from where they are exchanged and excreted *via* various body fluids. An idealized approach to check this hypothesis would rely on the comparison of VOC profiles from the different organs and body fluids of the same cancer patient and/or the same animal model. In this context, the simplest starting point would be a comparison between the VOC profiles in the headspace of cancer tumor tissue, (headspace of) blood samples, and breath samples. Due to not yet mature technical/experimental methods, no experimental results have been achieved with such an approach. Therefore, and given the unmet need to gain an understanding of the biochemical pathway of the cancer-related VOCs, we have simulated such an experiment with the help of thermodynamics viewpoint. In our simulation, we have targeted the *equilibrium concentrations* of a given VOC between “breath–blood–fat”, through estimation of the respective thermodynamic partition coefficients (see Fig. 2):

- Partition coefficient between fat and blood ($\lambda_{f:b}$): this coefficient is designed to estimate the equilibrium concentration of VOCs in fat tissue and (lipophilic) cell membranes with respect to blood.
- Partition coefficient between blood and air ($\lambda_{b:a}$): this coefficient is designed to simulate the equilibrium of VOCs between blood and exhaled air. If the respective VOC is systemic, the blood concentration can be estimated using the blood–air partition coefficients, $\lambda_{b:a}$.1,4,66,112–115 If experimentally determined $\lambda_{b:a}$ are not available, their values can be estimated by either theoretical molecular descriptors or on semi-empirical calculations using experimental physical properties (for example, water–air, rat-$\lambda_{b:a}$, or olive-oil–air partition coefficients).116–120

This partition-coefficient simulation is a straightforward and simple approach, which does not need elaborate modelling, as in the papers of King *et al.*121–129 To implement this approach, we have listed 115 VOCs that were reported in the literature as cancer biomarkers during the past 10 years, together with the constituent $\lambda_{b:a}$ and $\lambda_{f:b}$ (Table 1). The full list of 115 VOCs is divided into the following compound families: hydrocarbons, aromatic

compounds, alcohols, ketones, aldehydes, acids, esters, ethers, heterocyclic compounds, nitriles, sulfides, terpenes and others.

Based on the data presented in Table 1, the equilibrium concentrations in blood and fat are estimated (see Fig. 3 and Section 2.3), while waiving several normalization and standardization gaps that are present among the various published studies. Regarding the rest of these normalization and standardization gaps, we mention:

(a) variances and inconsistencies in the control groups of the clinical trials (healthy smokers, healthy non-smokers, age-matched groups, hospital personnel, relatives of the patients, *etc.*);

(b) differences in the instrumentations used for the identification of disease-related VOCs (*e.g.*, GC-MS,65,73 PTR-MS46,81);

(c) uncertainties in the identification of the VOCs, even though qualitative analysis by retention time and spectral library match is quite reliable in the case of GC-MS;42,43,45

(d) differences in the sampling procedures (*e.g.*, collection of mixed expiratory breath,81 CO_2-controlled sampling of end-tidal breath,65,130,131 sampling with Tedlar or Mylar bags,81,132 portable breath collection apparatus (BCA), 73 *etc.);*

(e) differences in the pre-concentration procedures used in conjugation with the mass spectrometry technique (*e.g.*, solid phase micro-extraction (SPME) fibers, 58,59 and thermal desorption units with cryo-focusing77);

(f) differences in the normalization procedures (*e.g.*, data normalization according to a specific VOC concentration in the examined sample59,81 or based on the difference in the examined sample and the inhaled air VOC concentrations); 72,73,77

(g) differences in the data analysis procedure (*e.g.*, peak identification and integration in the chromatograms of each sample,42–45 comparison among the quantitative data from different study groups,71,81,133 statistical analysis using regression and pattern recognition algorithms); and

(h) differences in the applied calibration standards between the groups involved in breath cancer studies; indeed, approximately 50% of the published breath-related studies still present qualitative data on potential VOC breath bio-markers for a variety of diseases, but with no quantification of their concentration levels.

Considering these variations, the current efforts might not provide precise or definite answers to the puzzling pathophysiological pathways of some cancer VOCs. Nevertheless, this effort would help stimulating constructive discussions and new ideas.

2.3 VOC exchange into the breath

The principle behind breath cancer detection is that cancer-related VOCs in the (fat) tissue are emitted to the blood and that the VOC blood chemistry is reflected in measurable changes in the breath through exchange *via* the lungs.134 It is found that some gases exchange in the airways, rather than in the alveoli, depending on the $\lambda_{b:a}$. Theoretical and experimental studies have shown that gases with low solubility in blood, mainly nonpolar VOCs ($\lambda_{b:a} < 10$; $\lambda_{b:a}$ in dimensionless units [mol L_b^{-1}/mol L_a^{-1}]), exchange almost exclusively in the alveoli, while VOCs that are well soluble in blood, *e.g.*, polar VOCs ($\lambda_{b:a} > 100$), tend to exchange also in the airways.113,121,127,128 Further studies predicting the location of the pulmonary gas exchange have shown that VOCs with $10 < \lambda_{b:a} < 100$ interact significantly both with the airways and with the alveoli.113 An important conclusion of these studies is that the airways play a more significant role in pulmonary gas exchange than previously assumed.127,128 Hence, the implications of pulmonary tests and breath tests might have to be re-evaluated.113 The VOC profile is also influenced by the retention of VOCs in the lungs, *viz.* the fraction of the molecules that remains in the respiratory tract at any time, after inhalation and exhalation, because of the $\lambda_{b:a}$.135 Thus, the final partition and exhalation of the VOCs depends on their physical and chemical properties, and on their interaction with the different alveolar clearance processes.135,136

We illustrate the blood–breath concentration relations using the examples of isoprene and acetone. Isoprene is more volatile and less soluble in blood, compared to acetone. This is expressed in that the $\lambda_{b:a}$ value for isoprene (~0.95114) is smaller than for acetone (~340137). Nevertheless, acetone has been reported to appear in noticeably higher concentrations in the breath, compared to isoprene. This difference is attributed to the fact that the concentration of acetone in the blood is generally more than three orders of magnitude higher than that of isoprene. This result might reflect the absence of direct out-gassing of marker VOCs into the airways, leading to low expression of the high boiling point (BP) VOCs that are not “picked up” in the breath analysis.138

In addition to $\lambda_{b:a}$, the $\lambda_{f:b}$ is a very important quantity. Together, these two physicochemical partition constants determine the *equilibrium concentration* of a given compound between breath, blood and fat. Most of the proposed cancer biomarkers are lipophilic, and, hence, can be expected to be stored in the fat compartment. For lipophilic compounds, a low concentration in exhaled breath (like ~1 ppb) can be associated with a relatively high concentration in the fat compartment.

For many VOCs, $\lambda_{b:a}$ and $\lambda_{f:b}$ are unknown.114,115 They may, however, be estimated based on the water:air partition coefficient ($\lambda_{w;a}$) and the octanol:water partition coefficient ($\lambda_{o:w}$) using the method of Poulin and Krishnan.139 If the $\lambda_{b:a}$ are not available in the literature, we estimate them by different methods. For alkanes, methylated alkanes and 1-alkenes, we use data from ref. 115 to estimate $\lambda_{b:a}$ by regression based on the number of carbon atoms, the BP and the molecular weights. For other VOCs, we use the estimate by Poulin and Krishnan139 given by the formula

$$\lambda_{b:a}=\lambda_{o:w}\cdot\lambda_{w:a}\cdot(a+0.3b)+\lambda_{w:a}\cdot(c+0.7b) \quad (1)$$

Here, $a \approx 0.0033$ is the fraction of neutral lipids in blood, $b \approx 0.0024$ is the fraction of phospholipids in blood, and $c \approx 0.82$ is the fraction of water in blood. The $\lambda_{o:w}$ values are taken from Scifinder (https://scifinder.cas.org). The $\lambda_{w:a}$ (Henry constants) at 25 °C are taken from the compilation of Sander,140 estimated using the EPI Suite™ software developed by the US Environmental Protection Agency (EPA, http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm). Alternatively, they are estimated by use of surrogate compounds, for which $\lambda_{w:a}$ is known, with correction by the quotient of the respective vapor pressures (of the compound in question and its surrogate compound). To estimate the Henry constants at 37 °C, we use the derivative $d\ln(\lambda_{w:a})/d(1/T)$ as given in the compilation by Sander,140 or the corresponding enthalpy of vaporization (ΔH_{vap}) divided by the gas constant, R. This is the standard procedure recommended by the US Environmental Protection Agency (EPA),141 for compounds whose data on temperature-dependence of the Henry constant are not accessible in the literature.141 The $\lambda_{f:b}$ values are computed from the $\lambda_{b:a}$ and the $\lambda_{f:a}$ using $\lambda_{f:b}=\lambda_{f:a}/\lambda_{b:a}$. If the $\lambda_{f:a}$ is not available in the literature, we use the estimate by Poulin and Krishnan139 given by the equation

$$\lambda_{f:a}=\lambda_{o:w} \cdot \lambda_{w:a} \cdot (A+0.3B)+\lambda_{w:a} \cdot (C+0.7B) \quad (2)$$

Here, $A \approx 0.798$ is the fraction of neutral lipids in adipose tissue (fat), $B \approx 0.002$ is the fraction of phospholipids in adipose tissue, and $C \approx 0.15$ is the fraction of water in adipose tissue.

Different VOCs carry different information about the various compartments of the human body. In particular, the storage capacity in the human body is quite different for various VOCs. Also, the time necessary to deplete storage for a certain compound is very different. Fig. 3 illustrates that different VOCs with the same concentration in exhaled breath may show very different concentrations in fat and blood (up to a factor of 108). In Fig. 3, the respective estimated concentrations in blood and fat are shown under the assumption that the concentration in breath is 1 ppb. The figure shows that different VOCs in blood or in the fat compartment might have very different concentrations, even though their concentration in exhaled breath is identical. Ethane, for example, with 1 ppb in exhaled breath is estimated to appear in blood at a concentration of 3.3×10^{-12} M and in fat at a concentration of 2.9×10^{-10} M. Tridecane, on the other hand, with 1 ppb in exhaled breath is estimated to appear in blood at a concentration of 1.0×10^{-8} M and in fat at a concentration of 3.3×10^{-6} M. Therefore, ethane and tridecane behave very differently in blood and fat, even when their concentrations in exhaled breath are identical (1 ppb).

The concentration information in Fig. 3 is most interesting, but rather limited. Much more detailed information than just partition coefficients and estimates of concentrations in blood and fat are available for some compounds like isoprene, which is the hydrocarbon displaying the highest concentration in exhaled breath. This more detailed information has been elucidated through *real-time* measurements of exhaled breath during exertion of an effort, and contains, in particular, the concentrations or amounts of isoprene in the periphery of the human body (*e.g.*, the muscles of the limbs). The isoprene stores in the body can be depleted by exertion of an effort, *e.g.*, on a stationary bicycle.121,124 After about 45 min of cycling,

a large part of the stored isoprene is exhaled and it takes about 1–2 h to re-synthesize isoprene in the body and fill up the stores. Similar information is available for acetone and, in part, for 2-pentanone. We expect similarly interesting effects for the other compounds presented in Table 1, with the $\lambda_{b:a}$ and $\lambda_{f:b}$ playing a central role.

When examining the variation of the VOCs' $\lambda_{b:a}$ according to a specific cancer type, some connections are revealed (Fig. 4). The results show that lung cancer, gastric cancer and liver cancer have rather similar values as seen from the median line, while breast cancer and head and neck cancer are similar. Colon cancer is different from the rest (see Fig. 4). While no obvious reason currently explains this difference, we can hypothesize that metabolic processes in cancer development and compound storage in tissue might be similar in these cancer types. In some of the cancers, a few VOCs are "outliers" with respect to the $\lambda_{b:a}$ within the general trend of the group. In breast cancer, three VOCs present a high $\lambda_{b:a}$ opposed to the rest: 2-amino-5-isopropyl-8-methyl-1-azulenecarbonitrile, which can be found in fragrances, 2,3-dihydro-1-phenyl-4(1*H*)-quinazolinone, which has been suggested as a cholecystokinin (CCK) antagonist,142 and 1-phenyl-ethanone (acetophenon), which can be found in fragrances, chewing gums, cigarettes and as an excipient. In head and neck cancer, two VOCs presented a high $\lambda_{b:a}$: 5-methyl-3-hexanone – a VOC that is found in human body fluids and feces143 – and 2,2-dimethyl-propanoic acid, which is an odiferous compound that exists in liquid phase at body temperature. Such "outliers", if confirmed and validated for a particular disease, could be particularly interesting due to very different concentration levels in blood, fat and breath, in comparison to other biomarkers of the disease.

2.4 The biochemical pathway of cancer VOCs

Different VOCs carry different information about the various compartments of the human body. In particular, the storage capacity of the human body is quite distinctive for different volatile compounds. Also the time necessary to deplete stores for a certain compound varies. With this in mind, we utilize the data and discussion presented in the previous sections to provide further insight into the biochemical pathways of the various chemical families of cancer VOCs, *viz.*, hydrocarbons (alkanes, branched-chain alkanes and branched-chain alkenes), primary and secondary alcohols, aldehydes and branched aldehydes, ketones, esters, nitriles, and aromatic compounds.

2.4.1 Hydrocarbons—The key mechanism that relates to hydrocarbon production in the body is oxidative stress (see Section 2.1). Alkanes are mainly produced by peroxidation of PUFA, found mainly in cellular and subcellular membranes (lipid peroxidation). Lipid peroxidation is responsible for damage of tissues *in vivo*. It may be a cause of cancer, inflammatory diseases, atherosclerosis, and aging. The human body tries to control and reduce lipid peroxidation by the use of anti-oxidants.4 Saturated hydrocarbons such as ethane and pentane are the end products of lipid peroxidation. Pentane and ethane in the breath have been extensively used as non-invasive *in vivo* indicators of lipid peroxidation. 144 Although the occurrence of other saturated hydrocarbons (*e.g.*, C_3–C_{11}) can be related to the lipid peroxidation process, in the case of branched hydrocarbons, this mechanism

seems to be irrelevant. Due to their low solubility in the blood, hydrocarbons that are not metabolized in the body are emitted into the breath within minutes.145,146

2.4.2 Alcohols—Alcohols can be absorbed from all parts of the gastrointestinal tract mainly by diffusion into the blood. Alcohols can as well be a product of hydrocarbon metabolism. Short-chain alcohols are absorbed rapidly in the blood due to their high affinity to water. Alcohol metabolism is prone to be affected by confounding factors in the body, mostly the changes in water and fat content among different people and genders.4 Possibly, enzymes such as alcohol dehydrogenase (ADH) and cytochrome p450 (CYP2E1, which predominantly works in the liver) are responsible for alcohol metabolism in the body. ADH can catalyze the oxidation of several different alcohols in humans; remaining VOCs are removed through the excretion of alcohol in breath, urine, sweat, feces, breast milk and saliva.4

2.4.3 Aldehydes—Aldehydes are produced in the body as part of common physiological processes. Some of the aldehydes are essential for functional processes. Others are thought to be cytotoxic intermediates with several functions, such as signal transduction, gene regulation and cellular proliferation.147,148 There are a number of sources of aldehydes in the body. The first source relates to metabolized alcohols. The second source of aldehydes in the body relates to the reduction of hydroperoxide by cytochrome p450 as a secondary product of lipid peroxidation.149 The third source for the aldehydes in the body relates to smoking. Saturated and unsaturated aldehydes such as formaldehyde, acetaldehyde, and acrolein are found in tobacco smoke.150 The fourth source for the aldehydes in the body is the detoxification process by cytochrome p450 as a result of the by-product of tobacco metabolism.151,152 Finally, aldehydes can also originate from dietary sources.153,154

2.4.4 Ketones—During cancer progression, an increase in the rate of fatty acid oxidation due to changes in metabolic conditions results in the formation of ketone bodies including acetone. Such compounds are also related to weight loss, which is, in turn, one of the symptoms of cancer.155 Acetoacetate and b-hydroxybutyrate are synthesized in the liver in significant quantities, followed by spontaneous decarboxylation of acetoacetate to yield acetone. Of the ketone bodies, acetone is produced in smaller quantities, and due to its high vapor pressure it can be secreted through the breath, urine and skin. Protein metabolism can result as well in ketone bodies. In the state of cachexia, typical in diseased conditions such as cancer, protein metabolism increases resulting in higher levels of ketone bodies.155 However, acetone is not suitable to be used as a cancer biomarker as its concentration levels in the breath are altered due to exercising, fasting and/or food consumption.156,157 Finally, other exogenous sources like food or chemical industries can result in ketone production that could eventually be absorbed in the body.15,34

2.4.5 Esters—This group of compounds can be found in natural fats and fatty oils, natural wax and fruit essential oils in large amounts. In humans, esterases hydrolyze esters into alcohol and acid at temperatures below 40 °C.158 One example of such an enzyme is lipase which catalyzes lipid hydrolysis as part of the natural metabolism in the body.

2.4.6 Nitriles and aromatic compounds—Nitriles and aromatic VOCs are usually considered to be pollutants of exogenous sources. Such sources include exposure to cigarette smoke, alcohol, pollution and radiation. While such compounds are most likely to be of exogenous origin, they could be of interest for cancer patient follow-up, since some are known to be carcinogens.4 These molecules are highly reactive, resulting in peroxidative damage to PUFA, proteins, and DNA. Such damage accumulates during a lifetime, while the natural fixing mechanisms in the body become less efficient, leading to age-dependent diseases such as cancer.110 These compounds are stored in the fatty tissues of the body; thus it is likely that cancer patients, previously exposed to continuous occupational pollutants or excessive smoking, could slowly release them in high concentrations through the exhaled breath. In addition, mechanical, cellular, and enzymatic defense mechanisms act to eliminate hazardous chemicals and xenobiotics by a two phase process, resulting in a more soluble and excretable form of molecule.4,159 One such compound is acetonitrile which is found in smokers. The pathway suggested for acetonitrile is the bio-transformation to cyanohydrin by cytochrome P450 monooxygenase, which spontaneously breaks down to hydrogen cyanide and formaldehyde. Because of the rather slow metabolism of acetonitrile in the body, substantial amounts of acetonitrile can be emitted as-is through exhaled breath and/or urine.4,160

2.5 Challenges and future directions for better understanding of cancer VOC biochemical pathways

In the following, we present a few proposals to improve the understanding of the metabolic pathways that generate potential cancer VOCs as well as to VOC production/consumption in the body:

- Many metabolic pathways such as glycolysis, apoptosis, loss of tumor suppressor genes, and angiogenesis are activated or over-activated in the case of cancer.161 These pathways may alter the production of VOCs in the body. To identify the exact change in the VOC pattern, we propose blocking such metabolic processes in various cell lines, each in a separate assay. This could be achieved by deactivating the specific enzyme (*e.g.*, hexokinase, pyruvate kinase dehydrogenase or matrix metallo-proteases) that initiates or is crucial to the process, in order to compare between the measured VOC profiles before and after the blocking. According to the specific blocking, the cancer VOCs can be associated with the different mechanisms occurring in the same cancer cell.
- The hypothesis that certain VOCs are associated with the cell metabolism *per se*, rather than with the microenvironment of the cancer or other indirect metabolic pathways in the body (human or animal), needs to be confirmed through direct observation. This hypothesis could be resolved by using cell lines from well-documented sources,42–45 so that they can be directly correlated to metabolic pathways without any confounding factors. In this context, using a variety of different cell lines, rather than replicas of the same cell line, could be helpful to simulate the natural

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diversity of cancer while eliminating potential confounding effects that are associated with clinical samples.

- Many cancer VOCs are related to non-cancer sources, such as environmental and tobacco compounds. Following inhalation, these molecules might affect the respiratory system, and later on, also the blood composition. In this case, the lipophilic species are stored in the fat compartment, with subsequent comparatively slow release through exhalation. Therefore, it is important to examine the effect on the blood and the fat compartment of inhaling these molecules, as well as examining the breath VOC profile. Using an animal model, such compounds could be introduced *via* inhalation or they could be directly introduced into the bloodstream to monitor the resulting breath VOC profile of the treated animals. In addition, oxidative stress could be determined through measuring the amount of glucose and the activity of G-6 PD. A comparison between the animal model and the introduction of the same molecules *in vitro* to cancer cells would allow gaining a detailed understanding of how these VOCs affect the body both at a cellular level and as a whole.
- It is hypothesized that a malignant tumor is a “free organ” having its own cancer stem cells (CSC). These cells present a chemotherapy-resistant population capable of self-renewal. Stem cells have high levels of ALDH activity, yet ALDH activity varies among different cells. A focused study on CSC, both *in vitro* and *in vivo*, might reveal variances in the patterns of VOCs that are released as a response to ALDH activity. This study could serve as a launching platform for developing a CSC (and/or ALDH activity) biomarker, namely a single VOC or a VOC pattern that could be indicative of recurring tumor initiation or metastasis initiation, thus aiding the prediction of patient prognosis, and tailoring personalized treatments.

These proposals can be implemented by means of mass-spectrometry techniques. A very promising approach in this endeavor is real-time analysis of exhaled breath by direct mass-spectrometric methods, such as proton-transfer-reaction mass spectrometry (PTR-MS),121–129,162,163 proton-transfer-reaction time-of-flight mass spectrometry (PTR-TOF-MS),164–171 or selected ion flow tube mass spectrometry (SIFT-MS).88,172–176 With these real-time techniques, exhaled breath is directly analyzed by mass spectrometry, without any need for sample storage or preconcentration. These techniques can even be carried out with breath-to-breath resolution. The mere possibility of real-time analysis (*e.g.*, when exerting an effort on a stationary bicycle or during sleep123,162) is a decisive advantage compared to investigations of blood samples. It allows detecting very fast processes, such as a quick release of isoprene during physical effort.124–126,163

Undoubtedly, conventional and real-time mass-spectrometry techniques are powerful tools that can provide qualitative and quantitative information on the cancer VOCs and, subsequently, enable extracting important information on the biochemical pathways of the release of cancer VOCs. However, to date, the use of these techniques has been impeded by

the need for expensive equipment, high levels of expertise required to operate such instruments, low speed required for sampling and analysis, and the need for preconcentration techniques. For cancer VOC testing to become a clinical reality, the advances in the knowledge of spectrometry-based specific cancer VOCs have to be translated to sensor development.

3 Sensors for testing cancer VOCs

Sensor matrices are likely to become a clinical and laboratory diagnostic tool, because they are significantly smaller, easier to use, and less expensive. An ideal chemical sensor for VOC analysis should be sensitive at very low VOC concentrations in the presence of water vapour, because the headspace of clinical samples is fully humidified. Furthermore, it should respond rapidly and differently to small changes in concentration, and provide a consistent output that is specific to a given exposure. When not in contact with the VOC, the sensor should return to its baseline state rapidly, or be simple and inexpensive enough to enable manufacturing large numbers of disposable units.

Sorption-based sensors are a candidate for low-power, compact chemical vapor detection for breath analysis. Such sensors combine a (semi-)selective transducer with chemo-selective materials that serve as a vapor concentrator, resulting in a highly sensitive detector that responds selectively to a particular class of chemical vapors. Among the choice of transducers are chemiresistors that monitor the resistance of polymers laced with conductive nanomaterials (see Fig. 6a and b) or conducting polymers (see Fig. 6c); chemiresistors or chemicapacitors based on metal oxide films that monitor changes of either resistance or dielectric properties (see Fig. 6d); mechanical oscillators and surface acoustic wave devices that respond to changes in mass (see Fig. 6e and g); and colorimetric sensors that monitor changes in optics (see Fig. 6f). Among these transducers, chemicapacitors and chemiresistors are best suited for low-power sensor arrays. Chemiresistors are simple to implement, but instability of the conductive particle/polymer interface can be a disadvantage. Chemicapacitors are more stable, but can take minutes to respond and recover. This slow response is limited by the time required to load and then remove the VOC from the relatively thick layers of chemo-selective dielectric (~1 μm) that are typically used.

In this review, we consider two complementary approaches to profile cancer-related VOCs by sensor matrices. The first approach relies on sensors with selective recognition characteristics, which aim to detect one or a few specific VOCs. The second approach uses cross-reactive (*i.e.*, semi-selective) sensors, which have a broad-spectrum of sensitivity to volatiles and gain their selectivity through pattern recognition.

3.1 Selective sensors for cancer VOCs

In the selective sensing concept, a highly selective receptor/detector is designed to specifically bind or detect the cancer VOC of interest.39 Sensor selectivity is defined here as higher sensitivity to a specific or mixture of gases/vapors in the presence of interfering gaseous species. This approach is suitable for detecting a well-defined target cancer VOC in the presence of interfering species and/or background (see Fig. 5, upper panel). In light of the difficulties to find distinctive cancer VOC(s), in the presence of controlled backgrounds

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and interferences, the development of selective sensors for cancer VOCs is still lagging. Additional limitation stems from the need to synthesize separate, highly selective nanomaterials for each VOC to be detected.177 Nevertheless, most currently available selective sensing techniques have aimed for non-volatile compounds.

3.2 Cross-reactive sensors for cancer VOCs

An emerging strategy that is complementary to the selective sensing approach is the cross-reactive sensor array.39 Bio-inspired, this approach performs detection through use of an array of broadly cross-reactive sensors in conjunction with pattern recognition methods.39 In contrast to the selective sensing approach, each sensor in this architecture produces a distinct fingerprint from the array of broadly cross-reactive sensors. This allows considerably widening the variety of compounds to which a given matrix is sensitive, to increase the degree of component identification and, in specific cases, to perform an analysis of individual components in complex multi-component (bio)-chemical media.89 Pattern recognition algorithms can then be used to obtain information on the identity, properties and concentration of the vapor exposed to the sensor array (see Fig. 5, lower panel).39,178 Although such sensor arrays are mostly qualitative or semi-quantitative in nature, such methodologies are ideal for rapid disease screening as the results can be obtained in minutes.39,179 Fig. 6 illustrates the schematic representation of different sensors used. An overview of some of them, in the context of detection of cancer VOCs, is presented.

3.2.1 Nanomaterial-based sensors—Distinct attention has been given in the past few years to approaches incorporating nanomaterial-based VOC/gas sensors (NMVSs). This is because NMVSs can lead to the development of sensitive, fast, and responsive diagnostic tools, though in relatively inexpensive manner.89 These advantages are the result of the nanoscale dimensions of the nanomaterials used, dimensions which provide them with superior physical, chemical, and optical properties, together with their high surface-to-volume ratio and low-priced fabrication. Thus, NMVSs allow high plasticity when fabricating sensors for breath analysis with the option to tailor them for specific disease related VOCs achieving high-level detection accuracy. Still, the choice of the breath analysis setup must take into consideration the potential restrictions of the applied sensor system, mainly because of potential gains and pitfalls in the NMVSs' breath analysis methodology (see Fig. 7). Nanoparticles, nanowires and carbon nanotubes are examples for nanomaterials that have been exploited for VOC sensing.

Nanomaterials combined with different molecular-sized organic functionalities has been used as sensitive transduction elements (see Fig. 8a and c).180 Examples of nanomaterial based transducers include field effect transistors (FETs) based on single-walled carbon nanotubes (CNTs)181,182 (see Fig. 8c) or nanowires (NWs) of various materials (see Fig. 8a),183–186 nanoelectromechanical oscillators,187–190 nanoporous chemi-optical materials,191,192 coaxial-chemicapacitors based on CNTs coated with nanoporous alumina193 and chemiresistors based on monolayer capped metal nanoparticle (MCNP) films,80,194,195 porous metal–oxide (*cf.* Fig. 6d),196 and random networks of single-walled CNTs183,197 or silicon NWs.198

The most common nanomaterial-based sensors are usually based on conductive inorganic nanomaterials (*e.g.*, metal nanoparticles, single walled carbon nanotubes, carbon black) that are capped with or in organic functionality.396970179197 In these films, the inorganic nanomaterials provide the electric conductivity and the organic film component provides sites for the sorption of VOCs (see Fig. 6a and b).89199 On exposure, VOCs reach the sensing surface or diffuse into the sensing film and react with the capping ligands or the functional groups that cap the inorganic nanomaterials. As a result of the latter, a volume expansion/shrinkage in the nanomaterial film occurs.3989 The connection between the inorganic nanomaterial blocks becomes lower/higher, and the conductivity decreases–increases.3989 In a few instances, exposure of the nanomaterial film to VOCs causes charge transfer from/to the inorganic nanomaterial, thus causing changes in the measured conductivity, even in the absence of any steric changes within the sensing film.3969194 The chemical diversity of the functional group(s) that cap the inorganic nanomaterial can be tailored for each sensor type, with the aim that each sensor responds to a particular fingerprint of VOCs in a different way. Consequently, a pattern of resistance changes is obtained from the sensor array to a given vapor.200

Clinical studies on breath samples with a cross-reactive array of MCNPs have shown the capability to distinguish lung cancer breath samples from control groups6869201 as well as distinguish between various types (lung cancer, colon cancer, breast cancer, prostate cancer, and head and neck cancer),63,68 in the presence of confounding factors. Peled *et al.*39,40 have shown that a cross-reactive MCNP array and a molecule-terminated single-walled carbon nanotube (SWCNT) array of chemiresistors discriminated between malignant and benign pulmonary nodules and between adeno- and squamous-cell carcinomas with 85–91% accuracy; additionally, it could also discriminate between early-stage and advanced-stage lung cancer with 86–90% accuracy.201 Similar results were achieved on cancer cell lines in an *in vitro* study.39–41 Another study included exhaled breath of 14 individuals with bronchogenic carcinoma and 45 control subjects without cancer using an array of chemiresistive films of polymer and carbon black.200 The sensor arrays detected lung cancer with 71.4% sensitivity and 91.9% specificity; positive and negative predictive values were 66.6% and 93.4%, respectively.200 Broza *et al.*202 included early-stage lung cancer (stages Ia, Ib and IIa) before and three weeks after tumor resection in their study. A modified array of MCNP-based sensors discriminated between pre-surgery and post-surgery lung cancer samples (80% accuracy), as well as between pre-surgery benign and lung cancer conditions (94% accuracy). In contrast, the same sensor array could not discriminate between pre-surgery and post-surgery benign states, nor between lung cancer and benign states post-surgery.202 These results point to the use of such MCNP-based chemiresistors array for short-term follow-up after lung cancer resection.202

Based on the effective classification of lung cancer, researchers studied malignant mesothelioma against an asbestos-related disease group and a control group. Breath analysis was carried out with an array of carbon black/polymer sensors enabling the discrimination of malignant mesothelioma from all other groups with 88% accuracy.203 The sensors could discriminate with 80.8% accuracy the malignant mesothelioma group from people with asbestos exposure and discriminate with 84.6% accuracy the malignant mesothelioma group from healthy controls.204 Haick, Liu and co-workers used an array of MCNP and SWCNT

sensors and showed excellent ability to differentiate between (i) gastric cancer and benign gastric conditions (90% accuracy); (ii) early stage gastric cancer (I–II) and late stage (III–IV) (92% accuracy); and (iii) ulcer and less severe cancer conditions (86% accuracy).15 The common effect between gastric disorders and respiratory disorders was recently studied using an array of polymers and carbon black chemiresistors.205 Study results presented an ability to differentiate between breath prints of obstructive lung disease patients without gastro-oesophageal reflux disease (GORD) and obstructive lung disease patients with GORD (with 67.6% accuracy), and asthmatic patients with reflux from asthmatics without GORD (85% accuracy). But in the case of patients with COPD and COPD with GORD, only 64% accuracy was achieved by the array.205 A larger prospective interventional study is needed as the results described were influenced by several different confounders.39,205

3.2.2 Colorimetric sensors—Colorimetric sensors are composed of a diverse range of chemically responsive dyes, whose colors depend on their chemical environment (see Fig. 6f).206,207 Since the measurable responses of the sensors are the color changes in each of the dyes, a colorimetric sensor array can easily be read out with the naked eye.206,207 Alternatively, auxiliary equipment such as a spectrometer is needed. Another advantage of colorimetric sensor arrays is their ease of fabrication: they can simply be printed on a variety of substrates using a disposable cartridge printer.

Colorimetric sensors array was applied successfully to lung cancer breath testing, using different classes of chemically responsive dyes.208 The colorimetric sensors are made from dye-containing metal ions (*e.g.*, metalloporphyrins) that respond to Lewis basicity, pH indicators that respond to Bronsted acidity/basicity, and dyes with large permanent dipoles that respond to polar breath VOCs. The sensitivity of the system is in the low ppmv range for many relevant VOCs, but it is not established for humid gas mixtures. An array of 24 colorimetric sensors was used in a clinical trial on 229 subjects (92 LC with different histology, 137 healthy controls).208 Results showed that better accuracies are achieved in the comparison of individual histologies and the control group (*e.g.*, squamous cell carcinoma, adenocarcinoma) than in the case of non-small cell lung cancer compared with the control group, which gave a sensitivity and specificity of 70% and 86%, respectively.39

3.2.3 Electro-acoustic sensors—Electro-acoustic sensors measure the electrical response to applied mechanical stress: mechanical stress generates a voltage in piezoelectric materials, and *vice versa*. An oscillating potential near the material's resonant frequency induces a variety of wave modes.209,210 Covering piezoelectric substrates with organic films provides the moderate chemical selectivity that is required for sensor array elements. The electro-acoustic sensors use either bulk acoustic waves (BAKs) or surface acoustic waves (SAWs).

3.2.3.1 Quartz microbalance (QMB) sensors: Quartz crystal microbalance (QCM) sensors constitute the simplest implementations of BAK sensors (see Fig. 6e).211–213 In a QCM, the acoustic wave propagates through the bulk of the crystal in a direction perpendicular to the surface.211–213 QCMs with chemo-active coatings have been widely used in gas and vapor sensing. Adsorption and desorption of the breath VOCs from the coated membrane cause changes in its mass, which, in turn, gives rise to shifts in the

resonator's frequency. The resonant frequency is also affected by variation in temperature and humidity, which could be important confounding factors during direct breath sampling. These two parameters should be controlled when using QCM sensor arrays for cancer breath testing, in order to minimize their effect during the exposure to the samples.

Lung cancer VOCs have been successfully demonstrated in a small-scale pilot study, using QCM sensor arrays with metalloporphyrin coatings.187188 These sensors presented decent sensitivity towards aromatic compounds, amines, alcohols, and ketones. Additionally, they have been shown to correctly classify breath prints of three groups of volunteers: (i) lung cancer patients before surgical treatment; (ii) control group including hospital staff; and (iii) lung cancer patients after the surgery. The accuracy of the array of QMB sensors was 90.3% with 100% correct classification of the lung cancer patients.39213

3.2.3.2 Surface Acoustic Wave (SAW) sensors: In a SAW device, wave motion occurs only at the surface, penetrating to a depth of approximately one acoustic wavelength into the crystal (see Fig. 6g).214 The direction of propagation is parallel to the surface, which can be covered with different chemiselective films. Adsorption and desorption of the breath VOCs from the coated membrane cause changes in its mass, resulting in a change in the mass (acoustic field of the SAW) and in the electrical conductivity (electric field of the SAW, associated with the acoustic field) of the chemical interface, influencing the SAW amplitude and phase velocity.214 SAW sensors have a higher sensitivity than QMB sensors to most VOCs and the devices offer better possibilities for surface modifications. Preliminary results showed promise for deriving a breath print marker for LC malignancy, using a pair of chemically modified (polyisobutylene) SAW sensors, but the study population was too small to draw far-reaching conclusions.

In a study on lung cancer, a pair of SAW sensors was used as detectors in breath analysis. The first sensor was coated using a poly(isobutylene) film and the other sensor was used as the reference.215 The study outline included a few steps: preconcentration of the breath samples with a solid-phase microextraction (SPME) fiber followed by their injection into a gas chromatography capillary column. The eluted VOCs were then introduced to the polymer-coated SAW sensor, one by one and measured as frequency change steps. The responses were evaluated by the back-propagation artificial neural network (ANN) algorithm. Results of 10 breath prints presented a diagnostic ability for lung cancer states with 80% sensitivity and specificity.39215

3.3 Challenges and future directions for detection of cancer VOCs

3.3.1 Tailoring advanced materials for improved detection of VOCs—Disease detection by breath analysis, particularly cancer, requires the capability to detect disease-related irregularities in the levels of breath VOCs regardless of characteristic variations in the levels of confounding VOCs.138 This requires comprehensive knowledge of breath composition and of possible factors that influence VOC breath levels. Standard exhaled breath samples contain nitrogen, oxygen, carbon dioxide, water vapor, argon, and a selection of thousands of VOCs, mostly at parts per billion levels.89138 Most VOC spectrum varies in abundance amongst different individuals in most breath samples of a given population. In

rare cases, a specific VOC could be uniquely found in the breath of diseased subjects as opposed to non-diseased subjects. Therefore, VOCs that indicate a clinical state generally display distinct levels and concentrations associated with the disease. The number of shared VOCs potentially indicative of a definite clinical state ranges from only a few to tens of VOCs.4216 Thus, constructing suitable sensors for the detection of a certain disease is challenging and should take into account these aspects: (i) the sensor's detection range based on the predicted VOC concentrations in breath; (ii) increasing specificity to relevant VOCs while reducing sensitivity to background noise;87 (iii) knowledge of the chemical identity of the target VOCs and their breath concentrations.

If initial VOC profiling for a given cancer reveals that a few specific marker VOCs are expected to appear at elevated concentrations (*e.g.*, methanol, acetone, and methane, up to a few ppmv)59,112217 in breath, then a sensing platform of semi-selective or highly selective sensors based on specific recognition would be suitable (see Fig. 9). Yet, when a varied composition of VOCs must be identified or when a doubt exists regarding the target VOCs' exact nature, a less specific sensing approach would be better (see Fig. 9). Sensor arrays based on chemiresistive layers of MCNPs or RN-CNTs are very attractive for such uses.138

High BP VOCs should be found in breath at low concentrations of single ppbv (for example, propofol)66 and even lower concentrations, especially the water soluble compounds (for example, indole218), due to a high $\lambda_{b:a}$. To enable sufficient limit of detections (LODs) for such compounds, their detection requires highly sensitive nanomaterial transducers, such as nanowire- or nanotube-based FETs as well as specific recognition features. If not, background VOCs "noise" from non-specific interactions would probably affect the signals of the target VOCs which can eventually result in positive false detection for the determination outlined in Fig. 9.180

When focusing efforts on fine-tuning an applied sensing technology for a specific clinical state, rough estimates are inadequate; an accurate picture of the indicative VOC print should be obtained. Therefore, analytical evaluations of the variances among the characteristic VOCs must be performed to distinguish breath composition patterns of healthy people against people suffering from a disease. The analytical evaluations should be done using standardized techniques, such as gas-chromatography mass spectrometry (GC-MS) or proton transfer reaction mass spectrometry (PTR-MS).59164

The physical and chemical characteristics of the target VOCs are very important for creating a suitable sensing platform to a given condition. Polarity is the main physical characteristic related to VOC sensing, while polar VOCs are generally easier to identify by sensors.8089 This easier detection is because polar VOCs are either directly detected through charge transfer between the sensing material and the molecule, or indirectly detected through a molecular layer as in the case of sensors based on functionalized single Si NW FETs or SWCNT FETs.184,185 Additionally, highly specific recognition elements are more available for polar VOCs because they offer a wider range of possible molecular interactions. For nonpolar VOCs, a sensing mechanism relies on indirect recognition through dielectric changes and steric interactions.138 Thus the size and shape of VOCs are vital factors for developing novel selective recognition for these chemicals. For instance,

molecular imprinted gold MCNP composites can serve as artificial biomimetic receptors (host–guest lock-and-key architecture) in conjugation with surface plasmon resonance (SPR) transduction to detect low (nM) concentrations of RDX in a selective manner.219 The current architecture of this approach is probably limited to sensing only large-sized compounds that can be accommodated through host–guest interactions within the interlinked MCNPs' matrix. An additional example in chemiresistive films would be the use of cube shaped MCNPs, as opposed to spherical MCNPs, that was shown to discriminate among VOCs based only on size.194,220 By applying this strategy, sensor selectivity can be tuned towards compound polarity characteristics based on the organic layer coating the MCNPs. Furthermore, the use of self-assembled polycyclic aromatic hydrocarbon (PAH) layers covering RN-CNT chemiresistive films was shown to provide the sensors with a selective response to polar and nonpolar VOCs in a changing humidity background.197 FETs based on single Si-NWs were successfully passivated to block silicon oxidation and functionalized with alkane-backbone silanes and alkenes, which enabled sensing straight alkanes by an "indirect" steric molecular gating mechanism.138184185

3.3.2 Overcoming confounding factors—To develop a detection system for real-world analysis, the system must be able to deal with various confounding factors. In particular, breath analysis sensors for trace-amount VOC detection must cope with chemical or physical factors such as ambient temperature and humidity or the instability of breath samples and sensing elements.216 From the first step of the breath analysis process, sampling, storage and transport of the exhaled breath to and into the sensor apparatus might result in VOC loss and/or involve considerable amounts of contaminants.221 These difficulties can be minimized by integrating appropriate sampling and preparation techniques for delivery of the sensors. Currently, a common technique used for sample storage involves the use of containers such as collection bags, vials, or canisters. These containers often introduce contamination and cause VOC loss during storage.222–224 A promising alternative option would be "trapping" the VOCs on a sorbent material (for example, Tenax® and/or Carbopack X and/or Carboxen) followed by thermal desorption (TD).424350178225–230 The latter can be accomplished by thermal desorption tubes or by needle trap devices.131231232 This technique allows using a semi-selective sorbent material that can trap a range of VOCs (see Fig. 10a). By performing pre-concentration of the breath sample, one can gain both a reduction of the sample volume (increased VOC concentration) and a decrease in its complexity. Because the various target VOCs are adsorbed/absorbed differently, an appropriate assessment should be performed on the choice of sorbent material.178225 The use of solid phase extraction can provide a breath sample storage solution up to a number of months, depending on the storage system. In addition, the solid phase extraction could allow integration of sensor systems with low volume delivery methods such as microfluidics. In this respect, microfluidics – the science and technology implementing microscale fluidic channels to manipulate microliter to nanoliter volumes – should be integrated with the TD system to optimize sample handling and delivery. Another important advantage of using sorbent material, especially ones with low breakthrough volumes for water (*e.g.*, Tenax), is the ability to trap high moisture content samples such as breath. The dehumidification of the sample improves the performance of the VOC sensor in most cases. By using a multi-capillary column (MCC), research studies could effectively

separate moisture from other breath components, by simply enabling higher chromatographic flow rates of up to 250 ml min^{-1} (ref. 233) allowing isothermal separation of VOCs at ambient temperature (see Fig. 10b).234–236 Besides the various dehumidification techniques that might cause the loss of VOCs,237 other approaches such as enhancing recognition element surface coverage238 and humidity calibration algorithms of the sensors can be applied to reduce the effects of humidity among samples (see Fig. 10c). 216 If the sensor responses to VOCs and water molecules are not independent due to competitive binding, this approach alone is limited and requires new recognition elements that are selective to the VOCs and to water vapors in the matrix.198219 Thus, practical sensing should always account for the VOC/humidity sensitivity ratios,216 which should be tested at humidity levels typical for the breath samples.

Another important aspect of breath analysis with sensing matrices is the working temperature. Breath samples, as well as most sensors, should be handled in a restricted range of operating temperatures.222 In the case of breath samples, the working temperature should not be too high, to protect VOCs from oxidation or thermo-degradation at high temperatures. Additionally, the short thermal desorption process of volatiles could lead to degradation of some compounds.239 Conversely, at low temperatures water condensation occurs in the storage containers causing polar VOCs to dissolve in the condensed humidity. To avoid condensation effects, breath samples in containers should be warmed to a temperature approximately ~40 °C before analysis. Unless the VOCs are extracted and transferred into an inert carrier gas (for example, nitrogen or argon), this approach limits using sensors based on metal oxide nanostructures that operate at high temperatures (for example, 260 °C),240 especially in the case of easily oxidizing compounds.80241 Maintaining a stable temperature throughout the measurement process is important and can be achieved by incorporating an on-chip embedded heating layer (see Fig. 10d).

Yet another aspect of breath analysis is the exposure of sensors to continuous thermal cycles as a result of multiple exposures of breath samples, which might enhance drift effects of the sensors. The drift effects can be overcome by doing sensitivity calibrations216 or by achieving stable sensing layers by inhibition of oxidation processes (see Fig. 10e).242 For stable sensor operation over time, an alternative option could be a long aging process (see Fig. 10f).216 Future breath testing technologies are expected to incorporate multidisciplinary approaches for minimizing the various limiting factors linked to breath analysis together with nanomaterials tailored specifically for target VOCs.

4 Conclusion and future perspective

In this review, we have discussed the possible cellular and biochemical origin of the cancer-related VOCs as well as the relation between the VOCs in the blood and in the exhaled breath. The data presented might not yet provide precise or definite answers to the puzzling pathophysiological pathways of cancer VOCs, but the data will help stimulating constructive discussions and new ideas. We have also discussed the important milestones that have been reached and those that still need to be achieved on the way to detecting a wide range of diseases by breath testing. The outcome of the comparative study we presented is based on cell biology, by means of one or a combination of the following biochemical pathways:

oxidative stress and cytochrome P450, liver enzymes, carbohydrate metabolism (glycolysis/gluconeogenesis pathways), and/or lipid metabolism.

Although the biological mechanisms discussed affect the concentration of the VOCs in both blood and breath, we believe that there is an enormous advantage of breath sampling compared to blood sampling. First, the blood and breath concentrations are related through the respective $\lambda_{b:a}$ of each VOC, so that in certain cases the breath concentration could be higher than the concentration of the same VOC in blood. Another aspect considers the reliability of the sampling technique. In the common process of blood sampling, VOCs are quickly released into the surrounding air. Hence, the sampling of VOCs from blood131 needs very careful preparation and processing of the sample to avoid degassing (and resultant loss) of the VOCs of interest and avoid contamination by VOCs present in the surrounding environment.

A third aspect relates to the analytical techniques. Measuring VOCs in gaseous samples is well developed and comparatively simple, because all the other (non-volatile) compounds do not interfere. However, measuring VOCs in blood samples (where they are surrounded by a much more complicated matrix) needs sampling of blood headspace (after equilibration).131

The last aspect concerns medical applications. Breath sampling is non-invasive and breath can be sampled as often as deemed necessary. Exhaled breath can even be sampled continuously during an ergometer challenge or during sleep,123,125 as opposed to blood, which cannot be sampled continuously.

With respect to current and future technologies for VOC analysis in general, and breath analysis in particular, comprehensive work has yet to be carried out. The exploration of new technologies and new biomarkers for basic and advanced disease detection is constantly gaining momentum. While highly sophisticated analytical methods and molecular methods are currently used in well-equipped clinical and professional laboratories, the future goal is to achieve fast and inexpensive personalized medicine that could be introduced to all parts of the globe including the developing world. As new communication technologies are invented every day, integration of nanoscale medical technologies into this framework would be highly desirable and would allow high-speed global diagnostics. Highly selective sensors could guarantee high sensitivity. Using arrays of cross-reactive sensors may limit the sensitivity, but on the other hand, would relax the stress constraints on the sensor design. The result could be a multi-purpose device with low to medium levels of sensitivity towards the VOCs of interest. In practice, most sensors suffer from some interference by responding to chemical species that are structurally or chemically similar to the desired VOC. This interference in the sensors could be overcome by using different (inorganic) material types and organic functionalities. The responses of the sensors to VOCs can be obtained from equilibrium or kinetic responses, with the latter often providing additional discriminating power. Both binding and solubility properties can be interrogated with advanced functional materials. For example, broadly responsive nanomaterials can be employed to allow a range of structurally similar molecules to bind, membranes could be used as size-selective sensors, and materials with highly selective functional groups could be employed to make selections on the basis of polarity. Often, all of these recognition mechanisms, along with others

described in this review, exist simultaneously in these systems but with different domination ratios. An array of sensors combining all these recognition approaches naturally is needed to yield a unique signal for complex but distinctive VOCs without requiring the mixture to be broken down into its individual components. This condition is a disadvantage when precise VOC composition of a complex mixture is required, but is advantageous when the only required information is the composition of the VOCs of interest.

Improved breath testing systems should combine various technologies that are highly sensitive to cancer-related VOCs and barely sensitive or not at all sensitive to parasitic responses that originate from different confounding factors. This could be achieved, for example, by pre-concentrating and dehumidifying the cancer-related VOCs, by means of the micro-adsorption process followed by TD,225 MEMS-based μ-preconcentrator,243 MCCs, 165,233,234,236,244 and micro-column gas chromatography (MCGC).245–247 The processed cancer-related VOCs are then delivered through a microfluidic system to highly sensitive and selective on-chip sensors that are integrated with a temperature control unit. Following the trend of miniaturization in the world of technology, a breath testing system should eventually be able to fit into a casing as small as a smart phone.

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Biographies

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Yoav Y. Broza received his PhD in Biotechnology & Food Engineering in 2009 from the Technion – Israel Institute of Technology. He has been a researcher in the Laboratory for Nanomaterial-Based Devices, headed by Prof. Hossam Haick, since 2009, and a researcher at the Alfred Mann Institute for biomedical devices between the years 2009 and 2012. Additionally Yoav holds an external lecturer position in the Technion. His current research interests include the identification of disease and infectious agents' biomarkers through volatile gas analysis by analytical methods and nano-arrays, and the development of novel technologies for gas sampling for clinical and non-clinical applications.

Pawel Mochalski is a senior postdoc at the Breath Research Institute of the Austrian Academy of Sciences led by Prof. Anton Amann. He received his PhD in Physical Sciences in 2004 from the Institute of Nuclear Physics Polish Academy of Sciences (IFJ PAN). His scientific interests embrace breath analysis, human scent analysis, volatile disease biomarkers, application of chemical analysis for the early detection of entrapped humans, and the development of analytical methods for the detection and identification of new volatile biomarkers (GC-MS, PTR-TOF-MS, IMS).

Vera Ruzsanyi is a post doc researcher at the Breath Research Institute of the Austrian Academy of Sciences and working in different projects at Innsbruck Medical University. She received her PhD in Bioengineering in 2006 from ISAS – Institute for Analytical Sciences. Since 2010 she has been an industrial researcher at the company G.A.S. mbH developing sensor systems based on ion mobility spectrometry. She received the Lise-Meitner fellowship from the Austrian Science Foundation in 2010. Her current scientific interest

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Anton Amann is director of the Breath Research Institute of the Austrian Academy of Sciences and professor at Innsbruck Medical University. He received his PhD degree in Natural Sciences from the Swiss Federal Institute of Technology (ETH-Zürich). Amann's current research interests include analysis of volatiles in exhaled breath and in the headspace of cells and bacteria. He is the recipient of the Marie Sklodowska Curie medal of the Polish Chemical Society.

Nomenclature

$\lambda_{b:a}$	Partition coefficient of 'blood:air'
$\lambda_{f:b}$	Partition coefficient of 'fat:blood'
$\lambda_{o:w}$	Partition coefficient of 'octanol:water'
$\lambda_{w:a}$	Partition coefficient of 'water:air'
ΔH_{vap}	Enthalpy of vaporization
ADH	Alcohol dehydrogenase
AHFP	2-(4-Aminophenyl)-1,1,13,3,3-hexafluoro-2-propanol
ALDH	Aldehyde dehydrogenase
ANN	Artificial neural network
BAK	Bulk acoustic waves
BCA	Breath collection apparatus
BP	Boiling point
CCK	Cholecystokinin
CNT	Carbon nanotubes
COPD	Chronic obstructive pulmonary disease
CSC	Cancer stem cells
CT	Computed tomography

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CYP	Cytochrome p450
DNA	Deoxyribonucleic acid
EPA	Environmental Protection Agency
FET	Field effect transistor
GC-MS	Gas chromatography – mass spectrometry
GORD	Gastro-oesophageal reflux disease
HPT	2-Heptanone
LC	Lung cancer
LOD	Limit of detection
MCC	Multi-capillary column
MCGC	Micro-column gas chromatography
MCNP	Monolayer capped metal nanoparticle
MEMS	Microelectromechanical system
MRI	Magnetic resonance imaging
NMVS	Nanomaterial based VOC sensor
NP	Nanoparticle
NW	Nanowire
PAH	Polycyclic aromatic hydrocarbon
PEI	Polyethyleneimine
PET	Positron emission tomography
ppbv	Parts per billion by volume
ppmv	Parts per million by volume
PTR-MS	Proton-transfer-reaction – mass spectrometry
PTR-TOF-MS	Proton-transfer-reaction time-of-flight mass spectrometry
PUFA	Polyunsaturated fatty acid
QCM	Quartz crystal microbalance
QMB	Quartz microbalance
RDX	Cyclotrimethylenetrinitramine
ROS	Reactive oxygen species

SAW	Surface acoustic wave
SEM	Scanning electron microscope
SIFT-MS	Selected ion flow tube mass spectrometry
SPME	Solid phase microextraction
SPR	Surface Plasmon resonance
SWCNT	Single-walled carbon nanotube
TD	Thermal desorption
TNT	Trinitrotoluene
VOC	Volatile organic compound

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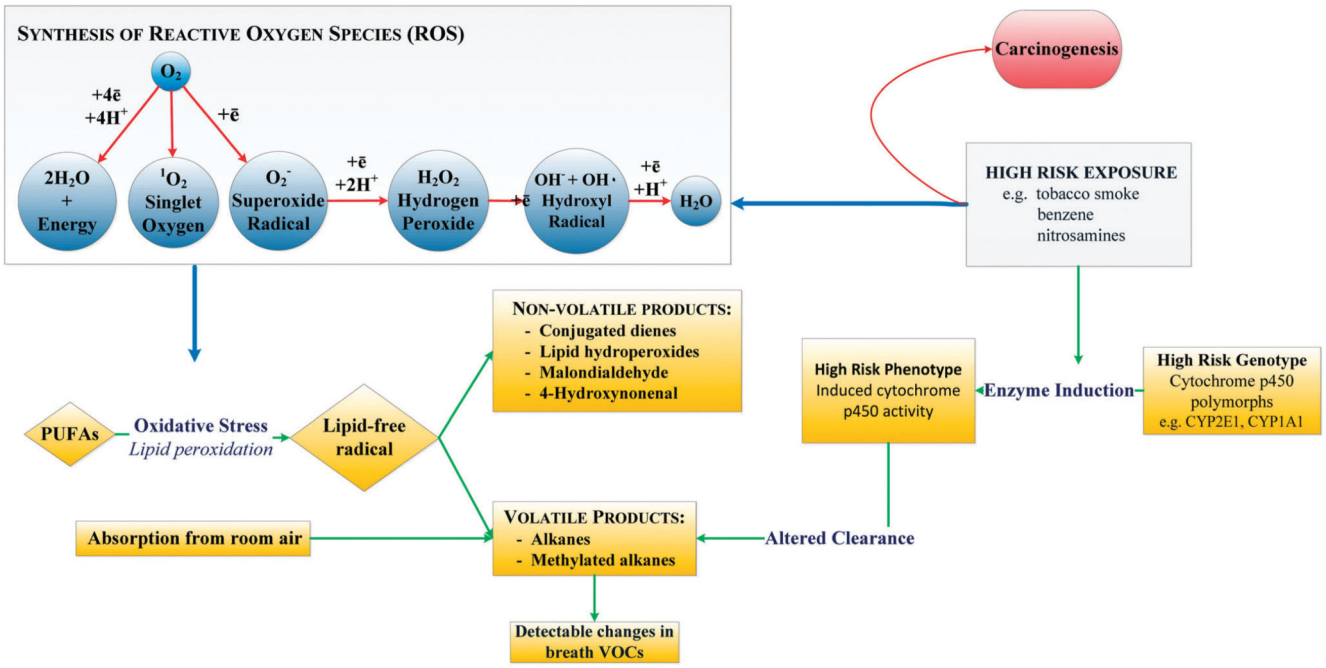

Fig. 1.

Hypothetical basis of the breath test for lung cancer: lung cancer could result from the interaction of hereditary and environmental factors. Several cytochrome p450 mixed oxidases are activated by exposure to environmental toxins such as tobacco smoke. The induced phenotype may increase the risk of lung cancer by increased conversion of precursors to carcinogens. An altered pattern of cytochrome p450 mixed oxidase activity could potentially modulate catabolism of endogenous VOC products of oxidative stress and generate an altered pattern of breath VOCs. Reprinted from ref. 4.

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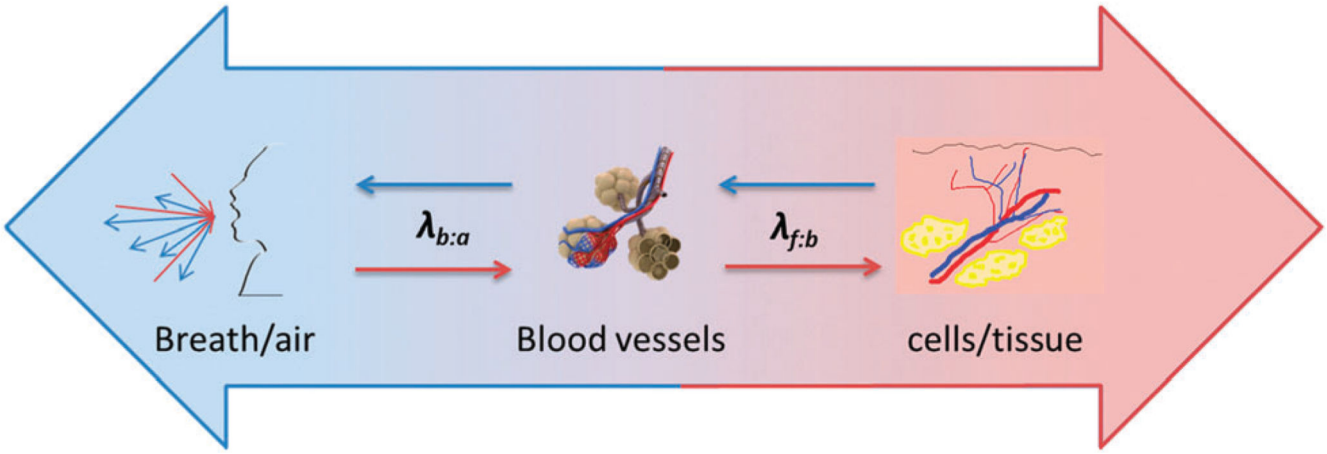

Fig. 2.

Simulation scheme of the two main thermodynamic parameters responsible for the diffusion of cancer VOCs between "breath–blood–fat": $\lambda_{f:b}$ – partition coefficient between fat and blood, which simulates the diffusion of VOC from the (cancer or healthy) tissue to the blood; and $\lambda_{b:a}$ – partition coefficient between blood and air, which simulates the diffusion of VOC from the blood to the exhaled air.

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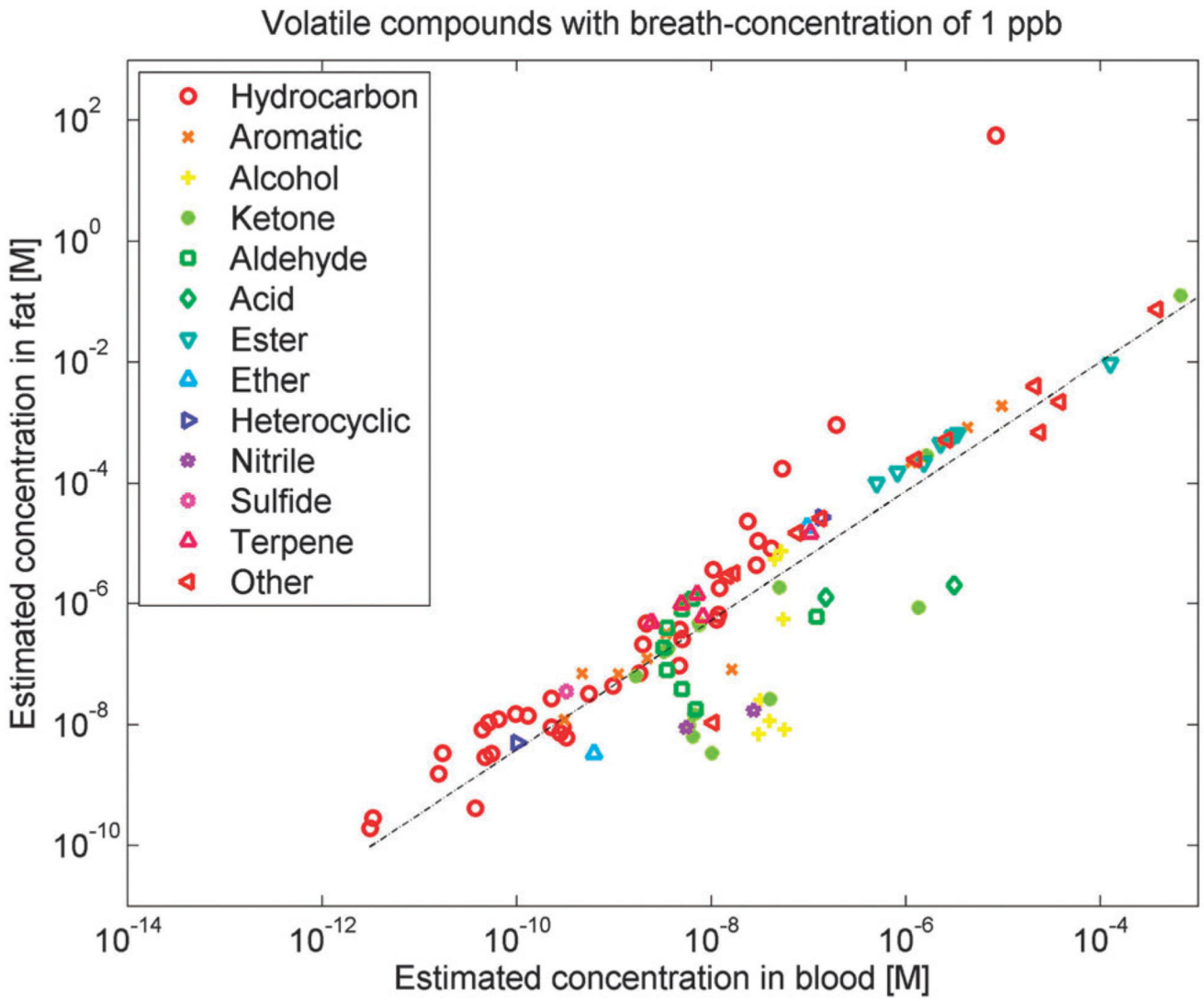

Fig. 3.

Estimated equilibrium concentrations in blood and fat for candidates of volatile cancer biomarkers published during the past decade.4,15,50,56,63,68,71,72,74–76,78,133,202,248,249 These equilibrium concentrations have been estimated under the assumption that the concentration in alveolar breath is 1 part-per-billion (ppb), based on the $\lambda_{b:a}$ (partition coefficient between blood and air) and $\lambda_{f:a}$ (partition coefficient between fat and blood) from Table 1. Hence, for different VOCs showing the same concentration in exhaled breath, the concentration in fat and blood may be very different (up to a factor of 10^8). Different VOCs, therefore, carry distinctive information on the various compartments of the human body. In the figure, various chemical classes of compounds (such as hydrocarbons or sulfides) are indicated by different symbols and colors.

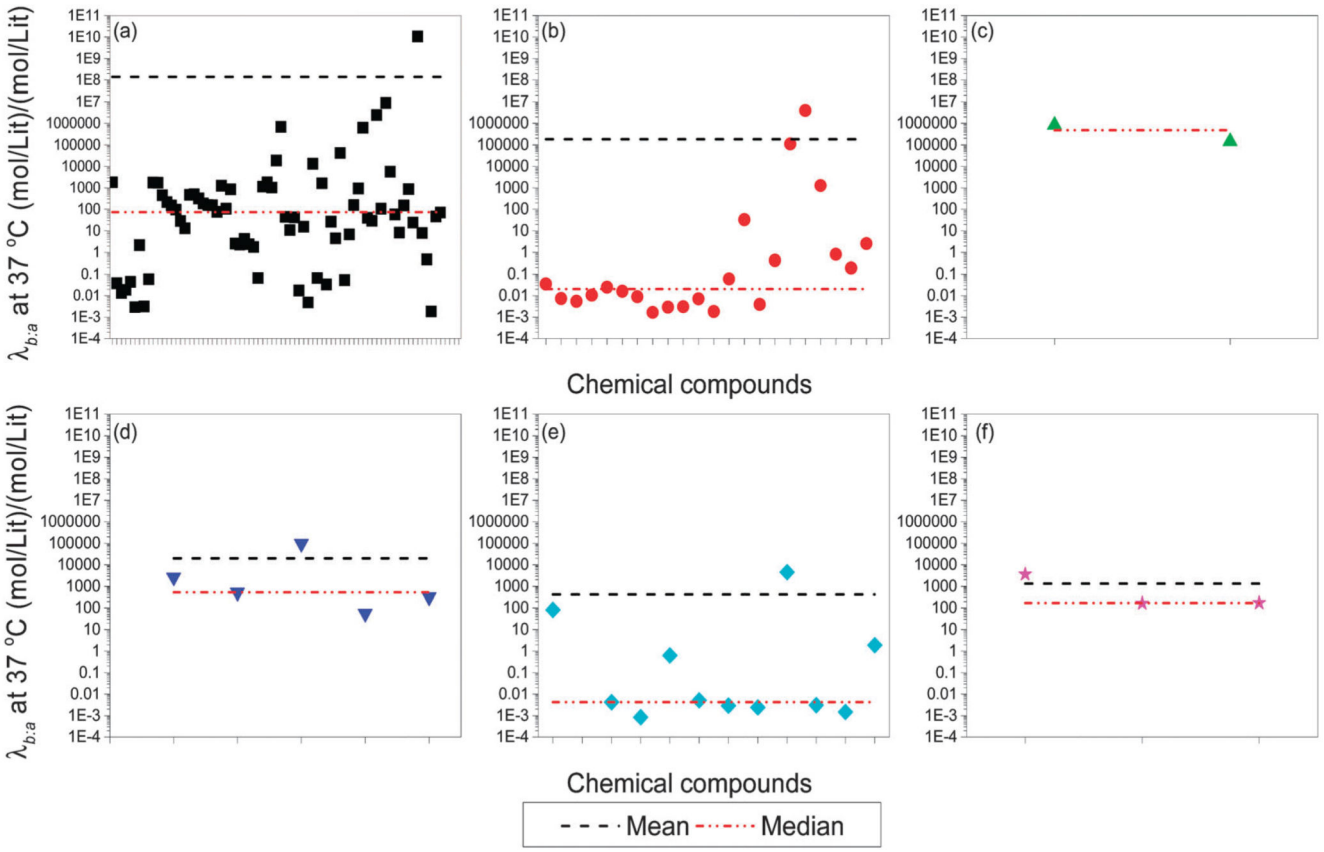

Fig. 4.

$\lambda_{b:a}$ as a function of the VOCs from different types of cancer: (a) lung cancer; (b) breast cancer; (c) colon cance ; (d) liver cancer; (e) head and neck cancer; (f) gastric cancer. Data show that lung cancer, gastric cancer and liver cancer have rather similar values as can be seen from the median line. On the other hand, breast cancer and head and neck cancer are similar and colon cancer is different from the rest.

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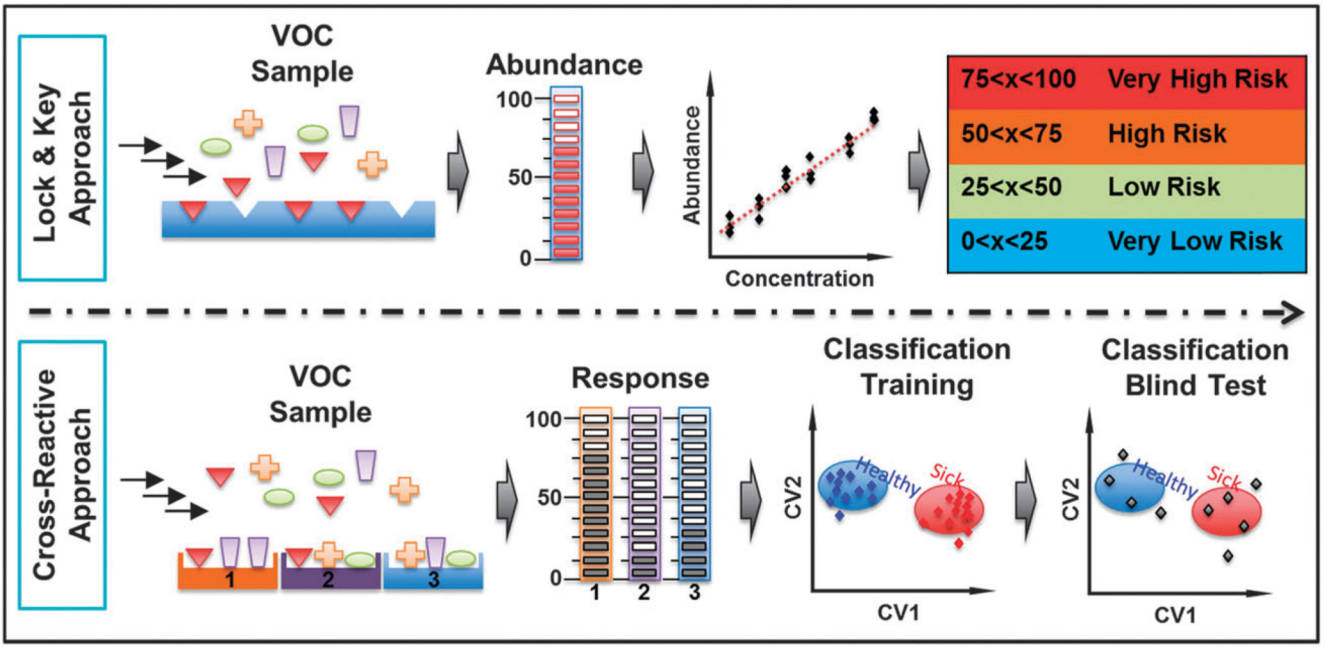

Fig. 5.

Schematic illustration of the selective sensing approach *versus* the cross-reactive sensing approach. Reconstructed from ref. 39.

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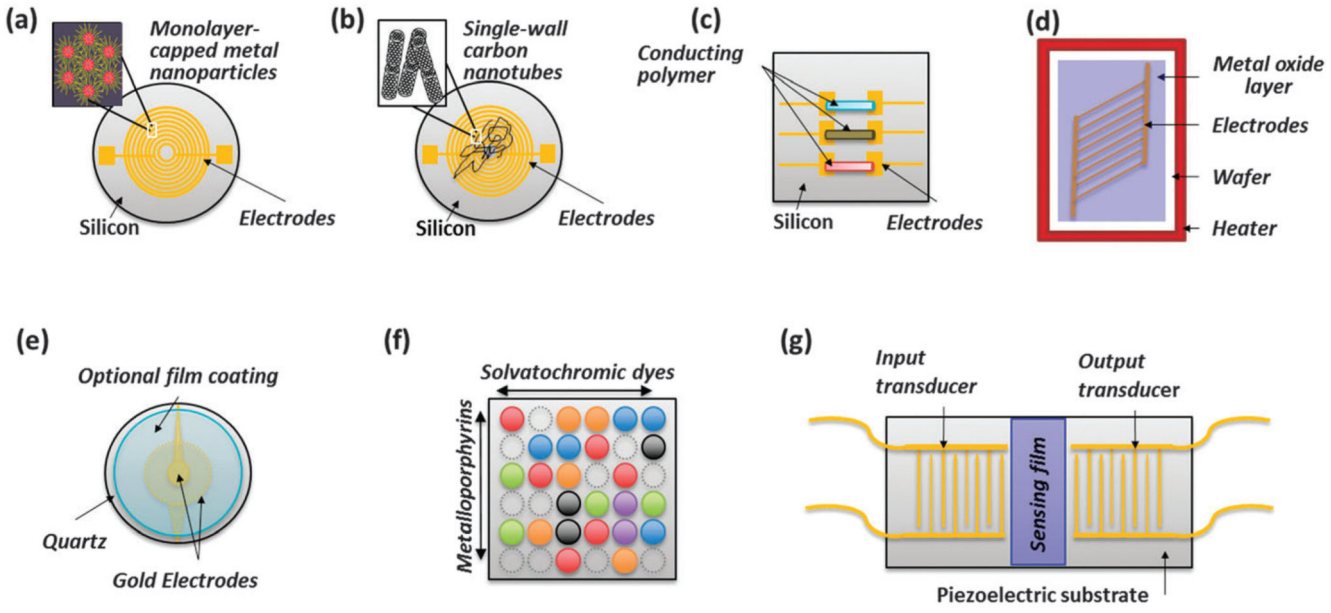

Fig. 6.

Schematic illustration of different nanomaterial-based sensors: (a) chemiresistor based on monolayer-capped metal nanoparticles; (b) chemiresistor based on single-walled carbon nanotubes; (c) chemiresistor based on conducting polymers; (d) chemiresistor or chemicapacitor based on metal-oxide films; (e) quartz microbalance (QMB) with selective coating; (f) colorimetric sensor; and (g) surface acoustic wave (SAW) sensor. Reconstructed from ref. 39.

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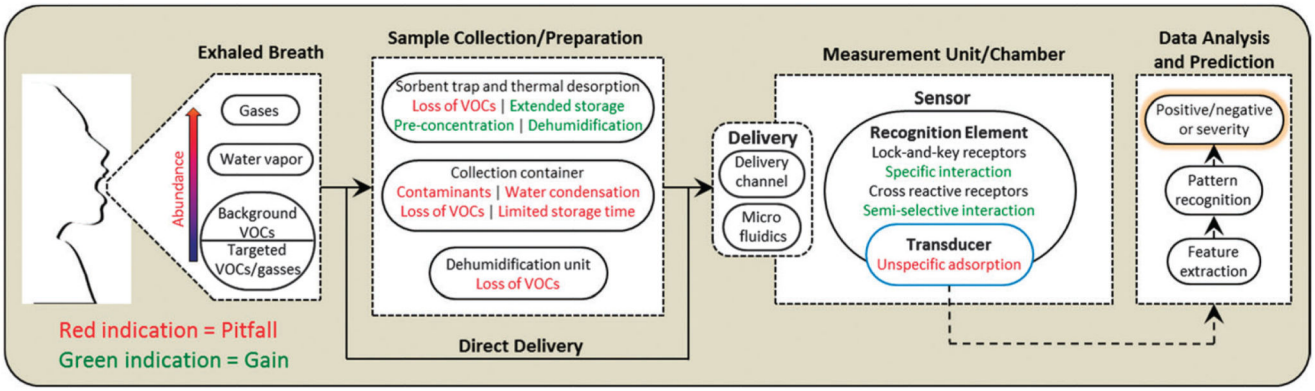

Fig. 7.

Overview of the processes involved in breath testing: exhaled breath is a complex mixture of gases, water vapor, and thousands of VOCs in which only a small number of specific VOCs and gases comprise the clinically significant breath print. In order to perform the breath test, a sample is prepared from the complex mixture of exhaled breath by “trapping” the breath components on a sorbent material (followed by thermal desorption for their release), within a collection container (for example, a bag, vial, or canister), a dehumidification unit, or a channeling unit for direct delivery. The sample is then delivered to a measurement chamber through a simple delivery channel or a microfluidic system. In the measurement chamber, the breath components interact with the recognition element of the NMVS, inducing a measurable change (that is, electrical or optical) in the transducer that is translated into an output signal. Data analysis is then performed on the output signals in order to make the clinical prediction of the breath test. Reconstructed from ref. 138.

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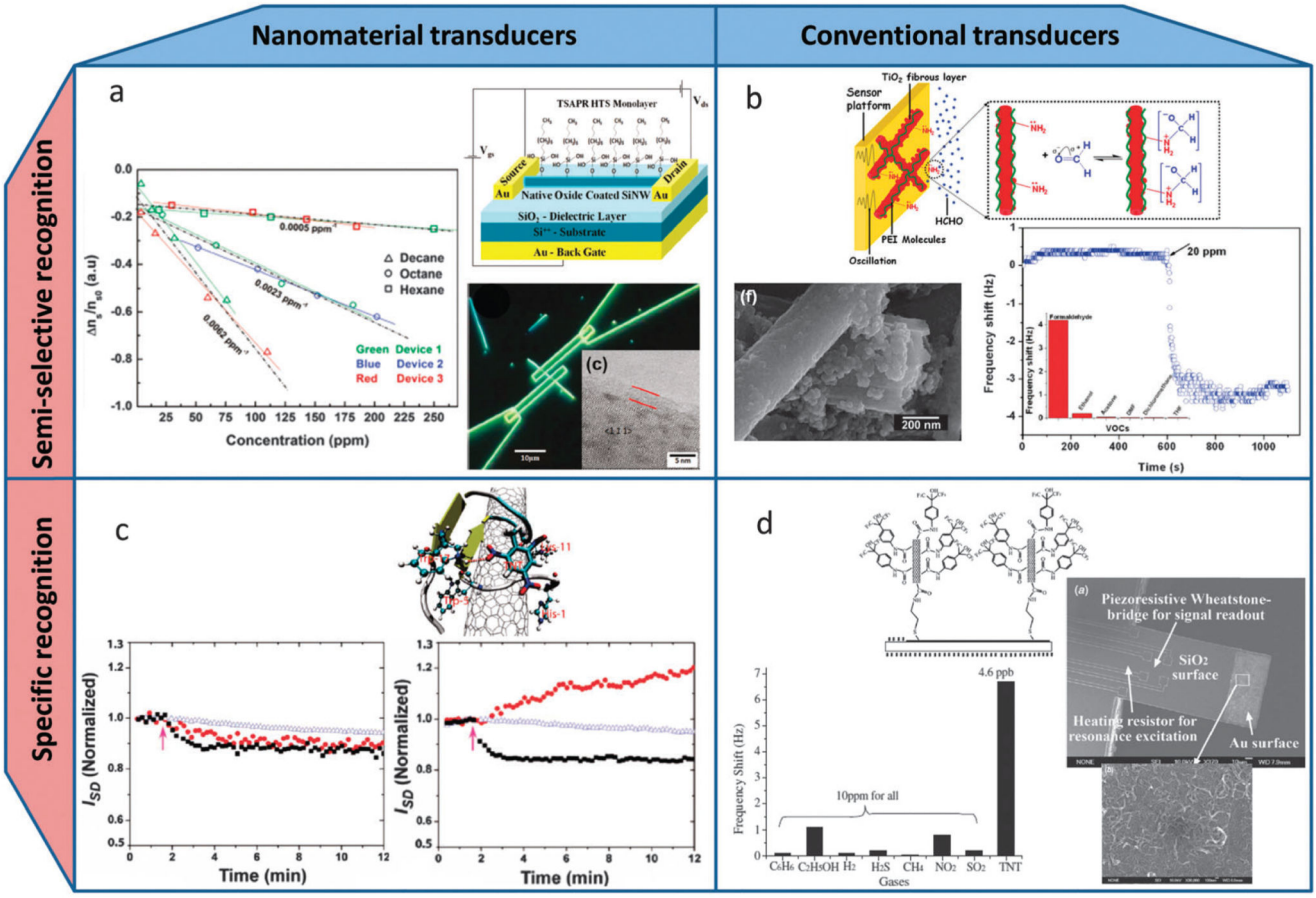

Fig. 8.

Nanomaterial-based VOC sensors can be divided into sensors based on nanomaterial transducers (left column, a and c) or conventional transducers (right column, b and d), with the recognition elements being either semi-selective (upper row, a and b) or specific (lower row, c and d), with the latter types typically more sensitive than the former. (a) Top right: schematic of a Si-NW FET configuration functionalized and passivated with an organic self-assembled monolayer of hexyltrichlorosilane. Bottom right: optical micrograph of a Si-NW FET with an inset showing a TEM image of a representative Si-NW. Left: semi-selectivity of the device shown by the relative surface-state density change ($\Delta n_s/n_{s0}$) as extracted from three different devices exposed to three different nonpolar VOCs (hexane, octane, and decane) at increasing concentrations.185 (b) Top: schematic of a QCM oscillator coated with a sensing layer of polyethyleneimine functionalized TiO_2 (PEI–TiO_2) nanoporous fibers. Bottom left: SEM image of a representative PEI–TiO_2 nanoporous fiber. Right: responses of QCM-based PEI–TiO_2 sensors on exposure to 20 ppmv formaldehyde. The inset shows the frequency shift of the sensor *versus* 20 ppmv of various VOCs demonstrating the increased selectivity (semi-selectivity) of the sensor towards formaldehyde.250 (c) Top: a computational modeling predicting the specific binding of TNT to a peptide–CNT hybrid through a H-bond with Trp17 of the peptide and **π**–**π** interaction with the CNT surface (as part of a SWCNT-FET sensor for TNT vapor). Bottom left and right: response of a bare and peptide-coated (respectively) CNT-FET sensor to vapors of TNT (red circles), RDX (blue

triangles), and HPT (black squares) showing the specific response to TNT. The arrow indicates when the vapor is introduced into the device.181 (d) Top: schematic of the surface modification of a gold-coated cantilever end with multi-walled CNTs functionalized with TNT-specific AHFP molecules. Right: SEM image of amicro-cantilever sensor immobilized with multi-walled CNTs. The inset shows a magnification of the random network of immobilized CNTs. Bottom left: response of a surface modified cantilever sensor, with HFIP functionalized multi-walled CNTs, to various interfering gases (all in about 10 ppmv concentration) in comparison with its response to 4.6 ppbv TNT vapor and demonstrating the specific response to TNT.251 Reconstructed from ref. 138.

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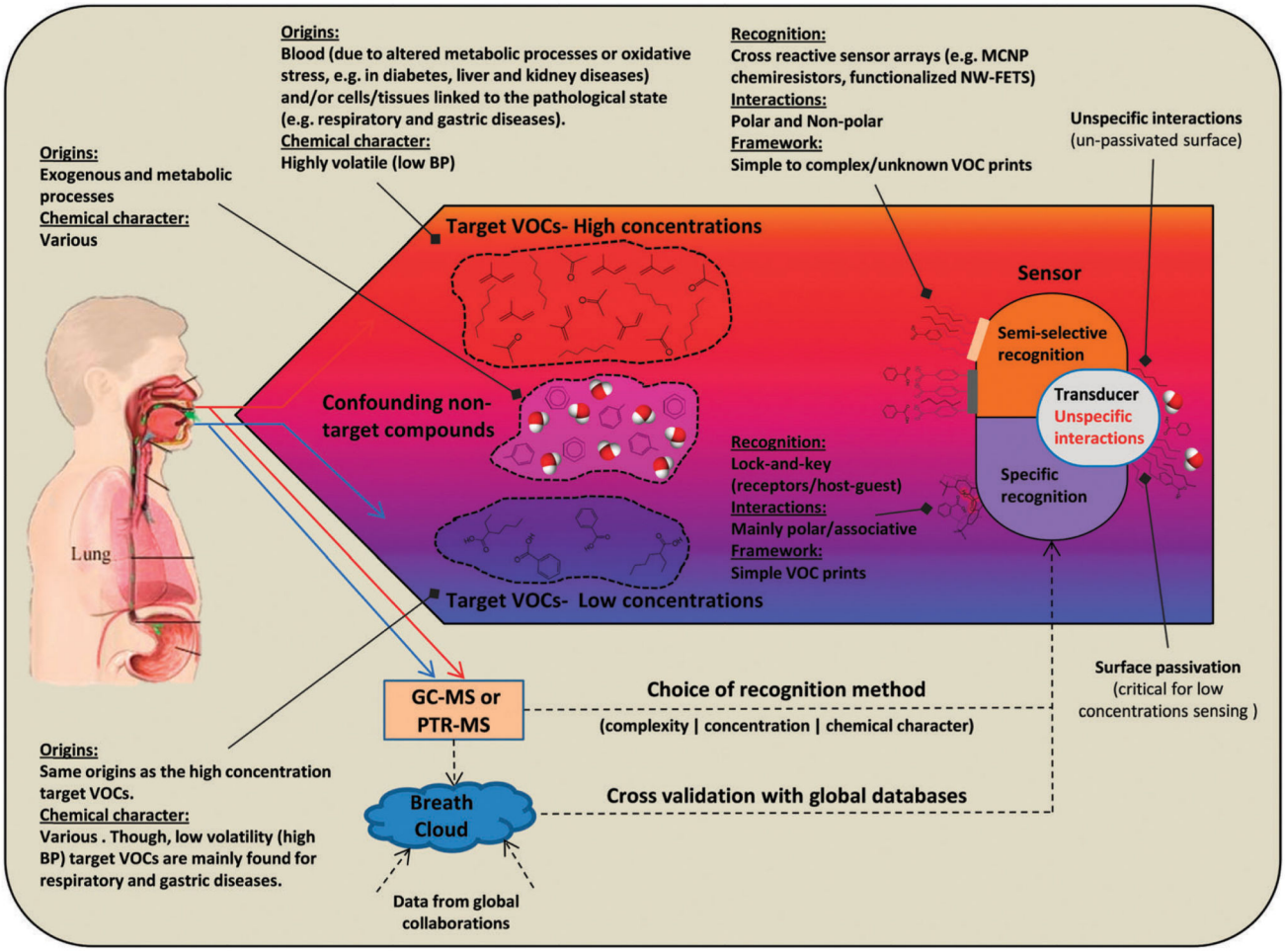

Fig. 9.

Illustration showing the two main sensing approaches (specific *vs.* cross-reactive approaches) and how they can be coupled to the different types of VOC prints originating from different types of clinical states. When the detection of a single or a few target breath markers is required, maximal selectivity is required from the NMVSs, so a lock-and-key approach is most suitable. This approach is especially important for compounds that tend to appear in breath at low concentrations, such as unvolatile (high boiling point) compounds. If the targeted breath print is composed of many compounds or their identity is unknown, an array of more semi-selective NMVSs should be used. Such a setup is especially suitable for volatile (low boiling point) compounds that tend to appear at more elevated levels. Reconstructed from ref. 138.

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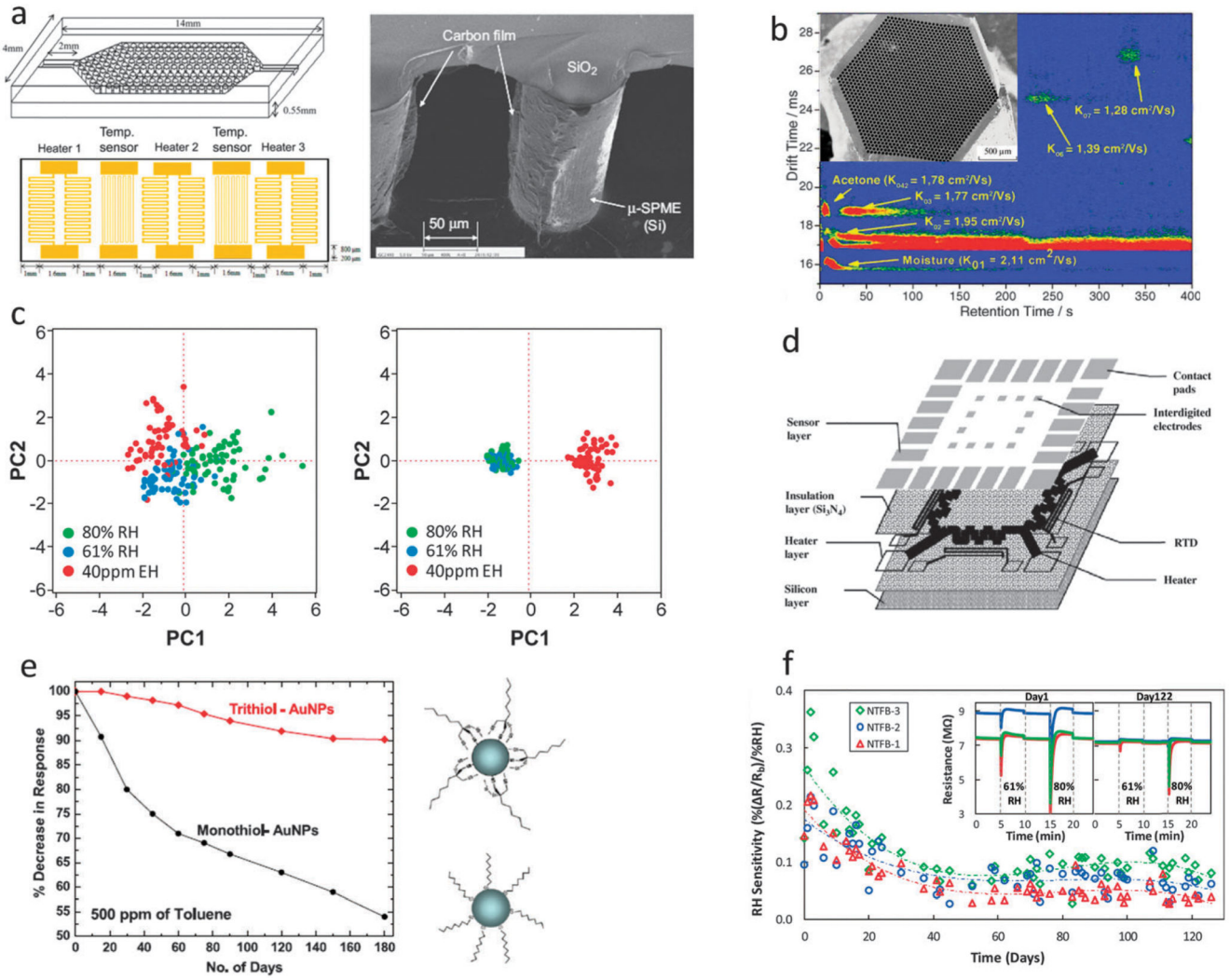

Fig. 10.

Means for tackling the implications of real-world confounding factors. (a) Top left: schematic diagram of a µ-preconcentrator chip that utilizes an array of solid-phase microextraction (SPME) needles coated with an *in situ*-grown carbon adsorbent film (as the sorbent material). Right: cross-section SEM image of an array of µ-SPME needles coated with the carbon film. Bottom left: schematic diagram of the heater and temperature sensors of the thermal desorption (TD) unit of the µ-preconcentrator chip.225 (b) A topographic plot of an ion mobility spectrometer (IMS) coupled to a multi-capillary column (MCC) from the breath of a patient suffering from lung infection. The plot shows on the bottom left hand side that the moisture of the breath sample is separated from the signals of the other breath components.234 The inset shows a micrograph of a transverse section of a MCC with ~1400 capillaries having a diameter of ~40 µm.244 (c) A comparison between the response patterns of an array of four gold-nanoparticle (Au-NP) based chemiresistors to clean moist air samples (blue and green closed circles) and air samples contaminated by ~40 ppm of 2-ethylhexanol before humidity compensation (left) and after humidity compensation (right). The plot shows the major improvement in the performance of the sensor array resulting from

the humidity compensation procedure.216 (d) A schematic view of the different layers composing a CNT-FET sensor integrated with an embedded heating layer situated between the substrate and the dielectric layer, which is useful for reducing the recovery time of the sensor by desorbing the bound molecules more rapidly.182 (e) Left: a plot showing the major improvement in the stability of the sensitivity to toluene of Au-NP based sensors capped by trithiols instead of monothiols, which is explained to be a result of slower oxidation of the thiolate groups in the case of the trithiol capping layer. Right: a schematic drawing showing the differences between the trithiol capped Au-NPs (top) and the monothiol capped Au-NPs (bottom).242 (f) A plot of the sensitivity of three identically fabricated Au-NP based chemiresistors towards water vapor over a period of ~124 days, which shows that their sensitivity drastically drifts down over the first few weeks and stabilizes after an aging period of ~40 days. The inset shows the resistance response profiles of the three sensors that become almost identical towards the end of the experiment.216

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Table 1

Candidates for cancer VOCs published during the past decade.4,15,50,56,63,68,71,72,74–76,78,133,202,248,249 Identification of these compounds was achieved through spectral library match. A number of authors validated their identification by comparison of retention time or retention index, *e.g.*, in ref. 1, 15, 42–45, 59, 65, 66, 131 and 133. For these VOCs the $\lambda_{b:a}$ and $\lambda_{f:b}$ are given in separate columns. If these partition coefficients are not known from the literature, they are estimated by regression from data in ref. 115 for hydrocarbons or estimated by the algorithms of Poulin and Krishnan139 for other compounds based on the partition coefficients for water:air($\lambda_{w:a}$) and for octanol:water ($\lambda_{o:w}$). All partition coefficients are given in dimensionless units [mol L^{-1}/mol L^{-1}]. Based on these physicochemical parameters, the equilibrium concentrations of VOCs in blood and fat can be estimated based on the concentration in alveolar breath

Chemical family	CAS number	Compound name	$\lambda_{b:a}$ at 37 °C in dimensionless units [(mol L^{-1})/(mol L^{-1})]	References for $\lambda_{b:a}$ at 37 °C	log[λ_{fb}]	References for $\lambda_{f:b}$ at 37 °C	Tentative origin	Comments	Ref.
Alkanes, branched-chain alkanes and branched-chain alkenes	74-84-0	Ethane	8.50×10^{-02}	Predicted252	1.934	120	Natural or petrol, product of lipid peroxidation	Lung cancer	4
	109-66-0	Pentane	4.16×10^{-01}	Measured115	1.998	253	Natural, possibly petrol, product of lipid peroxidation	Lung cancer	78
	142-82-5	Heptane	$2.71 \times 10^{+00}$	Measured115	2.190	253	Natural or petrol, plastics	Lung cancer	78
	111-65-9	Octane	$5.77 \times 10^{+00}$	Measured115	1.606	120	Natural or petrol	Lung cancer	56,78
	111-84-2	Nonane	$1.39 \times 10^{+01}$	Measured115	1.777	254	Natural or petrol	Breast cancer	74
	124-18-5	Decane	$2.48 \times 10^{+01}$	Predicted using data from ref. 115	1.656	254	Natural or petrol	Lung cancer	78
	1120-21-4	Undecane	$5.46 \times 10^{+01}$	Predicted using data from ref. 115	2.342		Natural or petrol	Breast cancer	76
	112-40-3	Dodecane	$1.20 \times 10^{+02}$	Predicted using data from ref. 115	1.901		Fuels	Lung cancer, breast cancer	76,248
	629-50-5	Tridecane	$2.67 \times 10^{+02}$	Predicted using data from ref. 115	2.540		Fuels	Breast cancer	76
	629-59-4	Tetradecane	$5.99 \times 10^{+02}$	Predicted using data from ref. 115	2.990			Breast cancer	76
	629-62-9	Pentadecane	$1.36 \times 10^{+03}$	Predicted using data from ref. 115	3.515			Breast cancer	76
	75-28-5	2-Methyl-propane	7.90×10^{-02}	Measured115	1.795		Refrigerant, contaminant from plastics, tubing, medical equipment	Breast cancer	74
	107-83-5	2-Methyl-pentane	4.73×10^{-01}	Measured115	2.295	253	Petrol	Lung cancer	78
	78-79-5	Isoprene	9.50×10^{-01}	Measured114	1.043	120	Mevalonic pathway – biosynthesis of cholesterol	Lung cancer and gastric cancer	15
	61141-72-8	4,6-Dimethyl-dodecane	$7.38 \times 10^{+02}$	Predicted	2.178		Kerosene fuel	Head and neck cancer	63

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Chemical family	CAS number	Compound name	$\lambda_{b:a}$ at 37 °C in dimensionless units [(mol L^{-1})/(mol L^{-1})]	References for $\lambda_{b:a}$ at 37 °C	log[λ_{fb}]	References for $\lambda_{f:b}$ at 37 °C	Tentative origin	Comments	Ref.
	17302-37-3	2,2-Dimethyl-decane	$1.18 \times 10^{+02}$	Predicted	1.316			Head and neck cancer	63
	473-19-8	2,2,3-Trimethyl-exobicyclo[2.2.1]heptane	$1.25 \times 10^{+02}$	Predicted	2.296			Head and neck cancer	63
	562-49-2	3,3-Dimethyl-pentane	$1.20 \times 10^{+00}$	Predicted	1.786			Breast cancer	68
	62185-53-9	5-(2-Methylpropyl)-nonane	2.86 ×10+02	Predicted	1.684			Breast cancer	68
	62238-15-7	2,3,4-Trimethyl-decane	$2.99 \times 10^{+02}$	Predicted	1.755			Breast cancer	68
	589-34-4	3-Methyl-hexane	$1.30 \times 10^{+00}$	Measured253	2.329	253	Unnatural, environmental contaminant	Head and neck cancer	63
	2213-23-2	2,4-Dimethylheptane	$7.55 \times 10^{+00}$	Predicted	1.492		High chance of mis-assignment of isomer, petrol	Head and neck cancer, lung cancer	4,63
	3221-61-2	2-Methyl-octane	$3.31 \times 10^{+00}$	Measured for rat blood254	2.040	254		Breast cancer	74
	2216-34-4	4-Methyl-octane	$8.21 \times 10^{+00}$	Predicted	1.284		Contaminant from plastics, tubing, medical equipment	Head and neck cancer, lung cancer	4,63
	54166-32-4	2,6,6-Trimethyl-octane	$4.64 \times 10^{+01}$	Predicted	1.593			Head and neck cancer	63
	5911-04-6	3-Methyl-nonane	$5.76 \times 10^{+00}$	Measured for rat blood254	2.079	254	Natural or petrol	Head and neck cancer	63
	16747-26-5	2,2,4-Trimethylhexane	$7.08 \times 10^{+00}$	Predicted	1.426		Petrol	Lung cancer	202
	25117-31-1	5-Methyl-tridecane	$7.67 \times 10^{+02}$	Predicted	2.559			Breast cancer	74
	1002-43-3	3-Methyl-undecane	$1.27 \times 10^{+02}$	Predicted	1.726			Breast cancer	74
	10105-38-1	6-Methyl-pentadecane	$4.86 \times 10^{+03}$	Predicted	3.689			Breast cancer	74
	6418-45-7	3-Methyl-nonadecane	$2.13 \times 10^{+05}$	Predicted	6.830			Breast cancer	74
	6117-97-1	4-Methyl-dodecane	$3.08 \times 10^{+02}$	Predicted	2.176			Breast cancer	74
	763-29-1	2-Methyl-1-pentene	$1.41 \times 10^{+00}$	Predicted	1.783		Contaminant from plastics, tubing, medical equipment	Lung cancer	202
	2847-72-5	Decane, 4-methyl-	$5.04 \times 10^{+01}$	Predicted	2.038			Lung cancer	71
	764-13-6	2,4-Hexadiene, 2,5-dimethyl-	$1.64 \times 10^{+00}$	Predicted	2.284			Lung cancer	71
	1515-79-3	5,5-Dimethyl-1,3-hexadiene	$1.13 \times 10^{+00}$	Predicted	2.281			Lung cancer	72
Primary and secondary alcohols	64-17-5	Ethanol	$1.50 \times 10^{+03}$	Measured252	−0.823	255	Natural, diet, disinfectants, intestinal bacterial flora	Liver cancer	50
	71-23-8	1-Propanol	$1.03 \times 10^{+03}$	Measured120	−0.532	255	Natural, disinfectants	Lung cancer	4,71

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Chemical family	CAS number	Compound name	$\lambda_{b:a}$ at 37 °C in dimensionless units [(mol L^{-1})/(mol L^{-1})]	References for $\lambda_{b:a}$ at 37 °C	log[λ_{fb}]	References for $\lambda_{f:b}$ at 37 °C	Tentative origin	Comments	Ref.
	67-63-0	2-Propanol	$8.30 \times 10^{+02}$	Measured252	−0.634	255	Natural, disinfectants	Lung and breast cancer	72,75
	71-36-3	1-Butanol	$9.33 \times 10^{+02}$	Measured252	−0.095	120	Natural, diet	Lung cancer	249
	104-76-7	2-Ethyl-1-hexanol	$1.31 \times 10^{+03}$	Predicted	2.156		Contaminant from tubing material	Lung cancer	4
	3391-86-4	1-Octen-3-ol	$1.13 \times 10^{+03}$	Predicted	2.089		Natural (produced in plants and fungi)	Liver cancer	56
	625-31-0	4-Penten-2-ol	$1.39 \times 10^{+03}$	Predicted	1.006			Lung cancer	72
Aldehydes and branched aldehydes	123-38-6	Propanal	$1.77 \times 10^{+02}$	Predicted	0.418		Natural or industrial waste product	Lung cancer	4
	123-72-8	Butanal	$1.27 \times 10^{+02}$	Predicted	0.894		Natural or industrial waste product, diet	Lung cancer	4
	110-62-3	Pentanal	$8.85 \times 10^{+01}$	Predicted	1.361		Natural, diet	Lung cancer	4,133
	66-25-1	Hexanal	$8.21 \times 10^{+01}$	Predicted	1.769		Natural, diet	Lung cancer, liver cancer	4,56,133
	111-71-7	Heptanal	$8.87 \times 10^{+01}$	Predicted	2.058		Natural or industrial waste product, diet,	Lung cancer, breast cancer	4,75
	124-13-0	Octanal	$1.26 \times 10^{+02}$	Predicted	2.209		Natural or industrial waste product diet	Lung cancer	4,133
	124-19-6	Nonanal	$1.63 \times 10^{+02}$	Predicted	2.268		Possibly natural	Lung cancer	4,133
	98-01-1	Furfural‖furaldehyde	$3.05 \times 10^{+03}$	Predicted	0.705		Natural or industrial waste product	Gastric cancer	15
Carboxylic acids	75-98-9	2,2-Dimethyl-propanoic acid	$3.82 \times 10^{+03}$	Predicted	0.930			Head and neck cancer	63
	64-19-7	Acetic acid	$7.96 \times 10^{+04}$	Predicted	−0.190		Natural or industrial waste product	Liver cancer	50
Ketones	67-64-1	Acetone	$3.40 \times 10^{+02}$	Measured137	−0.475	255	Fatty acids metabolism	Lung cancer	4
	78-93-3	2-Butanone	$1.64 \times 10^{+02}$	Measured120	−0.004	255	Diet, environmental contaminant	Lung cancer	4
	107-87-9	2-Pentanone	$1.50 \times 10^{+02}$	Measured256	0.206	120	Natural, diet	Lung cancer	4
	591-78-6	2-Hexanone	$1.68 \times 10^{+02}$	Measured120	0.356		Industrial waste product	Lung cancer	202
	106-35-4	3-Heptanone	$1.87 \times 10^{+02}$	Predicted	1.813		Natural, drugs	Lung cancer	202
	623-56-3	5-Methyl-3-hexanone	$9.15 \times 10^{+01}$	Predicted	1.702			Head and neck cancer	63
	7379-12-6	2-Methyl-3-hexanone	$8.28 \times 10^{+01}$	Predicted	1.702			Lung cancer	72
	110-93-0	6-Methyl-5-hepten-2-one	$1.92 \times 10^{+02}$	Predicted	1.779		Squalene oxidation	Gastric cancer	15
	513-86-0	3-Hydroxy-2-butanone	$1.02 \times 10^{+03}$	Predicted	−0.173			Lung cancer	249

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Chemical family	CAS number	Compound name	$\lambda_{b:a}$ at 37 °C in dimensionless units [(mol L^{-1})/(mol L^{-1})]	References for $\lambda_{b:a}$ at 37 °C	log[λ_{fb}]	References for $\lambda_{f:b}$ at 37 °C	Tentative origin	Comments	Ref.
	119-61-9	Benzophenone	$4.14 \times 10^{+04}$	Predicted	2.247		Industrial waste product (used as fragrance in soaps, in pharmaceuticals and ultraviolet absorbers – sunscreen)	Lung cancer	72
	3848-24-6	2,3-Hexanedione	$3.42 \times 10^{+04}$	Predicted	−0.189			Lung cancer	72
	565-80-0	3-Pentanone, 2,4-dimethyl-	$4.27 \times 10^{+01}$	Predicted	1.582			Lung cancer	71
Aromatic compounds	71-43-2	Benzene	$8.80 \times 10^{+00}$	Measured257	1.598	257	Petrol, smoking	Lung cancer	4
	108-88-3	Toluene	$1.39 \times 10^{+01}$	Measured257	2.180	257	Petrol, smoking	Lung cancer	4
	100-42-5	Styrene	$5.56 \times 10^{+01}$	Measured120	1.758	120	Natural, smoking	Lung cancer	4
	625-86-5	2,5-Dimethylfuran	$2.58 \times 10^{+00}$	Predicted	1.695		Smoking	Lung cancer	4,71
	106-42-3	*p*-Xylene	$3.89 \times 10^{+01}$	Measured120	0.709	257	Petrol, smoking	Head and neck cancer, prostate cancer	63,68
	496-16-2	2,3-Dihydro-benzofuran	$8.71 \times 10^{+01}$	Predicted	1.976			Liver cancer	50
	100-41-4	Ethylbenzene	$2.82 \times 10^{+01}$	Measured120	1.796	120	Petrol	Lung cancer	78
	98-86-2	1-Phenyl-ethanone	$1.27 \times 10^{+03}$	Predicted	1.573			Breast cancer	75
Nitrils	75-05-8	Acetonitrile	$6.98 \times 10^{+02}$	Predicted	−0.198		Smoking	Lung cancer	4
	107-13-1	2-Propenenitrile	$1.41 \times 10^{+02}$	Predicted	0.210		Smoking and car exhaust	Gastric cancer	15
	93946-48-6	2-Amino-5-isopropyl-8-methyl-1-azulenecarbonitrile	$9.65 \times 10^{+06}$	Predicted	2.294			Breast cancer	68,76
Terpens and terpenoids	138-86-3	DL-Limonene	$6.21 \times 10^{+01}$	Predicted	2.296		Industrial waste (used in food flavorings and cosmetics)	Breast cancer	76
	98-55-5	*p*-Menth-1-en-8-ol	$2.63 \times 10^{+03}$	Predicted	2.152		Cosmetics	Lung cancer	72
	21368-68-3	Camphor	$2.07 \times 10^{+02}$	Predicted	1.873		Natural	Lung cancer	72
Others	110-27-0	Isopropyl myristate	$5.75 \times 10^{+04}$	Predicted	2.298			Breast cancer	75
	124-63-0	Methane-sulfonyl chloride	$2.65 \times 10^{+02}$	Predicted	0.021		Transamination pathways (incomplete metabolism of methionine)	Liver cancer	50
	631-61-8	Ammonium acetate						Head and neck	63
	35242-43-4	2,3-Dihydro-1-phenyl-4(1*H*)-quinazolinone	$3.84 \times 10^{+07}$	Predicted	2.260			Breast cancer	68,75
	4282-42-2	1-Iodo-nonane	$3.72 \times 10^{+02}$	Predicted	2.298			Breast cancer	68
	24310-22-3	2-[(1,1-Dimethylethyl)thio]-acetic acid	$9.66 \times 10^{+05}$	Predicted	1.767			Colon cancer	68
	82406-83-5	4-(4-Propylcyclohexyl)-, 4′-cyano[1,1′-biphenyl]-4-yl ester benzoic acid	$1.77 \times 10^{+11}$	Predicted	2.298			Colon cancer	68

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	NIST 282650	2-Trifluoromethylbenzoic acid, 6-ethyl-3-octyl ester	$1.18 \times 10^{+05}$	Predicted	2.298			Breast cancer	68
	21064-19-7	1,5,9-Cyclododecatriene, 1,5,9-trimethyl-	$1.05 \times 10^{+03}$	Predicted	2.298			Lung cancer	71
	6846-50-0	Pentan-1,3-dioldiisobutyrate, 2,2,4-trimethyl	$8.50 \times 10^{+04}$	Predicted	2.293		Plasticizer	Lung cancer	71
	23676-09-7	Benzoic acid, 4-ethoxy-, ethyl ester	$2.07 \times 10^{+04}$	Predicted	2.266			Lung cancer	71
	74381-40-1	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	$7.68 \times 10^{+04}$	Predicted	2.293			Lung cancer	71
	494-19-9	10,11-Dihydro-5*H*-dibenz-[*b,f*]-azepine	$5.37 \times 10^{+05}$	Predicted	2.286			Lung cancer	71
	719-22-2	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	$1.69 \times 10^{+07}$	Predicted	2.283			Lung cancer	71
	101-84-8	Benzene, 1,1-oxybis-	$2.43 \times 10^{+03}$	Predicted	2.292			Lung cancer	71
	13049-35-9	1,1-Biphenyl, 2,2-diethyl-	$6.68 \times 10^{+04}$	Predicted	2.298			Lung cancer	71
	87-44-5	*trans*-Caryophyllene	$1.81 \times 10^{+02}$	Predicted	2.298			Lung cancer	71
	3910-35-8	1*H*-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	$3.22 \times 10^{+04}$	Predicted	2.298			Lung cancer	71
	84-66-2	1,2-Benzenedicarboxylic acid, diethyl ester	$3.85 \times 10^{+04}$	Predicted	2.154		Plasticizer	Lung cancer	71
	76-13-1	Ethane, 1,1,2-trichloro-1,2,2-trifluoro-	2.40×10^{-01}	Predicted	2.245			Lung cancer	72
	1634-04-4	Propane, 2-methoxy-2-methyl-	$1.59 \times 10^{+01}$	Predicted	0.728	258	Gasoline	Lung cancer	72
	42848-06-6	1-Propene, 1-(methylthio)-, (E)-	$8.18 \times 10^{+00}$	Predicted	2.043		Diet (onion, garlic)	Lung cancer	72
	824-22-6	1*H*-Indene, 2,3-dihydro-4-methyl-	$1.32 \times 10^{+02}$	Predicted	2.280			Lung cancer	72
	915392-37-9	5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	$5.97 \times 10^{+05}$	Predicted	1.473			Lung cancer	72
	127-51-5	Isomethyl ionone	$1.95 \times 10^{+03}$	Predicted	2.291			Lung cancer	72
	710336-76-8	2,2,7,7-Tetramethyltricyclo-[6.2.1.0(1,6)]undec-4-en-3-one	$4.33 \times 10^{+02}$	Predicted	2.274			Lung cancer	72
	24238-73-1	Bicyclo[3.2.2]nonane-1,5-dicarboxylic acid, 5-ethyl ester	$3.20 \times 10^{+06}$	Predicted	1.868			Lung cancer	72
	959016-51-4	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	$1.27 \times 10^{+04}$	Predicted	2.295			Lung cancer	72
	16204-36-7	1,2,4,5-Tetroxane, 3,3,6,6-tetraphenyl-	$9.14 \times 10^{+08}$	Predicted	2.295			Lung cancer	72
	6738-27-8	2,5-Cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-ethylidene-	$3.38 \times 10^{+03}$	Predicted	2.292			Lung cancer	72
	55162-49-7	Furan, 2-[(2-ethoxy-3,4-dimethyl-2-cyclohexen-1-ylidene)methyl]-	$3.47 \times 10^{+03}$	Predicted	2.297			Lung cancer	72
	7694-30-6	Benzene, 1,1-(1,2-cyclobutanediyl)bis, *cis*-	$2.85 \times 10^{+04}$	Predicted	2.296			Lung cancer	72
	53699-80-2	Benzene, 1,1-[1-(ethylthio)propylidene]bis-	$1.09 \times 10^{+05}$	Predicted	2.295			Lung cancer	72
	101580-33-0	Anthracene, 1,2,3,4-tetrahydro-9-propyl-	$2.45 \times 10^{+05}$	Predicted	2.298			Lung cancer	72

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	839-73-6	9,10-Anthracenediol, 2-ethyl-	$2.79 \times 10^{+11}$	Predicted	2.284			Lung cancer	72
	10224-91-6	Benzene, 1,1-ethylidenebis, 4-ethyl-	$6.30 \times 10^{+04}$	Predicted	2.298			Lung cancer	72