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 new fascinating and cost-effective clinical diagnostic procedures. Nevertheless, breath analysis is a very young field of research and has been facing challenges since 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 last decade, and classify them with respect to their "fat-to-blood" and "blood-to-air" partition coefficients. These partition coefficients provide estimation on the relative concentrations in alveolar breath, blood and the fat compartment of the human body. 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 basic science results as well as the mechanistic understanding to tools (sensors) that shall serve as point-of-care diagnostics of cancer. We end this review with conclusion and future perspective.

Keywords: Breath; cancer; volatile organic compound; partition coefficient; biochemical; pathophysiology; sensor.

1. Introduction

Cancer is a leading cause of mortality with more than 7.5 million deaths worldwide and more than 12 million new cases every year, according to WHO statistics for 2008.[1] While the lung cancer burden, 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.[1, 2] Approximately 30% of 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.[1] In few instances, the cause for cancer is hereditary.[1] 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 the less-developed countries.[1, 2]

1.1 Available Approaches for Cancer Diagnosis

Prognosis evaluation of cancer involves disease confirmation and disease staging.[3] Depending on the cancer type a variety of techniques for the diagnosis and staging are applied in clinical practice including: blood tests, X-Ray,[4] mammography,[5] colonoscopy,[6] computed tomography (CT),[7] magnetic resonance imaging (MRI),[8] positron emission tomography (PET),[9] and ultrasonography[10]. Although one or a combination of these techniques can show, to some or 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.[11] 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.[12] Furthermore, there is a possibility to miss small lesions, because the diseased areas may be patchy.[13] In few instances, such as in the lower stages of gastric mucosal atrophy,[13, 14] 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.[10, 12, 15]

Currently, there is a trend towards personalized medicine in cancer care, based on the molecular specification of the cancer cells, to optimize clinical response and to minimize toxicity.[3, 16-18] This trend towards personalized medicine drives the search for molecular cancer biomarkers that could complement the conventional diagnostic methods and improve their diagnostic yield.[16-30] Gene expression profiling and protein profiling are currently gaining importance for more accurate prediction of an individual patient's treatment response.[16-25] Microarray techniques profile gene expressions in cancer cells that have been associated with tumor heterogeneity and treatment outcome, and provide a global picture of cellular functioning. Protein profiling provides important additional information to the treating physician, as most targeted therapeutic agents are designed to inhibit the activity of proteins.[26-30] Even though 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, are often costly, time consuming, and not available in many medical facilities as described.[31-37]

1.2 Volatile Organic Compounds for Cancer Diagnosis

An evolving approach in cancer diagnostics is based on volatile organic compounds (VOCs), *viz.* organic compounds that have a high vapor pressure at ordinary room-temperature conditions that originate from the cell or disease location and enter the surrounding environment.[38] Cancer VOCs can be identified: **(i)** from the headspace of cancer cells lines (*i.e.*, the blend of VOCs confined above the cells in a sealed flask);[39-50] **(ii)** through the urine;[51] **(iii)** through the skin;[52, 53] **(iv)** through the blood;[54, 55] and/or **(v)** through the exhaled breath[14, 39-48, 50, 54, 56-80].

Generally, cancer-related samples contain thousands of VOCs that appear mostly at low concentrations. A major part of the VOC spectrum varies amongst different individuals while the rest of the VOCs could be found in all body fluids of a given population. Apart from rare cases, in which a specific VOC is uniquely linked with a disease state, disease-related VOCs are present in most body fluids, yet at distinct concentrations.[3] For example, a typical population of breath samples might contain around 3,000 different VOCs in total.[81] However, the number of common VOCs found in the breath of a specific population that share common health condition ranges from only a few to tens of VOCs.[82, 83,3]

The use of VOCs as a basis for a simple non-invasive diagnostic method has been supported by extensive empirical data.[3, 38, 84-87] Due to the fast advances in the methods for breath collection and gas-analysis, cancer-related VOCs monitoring may become a complementary approach for conventional clinical diagnostics.[49, 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 in references.[3, 38, 56, 57, 79, 86-90] Nevertheless, the pathophysiology underlying the alteration of the cancer VOCs has been vague to a large extent. In this review we shed a light on the pathophysiology

causing the metabolic changes of the VOCs levels and compositions in cancer. Towards this end, we have narrowed the wide spectrum of reported VOCs (*ca.* 3000, for most of which their significance is unknown)[91] to some hundred candidates for cancer-related VOCs. We have then used specific VOCs and combinations thereof to discuss important issues related to their possible biochemical origin and underlying pathophysiology causing (section 2) – a subject that has so far been insufficiently targeted.[3] 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 shall serve as point-of-care diagnostics of cancer (section 3). We end this review with conclusion and future perspective (section 4).

2. Assessing the Origin of Cancer VOCs

2.1 Why and How VOCs are emitted from Cancer Cell?

In normal and abnormal processes in the body, metabolite changes occur all the time. It has been shown, for example, that different liver enzymes affect the construction of cell membrane.[92, 93] In metabolic illnesses, such abnormal processes can alter the body's chemistry by either changing VOCs' concentrations or in cases even produce 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).[94] 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.[3, 71] 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* Figure 1).[3] In addition, the oxidation of organic chemicals that is catalyzed by cytochrome p-450 enzymes can be up-regulated by ROS molecules in human tissue.[95, 96] The latter enzyme family has been shown to be over expressed in human breast cancer tissue, namely aromatase which synthetizes estrogens.[97]

A complementary pathophysiologic model suggested that during the early stages of cancer development, some of the normal cells proliferating at prompt rates reach the oxygen diffusion boundary and become hypoxic (less than 0.1% oxygen in the gaseous phase).[98] Because of the increased demand for energy and macromolecular biosynthesis these cells prefer the use of glycolysis over oxidative phosphorylation (Warburg effect). This is associated with high rate of glycolysis and lactic acid fermentation,[99-102] thus allowing cell survival in the hypoxic micro-environment.[103, 104] The excessive lactate production causes the tissue to become acidic and eventually causes the breakage of the basement membrane. Moreover, the acidic surroundings defends the tumor from the immune system.[105] Tumor growth generally goes along with gene changes and/or protein changes.[106, 107] Individual alleles expression can create a unique VOC profile that is further secreted in body fluids.[108]

Although most models relate to VOCs which are produced endogenously, exogenous VOCs detected in breath are of great interest as well, mainly because they relate to carcinogens exposure of an individual. 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 age-dependent diseases as cancer.[109] 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.[110]

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 be to compare VOC profiles from the different sources (organs or body fluids) along this root in the same cancer patient and/or the same animal model. Within this approach, the simplest starting point would be a comparison between the VOC profiles in the headspace of cancer tumor tissue or cancer cells, in (headspace of) blood samples, and in breath samples. Due to pre-mature technical/experimental methods, no practical results have been achieved with such an approach. Therefore, and given the unmet need to gain an understanding on the biochemical pathway of the cancer–related VOCs, we have simulated such an experiment *via* thermodynamics approach. In our simulation, we have targeted the diffusion of cancer VOCs as well as the e*quilibrium concentration* of a given compound between "breath-blood-fat", through estimation of the respective thermodynamic partition coefficients (*see* Figure 2):

- **Partition coefficient between fat and blood ($\lambda_{f:b}$):** this coefficient is designed to estimate the equilibrium concentrations of VOCs in fat tissue and (lipophilic) cell membranes in 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.

To implement this approach, we have listed the 115 VOCs that were reported in the literature as cancer biomarkers during the past 10 years. The full list of 115 VOCs was divided into the following compound families: Hydrocarbons, aromatic, alcohols, ketones, aldehydes, acids, esters, ethers, heterocyclic compounds, nitriles, sulfides, terpenes and other. Table 1 lists the experimentally determined 115 cancer VOCs published during the past decade, together with the $\lambda_{b:a}$ and $\lambda_{f:b}$. Based on these partition coefficients, the equilibrium concentrations of VOCs in blood and fat can be estimated based on the concentration in alveolar breath (*see* Figure 3 and section 2.3).

Before proceeding further, we have to notify on evidence of lack of normalization and standardization – something that is expressed in significant variations in the VOC profiles and/or concentrations between the different studies reported in the literature. These inconsistencies can be attributed to:

(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) Variety of technological equipment used for disease-related VOCs (*e.g.*, GC-MS,[64, 72] PTR-MS,[45, 80] *etc*.). Even though in the case of GC-MS qualitative analysis by retention time and spectral library match is quite reliable, still, VOCs identification by GC-MS or PTR-MS is not 100% sure.[41, 42, 44]

(c) Different sampling procedures, *e.g.*: collection of mixed expiratory breath,[80] CO_2-controlled sampling of end-tidal breath,[64, 111, 112] sampling

with Tedlar or Mylar bags,[80, 113] portable breath collection apparatus (BCA), [72] *etc.*

(d) Different pre-concentration procedures, such as solid phase micro-extraction (SPME) fibers[57, 58], thermal desorption units with cryo-focusing[76], *etc*.

(e) Different normalization procedures. Data normalization is performed according to a specific VOC's concentration in the examined sample,[58, 80] or based on the difference in the examined sample and the inhaled air VOCs concentrations.[71, 72, 76] In some cases even non-normalized data are processed.

(f) Variation in data analysis procedure. For example, in the analysis of the GC-MS: **(i)** peak identification, and integration in the chromatograms of each sample. **(ii)** Quantitative analysis between different chromatograms based on the area under peak. This is done by using internal and/or external standards and calibration curves procedures. [41-44]. **(iii)** Comparison among the quantitative data from different study groups. [70] [80, 114] **(iv)** Statistical analysis of the derived data using regression and supervised or non-supervised pattern recognition algorithms, cluster analysis of VOC patterns.

(g) Reliability of applied calibration standards. In case of numerous VOCs it is not possible to obtain certified standard mixtures, or their price is extremely high. Moreover, the stability of such reference mixtures is limited. Consequently, necessary standards have to be produced on the spot using more or less reliable methods. Currently, there is no inter-

comparison of measurements between groups involved in breath cancer studies.

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

2.3 VOC Exchange into the Breath

The principle behind VOC analysis in general and breath cancer detection, in particular, is that cancer-related VOC changes in the (fat) tissue is emitted to the blood and that the VOC blood chemistry is reflected in measurable changes in the breath through exchange *via* the lungs.[115] It was found that some gases exchange in the airways, rather than 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/mol/L_a]), exchange almost exclusively in the alveoli, while well blood-soluble volatiles, *e.g.* polar VOCs ($\lambda_{b:a} > 100$), tend to exchange also in the airways.[116-119] 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.[116] An important conclusion of these studies is that the airways play a more significant role in pulmonary gas exchange than previously assumed.[118, 119] Hence, the implications of pulmonary tests and breath tests might have to be re-evaluated.[116] 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 $\lambda_{b:a}$.[120] 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.[120, 121]

Approximately 50% of the published breath-related studies still present qualitative data on potential VOC breath bio-markers for a variety of diseases, but no quantification of their concentration levels. We expect this to change in the near future. VOC concentrations in exhaled breath are now not more difficult to measure than the respective concentrations in blood. In addition, breath can be sampled continuously and measured in *real-time*.[122, 123] If the respective VOC is systemic, the blood concentration may be estimated using the blood-air partition coefficients $\lambda_{b:a}$, T[3, 65, 116, 124-127] If experimentally determined $\lambda_{b:a}$ are not available, their values can be estimated based either on 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).[128-132]

We illustrate the blood-breath concentration relations using the examples of isoprene and acetone as an example. Isoprene is more volatile and less soluble in blood, compared to acetone. This expressed in that the $\lambda_{b:a}$ value for isoprene (~0.95[126]) is smaller than acetone (~340[133]). 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, resulting in low expression of the high BP VOCs in breath, which in turn are then not "picked up" by analysis.[134]

In addition to the blood-breath partition coefficient $\lambda_{b:a}$, the partition coefficient $\lambda_{f:b}$ between fat and blood 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 ~1ppb) can be associated with a relatively high concentration in the fat compartment.

For many compounds, $\lambda_{b:a}$ and $\lambda_{f:b}$ are unknown.[126, 127] They may, however, be estimated based on water:air partition coefficient ($\lambda_{w:a}$) and octanol:water partition coefficient ($\lambda_{o:w}$) using the method by Poulin & Krishnan.[135] To get an overview over the concentration distribution within the body of the ~115 cancer VOCs, which have been published during the last decade,[3, 14, 49, 55, 62, 67, 70, 71, 73-75, 77, 114, 136-138] we have summarized the physicochemical information about these compounds in Table 1. If the $\lambda_{b:a}$ were not available from the literature, we estimated them by different methods. For alkanes, methylated alkanes and 1-alkenes, we used data from reference[127] to estimate $\lambda_{b:a}$ by regression based on the number of carbon atoms, the boiling points and the molecular weights. For other compounds, we used the estimate by Poulin & Krishnan[135] given by the formula:

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

Here a≈0.0033 is the fraction of neutral lipids in blood, b≈0.0024 the fraction of phospholipids in blood, and c≈0.82 the fraction of water in blood. The $\lambda_{o:w}$ have been taken from Scifinder (https://scifinder.cas.org). The $\lambda_{w:a}$ (Henry constants) at 25°C have either been taken from the compilation of Sander[139], estimated by the EPI Suite™ software developed at the US environmental protection agency (EPA, http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm) or 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 used the derivative $d\ln(\lambda_{w:a})/d(1/T)$ as given in the compilation by Sander[139], 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),[140] for compounds whose data on temperature-dependence of the Henry constant are not accessible in the literature[140]. The fat-blood partition coefficients $\lambda_{f:b}$ were computed from the $\lambda_{b:a}$ and the fat-air partition coefficient $\lambda_{f:a}$. by $\lambda_{f:b} = \lambda_{f:a}/\lambda_{b:a}$. If the $\lambda_{f:a}$ was not available from the literature, we used the estimate by Poulin & Krishnan[135] given by the equation:

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

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

Figure 3 illustrates that different volatile compounds with the same concentration in exhaled breath may show very different concentrations in fat and blood (up to a factor of 10^8). In Figure 3, the respective estimated concentrations in blood and fat are shown under the condition, that the **concentration in breath is 1 ppb**. Different VOCs, therefore, carry different information on the various compartments of the human body. In particular, the storage capacity of the human body is quite different for different volatile compounds. Also the time necessary to deplete stores for a certain compound is very different.

Relatively detailed information is available for isoprene, the hydrocarbon which displays the highest concentration in exhaled breath. The isoprene stores in the body can be depleted by exertion of an effort, *e.g.*, on a stationary bicycle.[117, 122] After

about 45 min of cycling, a large part of the stored isoprene is exhaled and it takes about 1-2h to re-synthesize isoprene in the body and fill up the stores. 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 biomarker's $\lambda_{b:a}$ according to the specific related cancer, some connections are revealed (Figure 4). Interestingly data shows that lung cancer, gastric cancer and liver cancer have rather similar values as can be seen from the median line, while breast cancer and head and neck cancer are similar and finally colon cancer which is different from the rest (*see* Figure 4). While no obvious reason currently explains this difference, we can hypothesis that metabolic processes as cancer process and compound storage in tissue might be similar within these types of cancer. In part 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 compounds presented 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(1H)-quinazolinone – was suggested as a Cholecystokinin (CCK) antagonist[141] thus might be a result of anti-anxiety medication; 1-phenyl-ethanone (Acetophenon) – can be found in fragrances, in 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 was found previously in human body fluids and feces;[142] 2,2-dimethyl-propanoic acid – this is an odiferous compound yet it is solid in room temperature but liquid in body temperature thus it's source in breath is not clear. 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 the other biomarkers of the disease.

2.4 The Biochemical Pathway of Cancer VOCs

The cancer marker VOCs reported in the literature can be classified into a number of chemical families: Hydrocarbons (alkanes, branched-chain alkanes and branched-chain alkenes); Primary and secondary alcohols; Aldehydes and branched aldehydes; Ketones; Esters; Nitriles; Aromatic compounds.

2.4.1. Hydrocarbons

The key mechanism which relates to hydrocarbons 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 antioxidants[3]. Saturated hydrocarbons such as ethane and pentane are the end products of lipid peroxidation. Pentane and ethane in the breath has been extensively used as a non-invasive *in-vivo* indicator of lipid peroxidation.[143] 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.[144, 145].

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 hydrocarbons 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.[3] Possibly, enzymes such as alcohol dehydrogenase (ADH) and cytochrome p450 (CYP2E1, which predominantly works in the liver) are responsible for the alcohols metabolism in the body. ADH can catalyse the oxidation of several different alcohols in humans, remaining cancer VOCs are removed through the excretion of alcohol in breath, urine, sweat, feces, breast milk and saliva [3].

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.[146, 147] 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.[148] The third source for the aldehydes in the body relates to smoking. Saturated and unsaturated aldehydes as formaldehyde, acetaldehyde, and acrolein, were found in tobacco smoke.[149] 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.[150, 151] Finally, aldehydes can also originate from dietary sources. [152, 153]

2.4.4. Ketones

During cancer progression an increase in the rate of fatty acid oxidation due to changes in metabolic conditions result with the formation of ketone bodies including

acetone, such compounds are also related to weight loss, that in turn is one of the symptoms of cancer.[154] Acetoacetate and β-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 and skin. Protein metabolism can result as well with ketone bodies. In the state of cachexia, typical in diseased conditions as cancer, protein metabolism increases resulting with higher levels of ketone bodies.[154] However, acetone is not suitable to be a cancer biomarker as its concentration levels in the breath are altered due to exercising, fasting and/or food consumption [155, 156] . Finally, other exogenous sources like food or chemical industries can result with ketones production that could eventually be absorbed in the body [152 3].

2.4.5. Esters

This group of compounds can be found in natural fats and fatty oils, natural waxes and fruit essential oils in large amounts. In humans, esterase hydrolyzes esters into alcohol and acid at temperatures below 40°C.[157]. One example of such 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 source. 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 patients follow-up since some are known to be carcinogens.[3] These molecules are highly reactive, resulting with peroxidative

damage to PUFA, proteins, and DNA. Such damage accumulates during life, while the natural fixing mechanisms in the body becomes less efficient, thus assumed to lead to age-dependent diseases as cancer.[109] 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 with a more soluble and excretable form of molecule.[3, 158] One such compound is acetonitrile which is found in smokers. The pathway suggested for acetonitrile is the bio-transformation to cyanohydrine by cytochrome P450 monooxygenase, which in turns spontaneously breaks down to hydrogen cyanide and formaldehyde. Because of the rather slow metabolism of acetonitrile in the body, substantial acetonitrile amounts can be emitted as-is through exhaled breath and/or urine.[3, 159]

2.5 Challenges and Future Directions for Better Understanding of Cancer VOC Biochemical Pathways

Open questions to be addressed are also the delineation of the metabolic pathways leading to the generation of potential biomarkers. With this in mind, we raise the following important issues in relation with cancer VOCs. We present ideas to investigate these issues with the aim to gain a better understanding of the mechanisms of VOC production/consumption in the body. **First issue:** Many metabolic pathways, such as glycolysis, apoptosis, loss of tumor suppressor genes, angiogenesis are activated or over-activated in the case of cancer.[160] These pathways may alter the production of VOCs in the body. In order 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 metalloproteases) 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. **Second issue:** 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 human's or animal's body, needs to be confirmed through direct observation. This issue could be resolved by using cell lines from well-documented sources,[41-44] 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 diversity of cancer while eliminating potential confounding effects that are associated with clinical samples. **Third issue:** Many cancer VOCs are related to environmental and tobacco compounds. Following inhalation, these molecules might affect the respiratory system, and later on also the blood composition. The lipophilic species will be stored in the fat compartment, with subsequent comparatively slow release through exhalation. Therefore it is important to examine the effect of inhaling these molecules on the blood and the fat compartment, as well as the breath VOC profile. Using an animal model, such compounds could be introduced either *via* inhalation, or they could be directly introduced into the blood stream, in order 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. Comparing between the animal model and the introduction of the same molecules *in-*

vitro to cancer cells would allow gaining a detailed understanding on how these VOCs affect the body both on a cellular level and as a whole. **Fourth issue:** 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 were found to have high levels of ALDH activity, yet there is a variance in ALDH activity between different cells. A focused study on CSC both *in-vitro* and *in-vivo*, might, therefore, reveal variances in the VOC patterns that are released as a response to different ALDH activity. This 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 for recurring tumor initiation, metastasis initiation, thus aiding the prediction of a patient's prognosis, and the tailoring of personalized treatments.

3. Sensors for Testing Cancer VOCs

Spectrometry and spectroscopy techniques are powerful tools for detecting VOCs and, thereafter, for 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, the high levels of expertise required to operate such instruments, the 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 specific cancer VOCs have to be translated to sensor development.

Important milestones have been reached in the field of breath testing for disease diagnostics. However, only few breath tests are currently being used in clinical practice. Examples include the ^{13}C-urea or ammonia breath tests for detecting *H.*

pylori infections and the nitric oxide test for detecting asthma.[59] This fact is primarily the result of the technological obstacles in trace amounts detection of definite breath biomarkers in a complex breath matrix. Although it remains unclear how much work needs to be done before comprehensive breath testing systems can be implemented as a major diagnostic tool in clinics, the technologies that offer potential solutions to these problems are expected to help close this gap.

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 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 serve as 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 vapor. Among the choice of transducers are: mechanical oscillators and surface acoustic wave devices that respond to changes in mass; chemicapacitors that detect changes in dielectric properties; and chemiresistors that monitor the resistance of conducting polymers or polymers laced with conductive particles. 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 article, 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 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.[38] Sensor selectivity is defined here as higher sensitivity to a given gas/vapor or class 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* Figure 5). In light of the difficulties to find unique cancer VOC(s), in the presence of controlled backgrounds and interferences, the development of selective sensors has been lagging. Additional limitation has stemmed from the need to synthesize separate, highly selective nanomaterials for each VOC to be detected [161]. Indeed, most 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, sensors array.[38] Bio-inspired, this approach performs detection through use of an array of broadly cross-reactive sensors in conjunction with pattern recognition methods.[38] In contrast to the selective sensing approach, each sensor in the cross-reactive array is broadly responsive to a variety of VOCs. In this architecture, each VOC produces a distinct fingerprint from the array of broadly cross-reactive sensors. This allows to considerably widen 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* Figure 5).[38, 162]

Although such sensors 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.[38, 163] Figure 6 illustrates the schematic representation of different sensors technologies used. We will overview here some of them in the context of detection of cancer VOCs.

3.2.1. Nanomaterial-based sensors

Distinct attention has been given to approaches incorporating nanomaterial based VOC/gas sensors (NMVSs) in the past few years as they can lead towards the development of sensitive, fast responsive, however relatively inexpensive detection systems.[89] These advantages are the result of the used nanomaterials' nano-scale dimensions that provides them with superior physical, chemical, and optical

properties, together with 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 of detection accuracy. However, 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* Figure 7). Nanoparticles, nanowires and carbon nanotubes are examples for nanomaterials that have been exploited for VOC sensing. Their nano-scale properties give them more than a few qualities, such as unique chemical, optical, and electrical properties together with high surface-to-volume ratio. The latter offers high sensitivity and low response and recovery times.[89]

Nanomaterials are used as sensitive transduction elements combined with different molecular-sized organic functionalizing chemicals that are used as recognition elements (*see* Figures 8a and c).[164] Examples of nanomaterials based transducers include field effect transistors (FETs) based on single-walled carbon nanotubes (CNTs)[165, 166] (*see* Figure 8c) or nano-wires (NWs) of various materials (*see* Figure 8a),[167-170] nano-electromechanical oscillators,[171-174] nano-porous chemioptical materials,[175, 176] coaxial-chemicapacitors based on CNTs coated by nano-porous alumina[177] and chemiresistors based on monolayer capped metal nanoparticle (MCNPs) films,[79, 178, 179] porous metal-oxide nanostructures,[180] and random networks of single-walled CNTs[167, 181] or silicon NWs.[182]

The most common nanomaterial-based sensors are based usually on conductive inorganic nanomaterials (*e.g.*, metal nanoparticle, single wall carbon nanotube, carbon black) that are capped with or in organic functionality.[38, 68, 69, 163, 181] In these films the inorganic nanomaterials provide the electric conductivity and the organic film component provides sites for the sorption of VOCs.[89, 183] Upon 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.[38, 89] As a consequence, the connection between the inorganic nanomaterial blocks becomes lower/higher, and the conductivity decreases/increases.[38, 89] In few instances, exposure of the nanomaterial film to VOCs cause a 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.[38, 68, 178] 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 will respond to particular fingerprint of VOCs in a different way. Consequently, a pattern of resistance changes is obtained from the sensor array to a given vapor.[184]

Clinical studies on breath samples with cross-reactive array of MCNP have shown the capability to distinguish lung cancer breath samples from healthy controls.[67, 68, 185] A similar MCNP-based sensor array was able to discriminate also among lung cancer, colon cancer, breast cancer, prostate cancer, and head and neck cancer, in the presence of confounding factors.[62, 67] Three additional clinical studies studied patients with suspected lung cancer that had pulmonary nodules detected by CT screening and underwent surgery.[137, 184, 185] In the first study, a cross-reactive MCNP and molecule-terminated single-walled carbon nanotubes (SWCNTs) 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 with 86-90% accuracy, between early-stage and advanced-stage lung cancer.[185] Similar results were achieved on cancer cell lines in an in vitro study.[39, 40] A second 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 [184]. The sensors array detected lung cancer with 71.4% sensitivity and 91.9% specificity; positive and negative predictive values were 66.6% and 93.4%, respectively.[184] The third study included early-stage lung cancer (stages Ia, Ib and IIa) before and 3 weeks after tumor resection.[137] 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 , nor amongst lung cancer and benign states conditions post-surgery [137]. These results point to the use of such MCNP-based chemiresistors array for short-term follow-up after lung cancer resection [137]. 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 done with an array of carbon black/polymer sensors enabling the discrimination of malignant mesothelioma from all other groups with 88% accuracy,[186] and 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.[187] Haick, Hu and coworkers using an array of MCNP and SWCNT sensors showed an excellent ability to differentiate amongst: (i) gastric cancer and benign gastric conditions, (90% accuracy); (ii) early stage gastric cancer (I-II) and late stage (III-IV) (92% accuracy); and between (iii) ulcer and less severe, (86% accuracy).[14] The common effect between gastric disorders and respiratory disorders was recently studied using an array of polymers and carbon black chemiresistors.[188] Study results presented an ability to differentiate between breath prints of obstructive lung disease patients

without Gastro-oesophageal reflux disease (GORD) from obstructive lung disease patients with GORD (with 67.6% accuracy), 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.[188] However, a larger prospective interventional study is needed as the described results were influenced by few different confounders. [38, 188]

3.2.2. Colorimetric Sensors

Colorimetric sensors are composed of a diverse range of chemically responsive dyes, whose colors depend on their chemical environment.[189, 190] Since the measurable responses of the sensors are the color changes in each of the dyes, a colorimetric sensor array can easily be red out with the naked eye.[189, 190] Alternatively, auxiliary equipment such as a spectrometer can be used. 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 sensor arrays have been applied successfully to LC breath testing, using different classes of chemically responsive dyes.[191] These were dyes containing metal ions (*e.g.* metaloporphyrins) 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 was in the low ppmv range for many relevant VOCs. However, it was 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).[191] Results showed that better accuracies were 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.[38]

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.[192, 193] 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.[194-196] In a QCM, the acoustic wave propagates through the bulk of the crystal in a direction perpendicular to the surface, with motion at the surface parallel to the surface.[194-196] QCMs with chemoactive coatings of their membranes have been widely used in gas and vapor sensing: Adsorption and desorption of the breath VOCs from the coated membrane causes changes in its mass, which, in turn, gives rise to shifts in the resonator's frequency. However, 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 breath testing, in order to minimize their effect during the exposure to the samples. Commercial QCM sensor systems are available out on the market. Most of them are indeed designated for moisture and inorganic gas detection; for example, the Model 3050 Moisture Analyzer from

Ametek is used for moisture trace detection.

Lung cancer VOCs has been successfully demonstrated in a small-scale pilot study, using QCM sensor arrays with metal-loporphyrin coatings.[187, 188] 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.[38, 196]

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.[197] 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 causes changes in its mass, results 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.[197] 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 for breath analysis. The first sensor was coated using a poly(isobutylene) film and the other was used as reference.[198] The study outline included few steps: pre-

concentration of the breath samples with a solid-phase microextraction (SPME) fiber followed by their injection into a gas chromatography capillary column. Then 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 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.[38, 198]

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.[134] This requires deep knowledge on the breath composition and the 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 in parts per billion levels.[89, 134] 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 opposed to non-diseased subjects. Therefore VOCs that can indicate a clinical state generally display distinct levels and conctrations that associate with the disease. The number of shared VOCs potentially indicative of a definite clinical state, ranges from only a few to tens of VOCs.[3, 199] Thus, constructing suitable sensors for the detection of a certain disease is challenging and should take into account few aspects: **(i)** the sensor's detection range based on the predicted VOC concentrations in breath; **(ii)** increasing

specificity to desired VOCs while reducing sensitivity to background noise;[86] **(iii)** knowledge of the chemical identity of the target VOCs and their breath concentrations.

With this in mind, if initial VOC profiling for a given sickness reveals that a few specific marker VOCs are expected to appear at elevated concentrations (up to a few ppmv *e.g.*, methanol, acetone, and methane),[58, 124, 200] in breath, then a sensing platforms of semi-selective or highly selective sensors based on specific recognition will be suitable (*see* Figure 9). Though, 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* Figure 9). Sensor arrays based on chemiresistive layers of MCNPs or RN-CNTs are very attractive for such uses.

On the other hand, high boiling point VOCs should be found in breath at low concentrations of single ppbv (for example, propofol)[65] and even lower, especially the water soluble compounds (for example, indole[201]), due to a high $\lambda_{b:a}$. In order to enable sufficient LODs for such compounds their detection requires highly sensitive nanomaterial transducers, such as nano-wire or nano-tube based FETs as well as, specific recognition features. If not, background VOCs "noise" from nonspecific interactions would probably affect the signals of the target VOCs which can eventually result with false positive detection (*see* Figure 9).[164]

When focusing efforts on fine tuning an applied sensing technology for a specific clinical state, rough estimates are inadequate and an accurate picture of the indicative VOC print should be obtained. Therefore, analytical evaluations of the variances among the characteristic VOCs have to be performed in order to distinguish breath composition patterns of non-diseased people against people suffering from a disease. The analytical valuations should be done using standardized techniques, as

gas-chromatography mass spectrometry (GC-MS) or proton transfer reaction mass spectrometry (PTR-MS).[58, 202] Because of numerous researches done worldwide a global breath VOC database, could help for enabling cross validation of the results (*see* Figure 9).

The physical and chemical characteristics of the target VOCs is very important for creating a suitable sensing platform to a given condition. Polarity is the main physical characteristic related to VOCs sensing, while polar VOCs are generally easier to identify by sensors.[79, 89] This easier detection is mainly because polar VOCs can be either directly detected through charge transfer between the sensing material and the molecule or indirectly through molecular such as in the case of sensors based on functionalized single Si-NW or SWCNT FETs.[168, 169] Additionally, highly specific recognition elements are more available for polar VOCs because they offer a wider range of possible molecular interactions. For non-polar VOCs, sensing mechanism rely on indirect recognition through dielectric changes and steric interactions[134]. Thus the size and shape of VOCs is a vital factor 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.[203] However, the current architecture of this approach is most likely limited for 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 shape MCNPs opposed to spherical shape MCNPs, that was shown to discriminate among VOCs based only on size.[178, 204] Buy applying this strategy sensors 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-CNTs chemiresistive films was shown to provide the sensors with a selective response to polar and non-polar VOCs in a changing humidity background.[181] 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.[134, 168, 169]

3.3.2. Overcoming Confounding Factors

In order to develop a detection system for real-world analysis it must be able to deal with the different confounding factors. Thus particularly breath analysis sensors for trace-amount VOC detection should cope with chemical or physical factors as the ambient temperature and humidity or the instability of breath samples and sensing elements.[199] From the very first step of the breath analysis process, sampling, storage and transport of the exhaled breath to and into the sensors apparatus can result with VOCs loss and/or involve considerable amounts of contaminants.[205] Such difficulties can be minimized by integrating proper sampling and preparation techniques with the sensor's delivery system. Currently, a common technique used for sample storage involves the use of a vessel such as collection bags, vials, or canisters. These solutions often introduce contaminations and causes VOC loss during storage.[206-208] An alternative promising 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).[41, 42, 49, 162, 209-214] the latter can be accomplished by thermal desorption tubes or by needle trap devices.[112, 215, 216] This technique allows the usage of a semi-selective sorbent material that can trap a range of VOCs (*see* Figure 10a).

By performing pre-concentration to the breath sample one can gain both a reduction of the sample volume (increased VOC concentration) and a decrease in its complexity. However, because different target VOCs are adsorbed/absorbed differently, a proper assessment should be performed on the choice of sorbent material.[162, 209] The use of solid phase extraction can provide a storing solution for breath samples up to a number of moths depending on the storage system. In addition it could allow integration of sensor systems with low volume delivery methods as microfluidics. In this respect, microfluidics – the science and technology implementing microscale fluidic channels to manipulate micro/nanolitre volumes – should be integrated with the TD system to optimize sample handling and delivery. Another important advantage of using sorbent material, especially those with low breakthrough volumes for water (*e.g.*, Tenax) is the ability to trap high moisture content samples as breath. The dehumidification of the sample, will improve the performance of the VOC sensor in most cases. By using a multi-capillary column (MCC) researches could effectively separate moisture from other breath components, by simply enabling higher chromatographic flow rates of up to 250ml/min[217] allowing isothermal separation of VOCs at ambient temperature (*see* Figure 10b).[218-220] Beside the various dehumidification techniques that might cause the loss of VOCs [221], other approaches such as enhancing recognition element surface coverage[222] and humidity calibration algorithms of the sensors can be applied to reduce the effects of humidity among samples (*see* Figure 10c).[199] However, if the sensors responses to VOCs and water molecules are not independent due to competitive binding this approach alone can be limited requiring new recognition elements that are selective to the VOCs and to water vapors in the matrix. [182, 203] Thus, practical sensing should always account for the VOC/humidity sensitivity ratios,[199] which should be tested at humidity levels

typical for the breath samples.

An alternative very promising method is *real-time* analysis of exhaled breath by direct mass-spectrometric methods, such as Proton-Transfer-Reaction Mass Spectrometry (PTR-MS) [117-119, 122, 123, 223-228], Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry (PTR-TOF-MS)[202, 229-235] or Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) [87, 236-240]. With these *real-time* techniques, exhaled breath is directly analyzed by mass spectrometry, without any need for sample storage or pre-concentration. This can even be done with breath-to-breath resolution. The mere possibility of real-time analysis (*e.g.*, when exerting an effort on a stationary bicycle or during sleep[123, 223]) is a decisive advantage in comparison to investigations of blood samples. It allows detecting very fast processes, such as a quick release of isoprene during physical effort[122, 224, 225, 227].

Another important aspect of breath analysis would be the working temperature. Breath samples as well as most sensors should be handled in a restricted range of operating temperatures.[206] 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 can lead to degradation of some compounds.[241] Conversely, at low temperatures water condensation will occur in the storage containers causing polar VOCs to dissolve in the condensed humidity. Therefore breath samples in containers should be warmed up to a temperature around ~40 °C before analysis to avoid condensation effects. 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 operates at high temperatures (for example, 260 °C[242]), especially in the case of easily oxidizing compounds.[79, 243] Thus, keeping a stable temperature throughout the

measurement process is important and can be reached by incorporating an on-chip embedded heating layer (*see* Figure 10d). Yet another aspect would be the exposure of sensors to continuous thermal cycles as a result of multiple breath samples exposures, which might enhance drift effects of the sensors. Such drift can be overcome by doing sensitivity calibrations[199] or by achieving stable sensing layers by inhibition of oxidation processes(*see* Figure 10e).[244] For stable sensor operation over time an alternative option could be a long aging process (*see* Figure 10f).[199]Future breath testing technologies is to be 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 presented data might not yet provide precise or definite answers to the puzzling pathophysiological pathways of cancer VOCs. However, it will help stimulate constructive discussions and new ideas. Furthermore, we have discussed the important milestones that have been reached and those that still need to be accomplished on the way towards detection of a wide range of diseases by breath testing. The outcome of the presented comparative study is based on cell biology, by means of one or combination of the following biochemical pathways: oxidative stress and cytochrome P450, liver enzymes, carbohydrates metabolism (glycolysis/ gluconeogenesis pathways), and/or lipid metabolism.

Although the biological mechanisms discussed above affect the concentration of the VOCs both in blood and breath, we presume that there is an enormous

advantage of breath sampling in comparison to blood sampling. Firstly, the blood and breath concentrations are related through the respective $\lambda_{b:a}$ of each compound, 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 blood[112] needs very careful preparation and processing of the sample to avoid degassing (and therefore the loss) of the compounds of interest and 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).[112] The last aspect concerns medical applications. Breath sampling is non-invasive and breath can be sampled as often as is desirable. Exhaled breath can even be sampled continuously during an ergometer challenge or during sleep,[123, 224] as opposed to blood, which cannot be sampled continuously.

In respect to the current and future technologies for VOC analysis in general and breath analysis in particular, comprehensive work has yet to be done. 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 communicational technologies are invented day by day and

are becoming an inseparable part of our life, integration of nanoscale medical technologies into this framework will be highly desirable and will 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 stressing constraints on the nanomaterial's and sensor's 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. Sensors can overcome this interference by utilizing different (inorganic) nanomaterial types and organic functionalities. The responses of the sensors towards 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 nanomaterials. For example, broadly responsive nanomaterials can be employed to allow a range of structurally similar molecules to bind, nanomaterial-made membranes may be used as size-selective sensors, and nanomaterials with highly-selective functional groups may 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 nanomaterial-based sensors combining all these recognition approaches naturally performs an integration 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 composite composition of the VOCs mixture of concern.

Improved breath testing systems should combine various technologies that are highly sensitive to cancer-related VOCs and barely (or not) sensitive for 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 micro-adsorption process followed by TD,[209] MEMS-based μ-preconcentrator,[245] MCCs,[217, 218, 220, 229, 246] and micro-column gas chromatography (MCGC).[247-249] The processed cancer-related VOCs will then delivered throught a microfluidic system to highly sensitive and selective on-chip sensors that are integrated with 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.

Acknowledgements

Hossam Haick gratefully acknowledges the funding from the FP7-Health Program under the LCAOS (grant agreement no. 258868) and the FP7's ERC grant under DIAG-CANCER (grant agreement no. 256639). Additionally, Hossam Haick acknowledge Gady Konvalina (Technion) for helpful discussions and the FP'7 Lung Cancer Artificial Olfactory System (LCAOS) partners for support. Anton Amann gratefully appreciates funding from the Austrian Federal Ministry for Transport, Innovation and Technology (BMVIT/BMWA, project 836308, KIRAS) and support from the Oncotyrol-project 2.1.1. The Competence Centre Oncotyrol is funded within the scope of the COMET - Competence Centers for Excellent Technologies through BMVIT, BMWFJ, through the province of Salzburg and the Tiroler Zukunftsstiftung/Standortagentur Tirol. The COMET Program is conducted by the Austrian Research Promotion Agency (FFG). Pawel Mochalski acknowledges support

from the Austrian Science Fund (FWF) under Grant No. P24736-B23. Veronika Ruzsanyi gratefully acknowledges a Lise-Meitner fellowship from the Austrian Science Fund (FWF) under Grant No. M1213-B18. A.A., P.M. and V.R. thank the government of Vorarlberg (Austria) for its generous support.

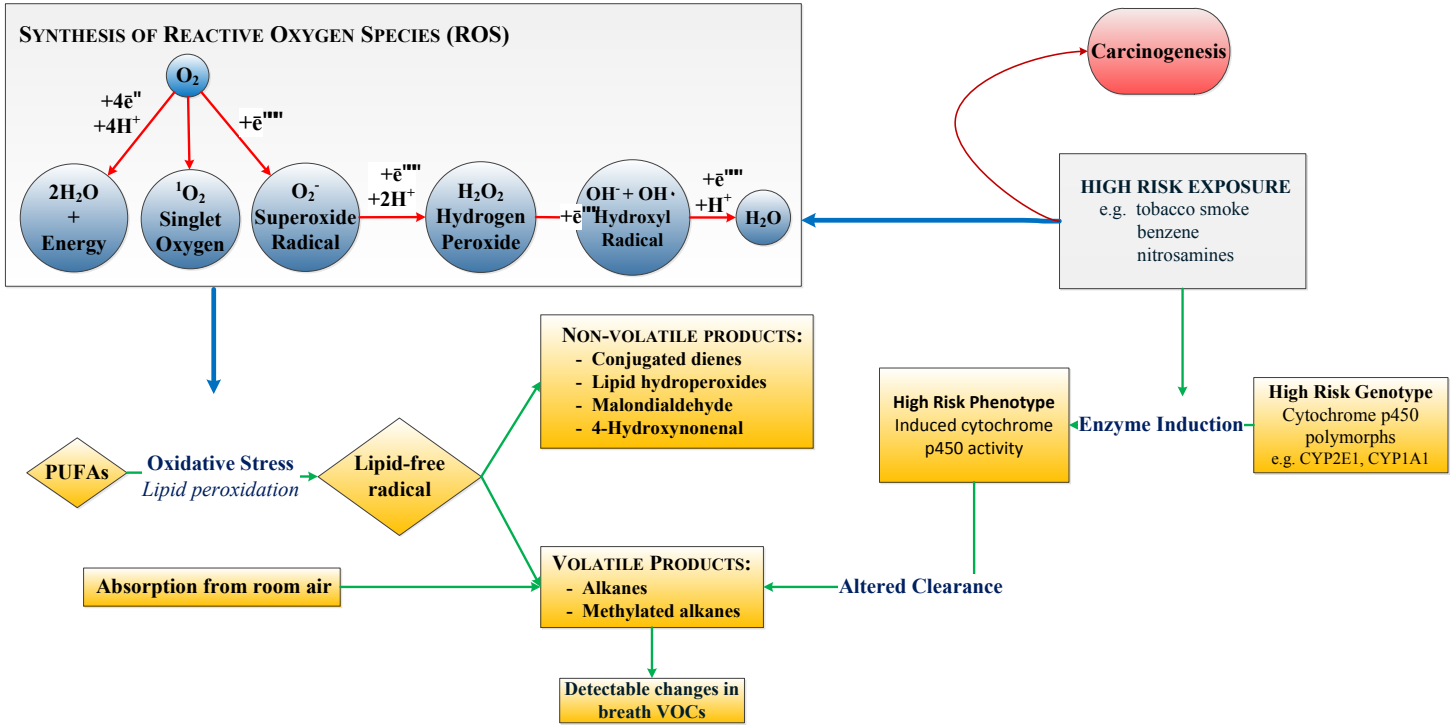

Figure 1: Hypothetical basis of the breath test for lung cancer: Lung cancer may result from the interaction of hereditary and environmental factors. Several cytochrome p450 mixed oxidases are activated by exposure to environmental toxins such as tobacco smoke. The induced phenotype may increase the 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. 3

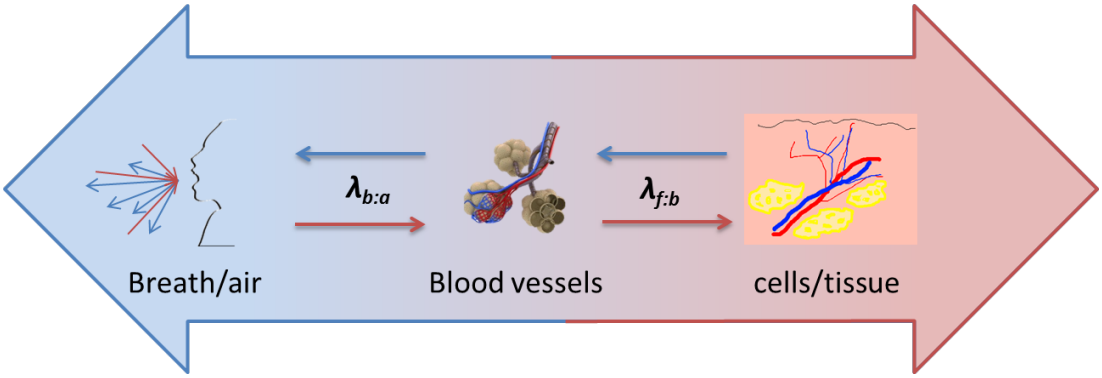

Figure 2: Simulation scheme of the main three thermodynamic parameters responsible for the diffusion of cancer VOCs between "breath-blood-fat": $\lambda_{f:b}$ - Partition coefficient between fat and blood: simulate the diffusion of VOC from the (cancer or healthy) tissue to the blood; and $\lambda_{b:a}$ - Partition coefficient between blood and air: simulate the diffusion of VOC from the blood to the exhaled air.

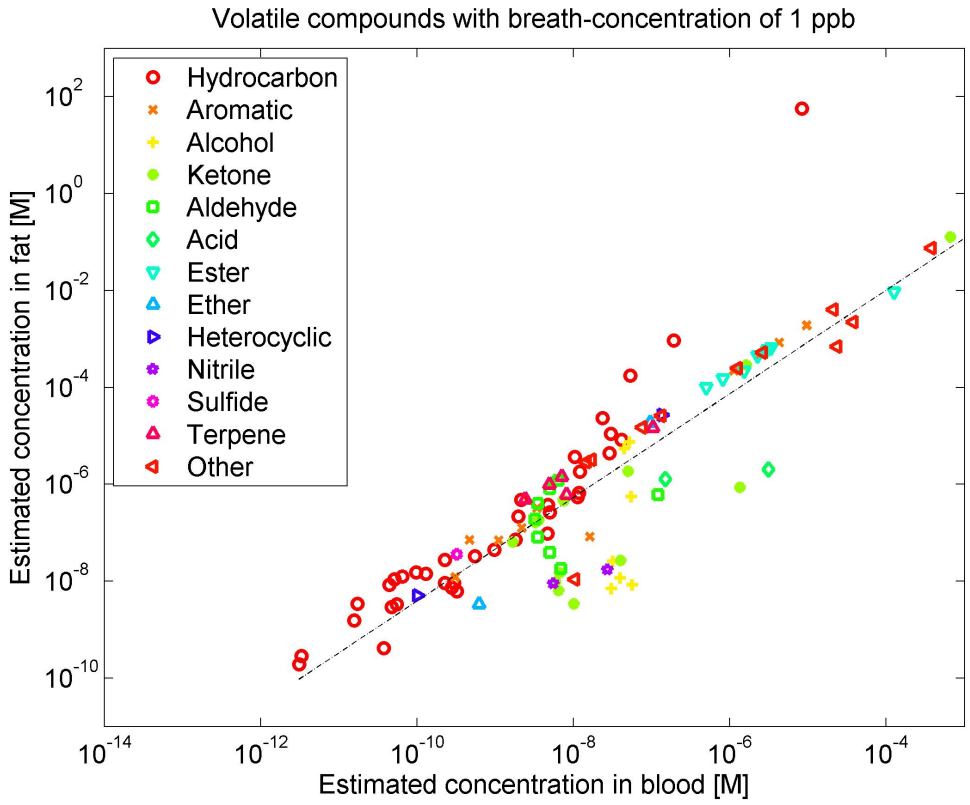

Figure 3: Estimated equilibrium concentrations in blood and fat for candidates of volatile cancer biomarkers published during the past decade. [3, 14, 49, 55, 62, 67, 70, 71, 73-75, 77, 114, 136-138] These equilibrium concentrations have been estimated under the assumption that *the concentration in 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 volatile compounds 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 volatile compounds therefore carry different information on the various compartments of the human body. In the figure, different chemical classes of compounds (such as hydrocarbons or sulfides) are indicated by different symbols and colors.

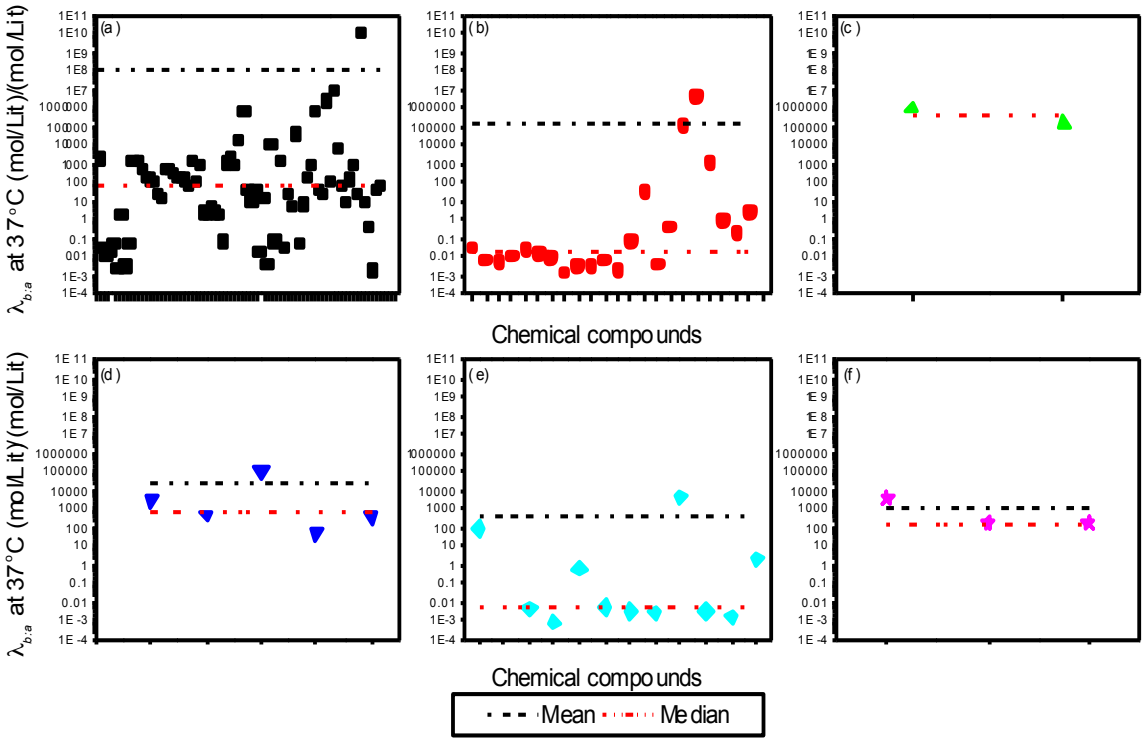

Figure 4. $\lambda_{b:a}$ as a function of the biomarkers from different types of cancer. **(a)** lung cancer; **(b)** breast cancer; **(c)** colon cancer; **(d)** liver cancer; **(e)** head and neck cancer; **(f)** gastric cancer. Data shows that lung cancer, gastric cancer and liver cancer have rather similar values as can be seen from the median line, while breast cancer and head and neck cancer are similar and finally colon cancer which is different from the rest, based on the physicochemical parameters from Table 1.

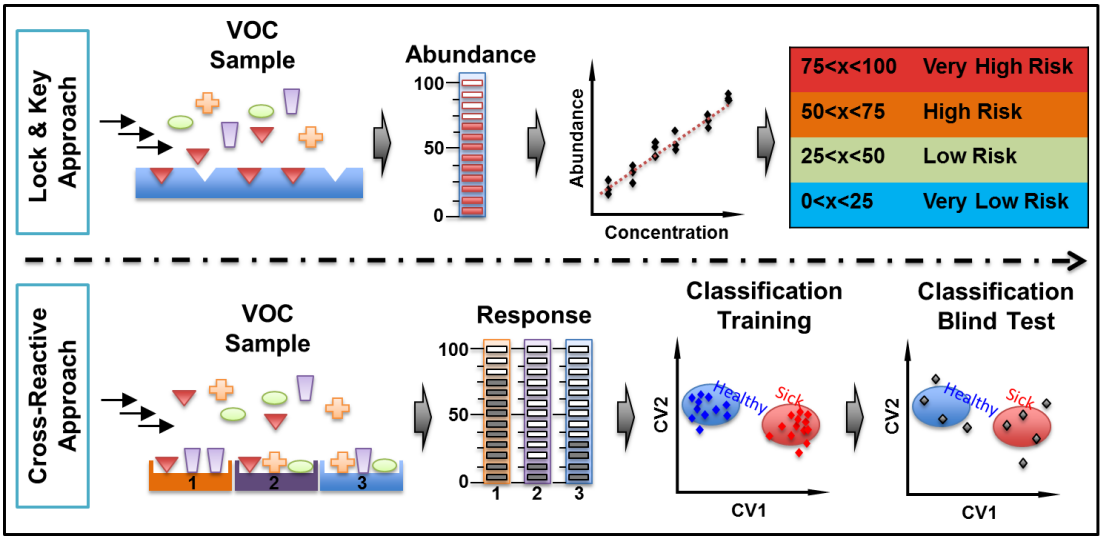

Figure 5: Schematic illustration of the selective sensing approach versus the cross-reactive sensing approach. Reconstructed from Ref. 38.

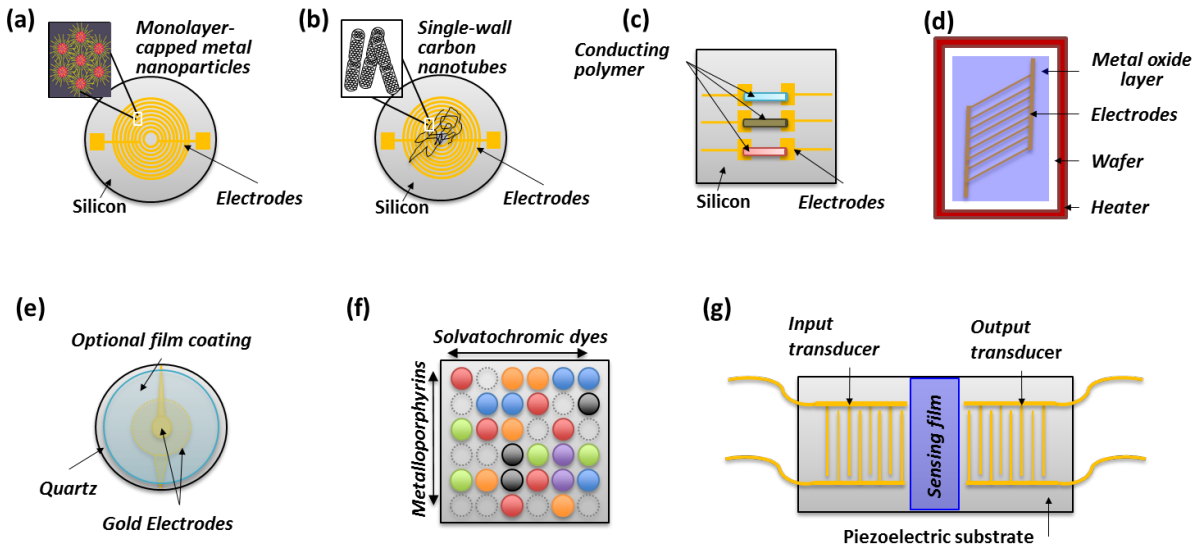

Figure 6: Schematic illustration of different nanomaterial-based sensors: **(a)** chemiresistor based on monolayer-capped metal nanoparticles; **(b)** chemiresistor based on single-wall carbon nanotubes; **(c)** chemiresistor based on conducting polymers; **(d)** chemiresistor or chemicapacitor based on metal-oxide film; **(e)** quartz microbalance (QMB) with selective coating; **(f)** colorimetric sensor; and **(g)** surface acoustic wave (SAW) sensor. Reconstructed from Ref. 38

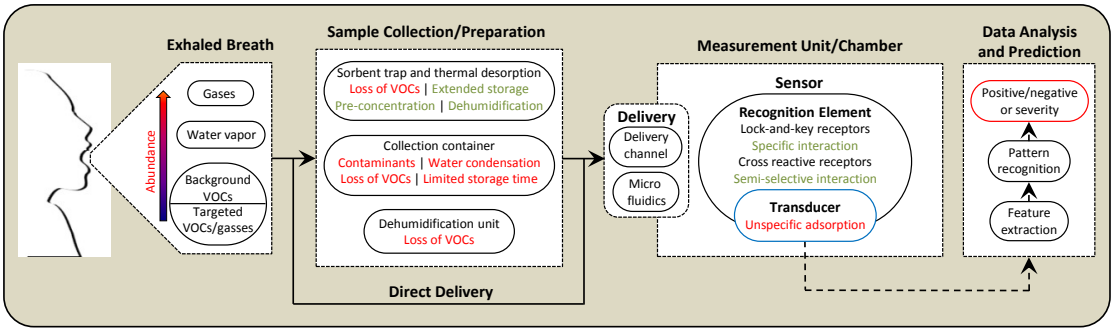

Figure 7. Overview of the processes involved in breath testing: Exhaled breath is a complex mixture of gases, water vapor, and thousands of VOCs in which only a small number of specific VOCs and gases comprise the clinically significant breath print. In order to perform the breath test, a sample is prepared 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. 134

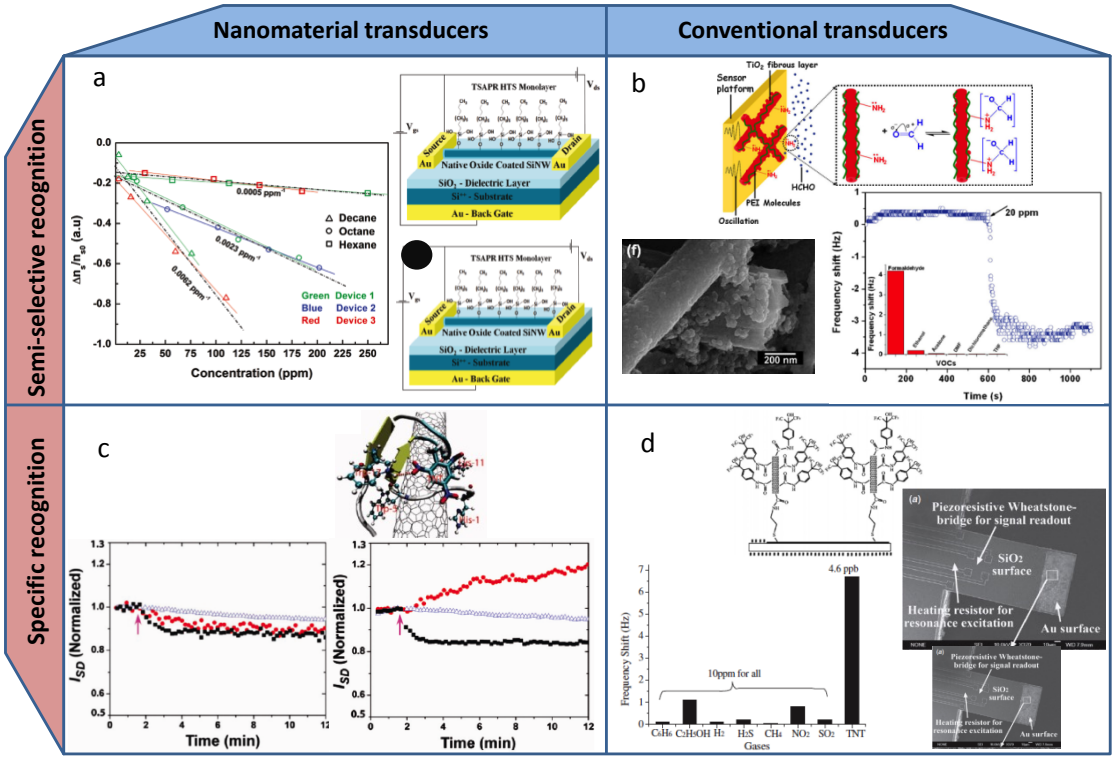

Figure 8. Different types of nanomaterial-based VOC sensors can be divided into sensors based on nanomaterial transducers (left column, a and c) or conventional transducers (right column, b and d), with the recognition elements being either semi-selective (upper row, a and b) or specific (lower row, 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.[169] **(b)** Top: schematic of a QCM oscillator coated with a sensing layer of polyethyleneimine functionalized TiO_2 (PEI-TiO_2) nano-porous fibers. Bottom left: SEM image of a representative PEI-TiO_2 nano-porous fiber. Right: responses of QCM-based PEI–TiO_2 sensors upon exposure to 20 ppm_v formaldehyde. Inset shows the frequency shift of the sensor versus 20 ppm_v 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 vapor of TNT (red circles), RDX (blue triangles), and HPT (black squares) showing the specific response to TNT. Arrow indicates when the vapor was introduced into the device.[165] **(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 a micro-cantilever sensor immobilized with multi-walled CNTs. Inset showing 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 ppm_v concentration) compared with to the response of 4.6 ppb_v TNT vapor and demonstrating the specific response to TNT.[251] Reconstructed from Ref.[134]

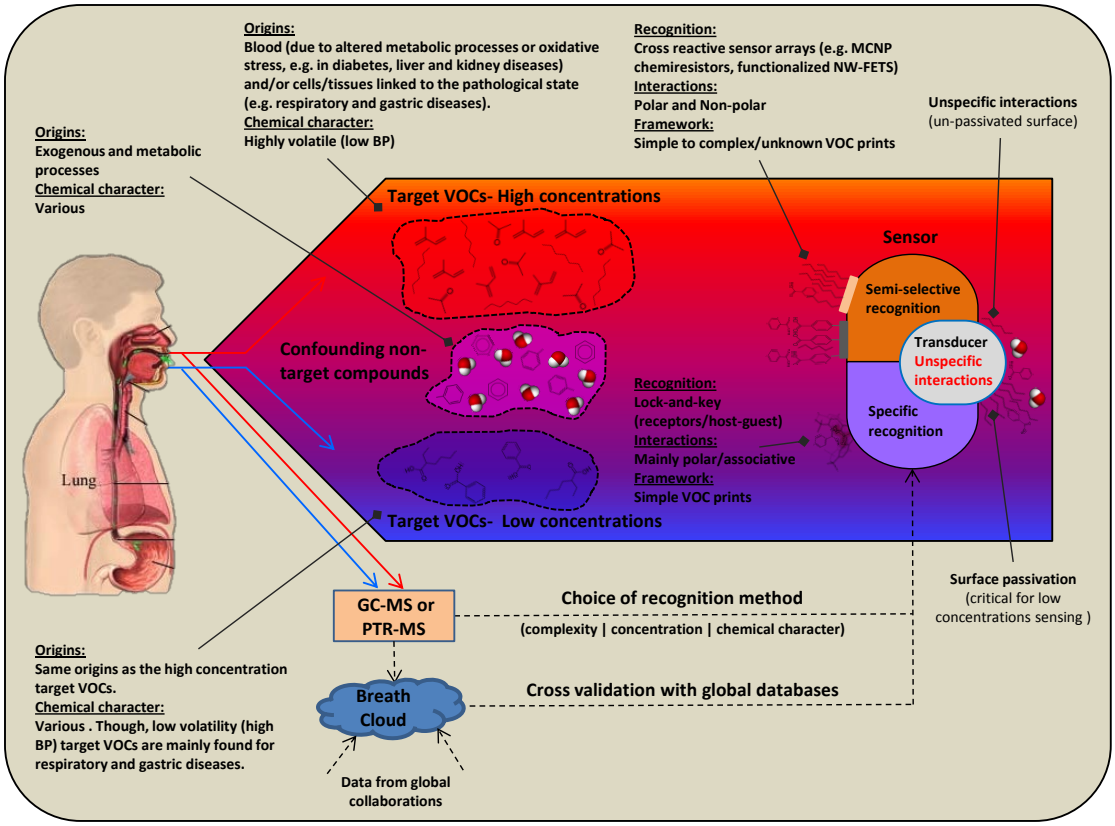

Figure 9: Illustration showing the two main sensing approaches (specific vs. cross-reactive approaches) and how they should be coupled to the different types of VOC prints originating from different types of clinical states. When the detection of a single or few target breath markers is required, maximal selectivity is required from the NMVSs, and therefore 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 un-volatile (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. 134

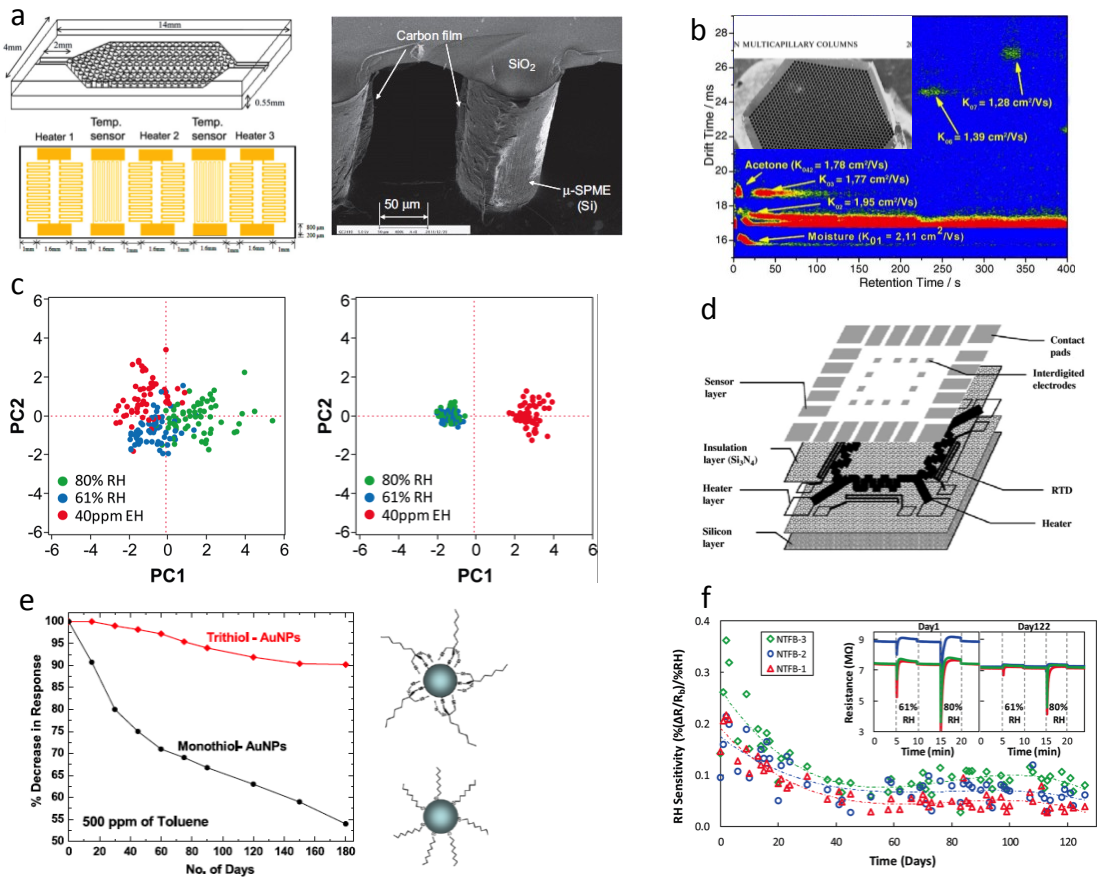

Figure 10: Means for tackling the implications of real-world confounding factors. **(a)** Top left: Schematic diagram of a μ-preconcentrator chip that utilizes an array of solid-phase microextraction (SPME) needles coated with an in-situ-grown carbon adsorbent film (as the sorbent material). Right: Cross-section 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.[209] **(b)** A topographic plot of an ion mobility spectrometer (IMS) coupled to a multi-capillary column (MCC) from the breath of a patient suffering lung infection. The plot shows on the bottom left hand side that the moisture of the breath sample was separated from the signals of the other breath components.[218] The inset shows a micrograph of a transverse section of a MCC with ~1400 capillaries having a diameter of ~40μm.[246] **(c)** A comparison between the response patterns of an array of four gold-nanoparticle (Au-NPs) based chemiresistors to clean moist air samples (blue and green closed circles) and air samples contaminated by ~40ppm 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.[199] **(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.[166] **(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 monothiols capped Au-NPs (bottom).[244] **(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.[199]

Table 1: Candidates for cancer VOCs published during the past decade. [3, 14, 49, 55, 62, 67, 70, 71, 73-75, 77, 114, 136-138] 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 [127] for hydrocarbons or estimated by the algorithms of Poulin and Krishnan[135] 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/Liter /mol/Liter]. 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/Lit)/(mol/Lit)]	References for $\lambda_{b:a}$ at 37°C	log[$\lambda_{f:b}$]	References for $\lambda_{f:b}$ at 37°C	Tentative origin	Comments	References
Alkanes, branched-chain alkanes and branched-chain alkenes	74-84-0	Ethane	8.50E-02	predicted [252]	1.934	132	Natural or petrol, product of lipid peroxidation	Lung Cancer	3
	109-66-0	Pentane	4.16E-01	measured [127]	1.998	253	Natural, possibly petrol, product of lipid peroxidation	Lung Cancer	77
	142-82-5	Heptane	2.71E+00	measured [127]	2.190	253	Natural or petrol, plastics,	Lung Cancer	77
	111-65-9	Octane	5.77E+00	measured [127]	1.606	132	Natural or petrol	Lung Cancer	55, 77
	111-84-2	Nonane	1.39E+01	measured [127]	1.777	254	Natural or petrol	Breast Cancer	73

124-18-5	Decane	2.48E+01	predicted using data from Ref [127]	1.656	254	Natural or petrol	Lung Cancer	[77]
1120-21-4	Undecane	5.46E+01	predicted using data from Ref [127]	2.342		Natural or petrol	Breast Cancer	[75]
112-40-3	Dodecane	1.20E+02	predicted using data from Ref [127]	1.901		Fuels	Lung Cancer, Breast Cancer	[75, 136]
629-50-5	Tridecane	2.67E+02	predicted using data from Ref [127]	2.540		Fuels	Breast Cancer	[75]
629-59-4	Tetradecane	5.99E+02	predicted using data from Ref [127]	2.990			Breast Cancer	[75]
629-62-9	Pentadecane	1.36E+03	predicted using data from Ref [127]	3.515			Breast Cancer	[75]
75-28-5	2-Methyl-propane	7.90E-02	measured [127]	1.795		Refrigerant, contaminant from plastics, tubing, medical equipment,	Breast Cancer	[73]
107-83-5	2-Methyl-pentane	4.73E-01	measured [127]	2.295	253	Petrol	Lung Cancer	[77]
78-79-5	Isoprene	9.50E-01	measured [126]	1.043	132	Mevalonic pathway-biosynthesis of cholesterol	Lung Cancer & Gastric Cancer	[14]
61141-72-8	4,6-Dimethyl-dodecane	7.38E+02		2.178		Kerosene fuel	Head and Neck Cancer	[62]
17302-37-3	2,2-Dimethyl-decane	1.18E+02		1.316			Head and Neck Cancer	[62]
473-19-8	2,2,3-Trimethyl-, exobicyclo[2.2.1]heptane	1.25E+02		2.296			Head and Neck Cancer	[62]
562-49-2	3,3-Dimethyl-pentane	1.20E+00		1.786			Breast Cancer	[67]

62185-53-9	5-(2-Methylpropyl)-nonane	2.86E+02		1.684			Breast Cancer	67
62238-15-7	2,3,4-Trimethyl-decane	2.99E+02		1.755			Breast Cancer	67
589-34-4	3-Methyl-hexane	1.30E+00	measured [253]	2.329	253	Un-natural, environmental contaminant	Head and Neck Cancer	62
2213-23-2	2,4-Dimethylheptane	7.55E+00		1.492		High chance of mis-assignment of isomer, petrol	Head and Neck Cancer Lung Cancer	3 62
3221-61-2	2-Methyl-octane	3.31E+00	measured for rat blood [254]	2.040	254		Breast Cancer	73
2216-34-4	4-Methyl-octane	8.21E+00		1.284		Contaminant from plastics, tubing, medical equipment,	Head and Neck Cancer, Lung Cancer	3 62
54166-32-4	2,6,6-Trimethyl-octane	4.64E+01		1.593			Head and Neck Cancer	62
5911-04-6	3-Methyl-nonane	5.76E+00	measured for rat blood [254]	2.079	254	Natural or petrol	Head and Neck Cancer	62
16747-26-5	2,2,4-Trimethylhexane	7.08E+00		1.426		Petrol	Lung Cancer	137
25117-31-1	5-Methyl-tridecane	7.67E+02		2.559			Breast Cancer	73
1002-43-3	3-Methyl-undecane	1.27E+02		1.726			Breast Cancer	73
10105-38-1	6-Methyl-pentadecane	4.86E+03		3.689			Breast Cancer	73
6418-45-7	3-Methyl-nonadecane	2.13E+05		6.830			Breast Cancer	73

	6117-97-1	4-Methyl-dodecane	3.08E+02		2.176			Breast Cancer	73
	763-29-1	2-Methyl-1-pentene	1.41E+00		1.783		Contaminant from plastics, tubing, medical equipment,	Lung Cancer	137
	2847-72-5	Decane, 4-methyl-	5.04E+01		2.038			Lung Cancer	70
	764-13-6	2,4-Hexadiene, 2,5-dimethyl-	1.64E+00		2.284			Lung Cancer	70
	1515-79-3	5,5-Dimethyl-1,3-hexadiene	1.13E+00		2.281			Lung Cancer	71
Primary and secondary alcohols	64-17-5	Ethanol	1.50E+03	measured [252]	-0.823	255	Natural, diet, disinfectants, intestinal bacterial flora	Liver Cancer	49
	71-23-8	1-Propanol	1.03E+03	measured [132]	-0.532	255	Natural, disinfectants	Lung Cancer	3, 70
	67-63-0	2-Propanol	8.30E+02	measured [252]	-0.634	255	Natural, disinfectants	Lung and Breast Cancer	71, 74
	71-36-3	1-Butanol	9.33E+02	measured [252]	-0.095	132	Natural, diet	Lung Cancer	138
	104-76-7	2-Ethyl-1-hexanol	1.31E+03		2.156		Contaminant from tubing material	Lung Cancer	3
	3391-86-4	1-Octen-3-ol	1.13E+03		2.089		Natural (produced in plants and Fungi)	Liver Cancer	55
	625-31-0	4-Penten-2-ol	1.39E+03		1.006			Lung Cancer	71
Aldehydes and branched aldehydes	123-38-6	Propanal	1.77E+02		0.418		Natural or industrial waste product	Lung Cancer	3
	123-72-8	Butanal	1.27E+02		0.894		Natural or industrial waste product, diet	Lung Cancer	3
	110-62-3	Pentanal	8.85E+01		1.361		Natural, diet	Lung Cancer	3, 114
	66-25-1	Hexanal	8.21E+01		1.769		Natural, diet	Lung Cancer, Liver Cancer	3, 55, 114
	111-71-7	Heptanal	8.87E+01		2.058		Natural or industrial waste product, diet,	Lung Cancer, Breast Cancer	3, 74
	124-13-0	Octanal	1.26E+02		2.209		Natural or industrial waste	Lung Cancer	3, 114

							product, diet,		
	124-19-6	Nonanal	1.63E+02		2.268		Possibly natural	Lung Cancer	[3, 114]
	98-01-1	Furfural || Furaldehyde	3.05E+03		0.705		Natural or industrial waste product	Gastric Cancer	[14]
carboxylic acids	75-98-9	2,2-Dimethyl-propanoic acid	3.82E+03		0.930			Head and Neck Cancer	[62]
	64-19-7	Acetic acid	7.96E+04		-0.190		Natural or industrial waste product	Liver Cancer	[49]
Ketones	67-64-1	Acetone	3.40E+02	measured [133]	-0.475	255	Fatty acid metabolism	Lung Cancer	[3]
	78-93-3	2-Butanone	1.64E+02	measured [132]	-0.004	255	Diet, environmental contaminant	Lung Cancer	[3]
	107-87-9	2-Pentanone	1.50E+02	measured [256]	0.206	132	Natural, diet	Lung Cancer	[3]
	591-78-6	2-Hexanone	1.68E+02	measured [132]	0.356		Industrial waste product	Lung Cancer	[137]
	106-35-4	3-Heptanone	1.87E+02		1.813		Natural, drugs	Lung Cancer	[137]
	623-56-3	5-Methyl-3-hexanone	9.15E+01		1.702			Head and Neck	[62]
	7379-12-6	2-Methyl-3-hexanone	8.28E+01		1.702			Lung Cancer	[71]
	110-93-0	6-Methyl-5-hepten-2-one	1.92E+02		1.779		Squalene oxidation	Gastric Cancer	[14]
	513-86-0	3-Hydroxy-2-butanone	1.02E+03		-0.173			Lung Cancer	[138]
	119-61-9	Benzophenone	4.14E+04		2.247		Industrial waste product (used as fragrance in soaps, in pharmaceuticals and ultraviolet absorbers- sunscreen)	Lung Cancer	[71]
	3848-24-6	2,3-Hexanedione	3.42E+04		-0.189			Lung Cancer	[71]
	565-80-0	3-Pentanone, 2,4-dimethyl-	4.27E+01		1.582			Lung Cancer	[70]

Aromatic compounds	71-43-2	Benzene	8.80E+00	measured [257]	1.598	[257]	Petrol, smoking	Lung Cancer	[3]
	108-88-3	Toluene	1.39E+01	measured [257]	2.180	[257]	Petrol, smoking	Lung Cancer	[3]
	100-42-5	Styrene	5.56E+01	measured [132]	1.758	[132]	Natural, smoking	Lung Cancer	[3]
	625-86-5	2,5-Dimethylfuran	2.58E+00		1.695		Smoking	Lung Cancer	[3, 70]
	106-42-3	*p*-Xylene	3.89E+01	measured [132]	0.709	[257]	Petrol, smoking	Head and Neck Cancer, Prostate cancer	[62, 67]
	496-16-2	2,3-Dihydro-benzofuran	8.71E+01		1.976			Liver Cancer	[49]
	100-41-4	Ethylbenzene	2.82E+01	measured [132]	1.796	[132]	Petrol	Lung Cancer	[77]
	98-86-2	1-Phenyl-ethanone	1.27E+03		1.573			Breast Cancer	[74]
Nitrils	75-05-8	Acetonitrile	6.98E+02		-0.198		Smoking	Lung Cancer	[3]
	107-13-1	2-Propenenitrile	1.41E+02		0.210		Smoking and car exhaust	Gastric Cancer	[14]
	93946-48-6	2-Amino-5-isopropyl-8-methyl-1-azulenecarbonitrile	9.65E+06		2.294			Breast Cancer	[67, 75]
Terpens and Terpenoids	138-86-3	D-Limonene	6.21E+01		2.296		Industrial waste (used in food flavorings and cosmetics)	Breast Cancer	[75]
	98-55-5	*p*-Menth-1-en-8-ol	2.63E+03		2.152		Cosmetics	Lung Cancer	[71]
	21368-68-3	Camphor	2.07E+02		1.873		Natural	Lung Cancer	[71]
Esters	110-27-0	Isopropyl myristate	5.75E+04		2.298			Breast Cancer	[74]
	124-63-0	Methane-sulfonyl chloride	2.65E+02		0.021		Transamination pathways (incomplete metabolism of methionine)	Liver Cancer	[49]
	631-61-8	Ammonium acetate						Head and Neck	[62]
	35242-43-4	2,3-Dihydro-1-phenyl-4(1H)-quinazolinone	3.84E+07		2.260			Breast Cancer	[67, 74]

	4282-42-2	1-Iodo-nonane	3.72E+02		2.298			Breast Cancer	[67]
	24310-22-3	2-[(1,1-Dimethylethyl)thio]-acetic acid	9.66E+05		1.767			Colon Cancer	[67]
	82406-83-5	4-(4-Propylcyclohexyl)-, 4'-cyano[1,1'-biphenyl]-4-yl ester benzoic acid	1.77E+11		2.298			Colon Cancer	[67]
	NIST 282650	2-Trifluoromethylbenzoic acid, 6-ethyl-3-octyl ester	1.18E+05		2.298			Breast Cancer	[67]
	21064-19-7	1,5,9-Cyclododecatriene, 1,5,9-trimethyl-	1.05E+03		2.298			Lung Cancer	[70]
	6846-50-0	Pentan-1,3-dioldiisobutyrate, 2,2,4-trimethyl	8.50E+04		2.293		Plasticizer	Lung Cancer	[70]
	23676-09-7	Benzoic acid, 4-ethoxy-, ethyl ester	2.07E+04		2.266			Lung Cancer	[70]
	74381-40-1	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	7.68E+04		2.293			Lung Cancer	[70]
	494-19-9	10,11-Dihydro-5H-dibenz-[*b,f*]-azepine	5.37E+05		2.286			Lung Cancer	[70]
	719-22-2	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	1.69E+07		2.283			Lung Cancer	[70]

	101-84-8	Benzene, 1,1-oxybis-	2.43E+03		2.292			Lung Cancer	70
	13049-35-9	1,1-Biphenyl, 2,2-diethyl-	6.68E+04		2.298			Lung Cancer	70
	87-44-5	trans-Caryophyllene	1.81E+02		2.298			Lung Cancer	70
	3910-35-8	1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	3.22E+04		2.298			Lung Cancer	70
	84-66-2	1,2-Benzenedicarboxylic acid, diethyl ester	3.85E+04		2.154		Plasticizer	Lung Cancer	70
	76-13-1	Ethane, 1,1,2-trichloro-1,2,2-trifluoro-	2.40E-01		2.245			Lung Cancer	71
	1634-04-4	Propane, 2-methoxy-2-methyl-	1.59E+01		0.728	258	Gasoline	Lung Cancer	71
	42848-06-6	1-Propene, 1-(methylthio)-, (E)-	8.18E+00		2.043		Diet (onion, garlic)	Lung Cancer	71
	824-22-6	1H-Indene, 2,3-dihydro-4-methyl-	1.32E+02		2.280			Lung Cancer	71
	915392-37-9	5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	5.97E+05		1.473			Lung Cancer	71
	127-51-5	Isomethyl ionone	1.95E+03		2.291			Lung Cancer	71
	710336-76-8	2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one	4.33E+02		2.274			Lung Cancer	71
	24238-73-1	Bicyclo[3.2.2]nonane-1,5-dicarboxylic acid, 5-ethyl ester	3.20E+06		1.868			Lung Cancer	71

	959016-51-4	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	1.27E+04		2.295			Lung Cancer	71
	16204-36-7	1,2,4,5-Tetroxane, 3,3,6,6-tetraphenyl-	9.14E+08		2.295			Lung Cancer	71
	6738-27-8	2,5-Cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-ethylidene-	3.38E+03		2.292			Lung Cancer	71
	55162-49-7	Furan, 2-[(2-ethoxy-3,4-dimethyl-2-cyclohexen-1-ylidene)methyl]-	3.47E+03		2.297			Lung Cancer	71
	7694-30-6	Benzene, 1,1-(1,2-cyclobutanediyl)bis, *cis*-	2.85E+04		2.296			Lung Cancer	71
	53699-80-2	Benzene, 1,1-[1-(ethylthio)propylidene]bis-	1.09E+05		2.295			Lung Cancer	71
	101580-33-0	Anthracene, 1,2,3,4-tetrahydro-9-propyl-	2.45E+05		2.298			Lung Cancer	71
	839-73-6	9,10-Anthracenediol, 2-ethyl-	2.79E+11		2.284			Lung Cancer	71
	10224-91-6	Benzene, 1,1-ethylidenebis, 4-ethyl-	6.30E+04		2.298			Lung Cancer	71

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