Assessment of the Exhalation Kinetics of Volatile Cancer Biomarkers Based on their Physicochemical Properties

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Abstract:

The current review provides an assessment of the exhalation kinetics of volatile organic compounds (VOCs) that have been linked with cancer. Towards this end, we evaluate various physicochemical properties, such as 'breath:air' and 'blood:fat' partition coefficients, of 112 VOCs that have been suggested over the past decade as potential markers of cancer. With these data, we show that the cancer VOC concentrations in the blood and in the fat span over 12 and 8 orders of magnitude, respectively, in order to provide a specific counterpart concentration in the exhaled breath (e.g., 1 ppb). This finding suggests that these 112 different compounds have different storage compartments in the body and that their exhalation kinetics depends on one or combination of the following factors: **(i)** the VOC concentrations in different parts of the body; **(ii)** the VOC synthesis and metabolism rates; **(iii)** the partition coefficients between different tissues with blood and air; and **(iv)** the VOCs' diffusion constants. Based on this analysis, we discuss how this knowledge allows modeling and simulation of the behavior of a specific VOC under different sampling protocols (with and without exertion of effort). We end this review by a brief discussion on the potential role of these scenarios in screening and therapeutic monitoring of cancer.

1. Introduction

1.1. Background

Volatile organic compounds (VOCs) of cancer have been found in breath, blood [1], headspace of cancer cells [2-10], and in headspace of resected cancer tissues [11]. Exhaled breath, which may change its chemical signature depending on the physiological or pathophysiological state of cancer [12-24], is considered as one of the most fascinating body fluids/sources. Sampling of breath is non-invasive and can be used for screening, at an intensive care unit (ICU) [25, 26], during surgery [27-29], or monitoring pre and post-surgery [30]. Volatile compounds that do not appear normally in exhaled breath can be used for detection of bacterial or fungal infection in the lungs [31-35]. Also hydrogen and methane [36] are produced by bacteria in the gut, and show high concentrations in persons with fructose or lactose malabsorption after ingestion of these carbohydrates [37, 38]. Other volatile compounds appear after ingestion of drugs, an example being 3-heptanone during valproate therapy [39]. ^{13}C-labeled compounds such as ^{13}C-uracil, ^{13}C-dextromethorphan or ^{13}C-pantoprazol are specifically administered to measure enzyme activity through the respective potential to metabolize these precursors with production of $^{13}CO_2$ measured in exhaled breath [40-45]. Volatile compounds do not only appear in exhaled breath, but also in skin emanations [46, 47], urine [48], blood [1] and saliva [49]. In addition to volatile organic compounds, also small inorganic molecules like hydrogen, nitric oxide [50-61] or carbon monoxide [62] are most interesting. Another most interesting application of volatiles is their use in search operations [63, 64]. The potential of volatiles and of breath analysis for clinical diagnosis and therapeutic monitoring is enormous, even though at the present stage there are only very few breath tests which have got approval by FDA or by the European Medicines Agency (EMA). In particular, no breath tests based on endogenously produced volatile biomarkers for cancer are FDA- or EMA-approved.

The main reasons for the pre-maturity of cancer breath analysis in real clinical settings is related, in general, to the lack of standardization and to the poor knowledge of the biochemical pathways and exhalation kinetics of the cancer-related VOCs. The standardization aspects and the biochemical pathways of the cancer-related VOCs were presented and discussed in earlier papers [18, 65-67]. In the present review, we focus on the discussion of the exhalation kinetics of the cancer-related VOCs. The exhalation kinetics can be determined by actual *real-time* measurements under different conditions [68-76] or by simulation of the flow of some compound within the body [70, 77-83]. With this in mind, we present a list of 112 tentative cancer-related VOCs published in the literature during the last decade. Then, we classify the 112 cancer-related VOCs with respect to their “fat-to-blood” and “blood-to-air” partition coefficients and show how these partition coefficients provide estimation on the relative concentrations in alveolar breath, blood and the fat compartment of the human body. Based on the generated ensemble of the physicochemical data, we discuss how the balance between all of these factors determines the *exhalation kinetics* of cancer VOCs.

1.2. Exhalation kinetics of cancer VOCs

To illustrate the exhalation kinetics of cancer VOCs, we present isoprene as a representative example. Isoprene is the most prominent hydrocarbon in exhaled breath, appearing at ~100 ppb in healthy persons who do not exert any effort [1, 84]. In humans it is believed to be synthesized in the

mevalonate pathway [85-87]. It can be measured in *real-time* during an experiment performed at a stationary bicycle [69, 79, 82] or even during sleep [68, 72].

Recently, it was shown that isoprene appears at lower concentration in exhaled breath of lung cancer [88] and breast cancer patients [89], compared to healthy volunteers. So far, this result is corroborated by the observation that the decrease in concentration of isoprene is correlated with the immune activation as measured by the neopterin concentration in blood [90]. While these findings offer isoprene as a potential cancer biomarker, it is important to know that the concentration of isoprene depends very much on the specific sampling protocol [69-72, 78, 81, 82, 91]. In either case, the clear isoprene's *in-situ* signals achieved by on-line mass-spectrometry techniques make it an ideal candidate for the investigation of cancer-related VOC exhalation kinetics.

Figure 1 shows the output of isoprene (in nmol/L) during an experiment with a healthy volunteer on a stationary bicycle. For the sake of comparison, the output of acetone (in arbitrary units) and CO_2 (in L/min) are presented on the same figure, too. The specific investigations of isoprene and acetone have the advantage that the respective concentrations in exhaled breath are high (~100 ppb and ~400 ppb, respectively), which makes measurement and demarcation of exhaled vs. inhaled concentrations comparatively easy. The protocol of this experiment changes between rest phases and 75 W workload pedaling phases. During the first pedaling phase, the output of isoprene increases by a factor of ~10, and subsequently decreases exponentially (still during pedaling phase), with a further decrease back to the baseline concentration during the rest phase. When the volunteer starts to pedal again, the increase in isoprene output is much smaller than during the first pedaling phase. After another rest phase, resumption of the pedaling increases the isoprene output, but at a rather low level. It takes approximately 2 hours to resynthesize isoprene in the body and to fill up the isoprene stores in such a way that the huge increase in isoprene output by a factor of ~10 appears again. Putting these findings in a more specific perspective, it is likely that isoprene is produced and stored in the periphery of the human body, probably in the muscles [79, 91]. During exertion of an effort, the blood flow through the muscles increases and leads to transport of isoprene from the muscles to the lungs, where it is exhaled. The exponential decay of isoprene output (still during pedaling phase) is a consequence of the depletion of isoprene in the muscles. During the rest phase, the blood flow through the muscles goes back to baseline and so does the isoprene concentration. It is important to note in this context that the increase of concentration in breath during exertion of an effort indicates that the specific choice of the breath sampling protocol (e.g., quietly sitting, standing, after a period of rest, etc.) is of great importance for both healthy and cancer states [92]. In particular, different sampling protocols may result in very different isoprene concentrations (up to a factor of ~5). We expect that a similar situation is true for many volatile cancer markers [70].

To get a more thorough look on the exhalation kinetics of VOCs, it is important to know which VOCs behave according to the *Farhi equation* [93]:

$$C_{\text{alveolar}} = \frac{C_{\bar{v}}}{\lambda_{b:a} + \dot{V}_A / \dot{V}_Q} \qquad (1)$$

The Farhi equation is a first very important step in modeling of the exhalation kinetics [69-72, 78, 80-82, 91]. This equation relates the alveolar concentration ($C_{alveolar}$) of a VOC to the concentration in mixed venous blood ($C_{\bar{v}}$). As seen in this equation, a *decrease* in the concentration during exertion of an effort happens because the alveolar ventilation ($\dot{V}_A$) usually increases more strongly than the cardiac output ($\dot{V}_Q$). An example of a compound that decreases in concentration during effort (in line with Farhi's equation) is *butane.*[70]

An important physicochemical constant in the Farhi equation is the 'blood:air' partition coefficient ($\lambda_{b:a}$). For isoprene, this partition coefficient is approximately **0.95 (mol/L)/(mol/L)** [94, 95]. This means that the concentration of isoprene in blood is ~95% of the concentration in alveolar air. Incidentally, the 'blood:air' partition coefficient can be quite different from the 'water:air' partition coefficient ($\lambda_{w:a}$) at 37 °C. In case of isoprene, the $\lambda_{w:a}$ is approximately 0.28. The reason for this difference is that blood contains lipids, which take up a larger amount of isoprene than water.

Another important physicochemical constant is the 'fat:blood' partition coefficient ($\lambda_{f:b}$). For isoprene, this $\lambda_{f:b}$ is approximately **82.0 (mol/L)/(mol/L)** [96]. This means that the equilibrium concentration of isoprene in fat is about 82 times the concentration of isoprene in blood. For an isoprene concentration of 150 ppb (~5.8×10^{-9} mol/L) in alveolar air, the estimated equilibrium concentration in blood is ~5.5×10^{-9} mol/L (=$0.95*5.8\times10^{-9}$ mol/L) and the estimated concentration in fat is 4.5×10^{-7} mol/L (=$82*5.5\times10^{-9}$ mol/L).

For a 70 kg person of 1.80 m height, the volume of blood is ~6 L and the amount of fat tissue is estimated to be 12.78 L [97]. In 6 L of **blood** and 12.78 L of **fat,** we estimate the amount of isoprene to be **~3.3×10^{-8} mol** (= $6\times5.5\times10^{-9}$ mol) and **~5.8×10^{-6} mol** (=$12.78\times4.5\times10^{-7}$ L), respectively. Hence the amount of isoprene in fat is estimated to be ~175 times the amount in blood. With this in mind, it is reasonable to claim that the exhalation kinetics of cancer-related VOCs depends on the concentration of the respective VOC in different compartments of the body, on the partition coefficients between the different compartments (e.g., $\lambda_{b:a}$ and on the fat:air partition coefficient $\lambda_{f:a}$), and on the diffusion kinetics between different compartments. In the following section, we will present ways to obtain each of these parameters and discuss the inter-relationship between these parameters as well as with the exhaled VOC-related cancer.

2. Estimating the $\lambda_{b:a}$ and $\lambda_{f:b}$

For the majority of VOCs of interest, the $\lambda_{b:a}$ and $\lambda_{f:b}$ have not been measured. Nevertheless, a number of papers present relevant data on $\lambda_{b:a}$, $\lambda_{f:a}$ and $\lambda_{f:b}$ of VOCs [94, 95, 98-106]. In addition, the $\lambda_{b:a}$ of many hydrocarbons can be estimated based on the respective $\lambda_{b:a}$ of similar (but not identical) alkanes and isoalkanes which have been investigated in Ref. [95]. Finally, one may estimate $\lambda_{b:a}$ using the formula given by Poulin & Krishnan [107]

$$\lambda_{b:a} = \lambda_{o:w} \cdot \lambda_{w:a} \cdot (a+0.3b) + \lambda_{w:a} \cdot (c+0.7b) \qquad (2)$$

Here, a≈0.0033 is the fraction of neutral lipids in the blood, b≈0.0024 is the fraction of phospholipids in the blood, and c≈0.82 is the fraction of water in the blood. The octanol:water partition coefficients ($\lambda_{o:w}$) can be compiled from Scifinder (https://scifinder.cas.org), whereas the water:air partition coefficients ($\lambda_{w:a}$; Henry's constants) at 25°C can be taken from the compilation of Sander [108] or estimated by the EPI Suite™ software developed at the US environmental protection agency (EPA, http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm). Otherwise, $\lambda_{w:a}$ can be 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). Furthermore, in order to estimate the Henry constants at 37°C, the derivative $d\ln(\lambda_{w:a})/d(1/T)$ as given in the compilation by Sander [108] can be used, or the corresponding enthalpy of vaporization (ΔH_{vap}) divided by the gas constant, R.

Similar to the $\lambda_{b:a}$, one may estimate $\lambda_{f:a}$ using the method of Poulin & Krishnan,[107] given by the equation:

$$\lambda_{f:a} = \lambda_{o:w} \cdot \lambda_{w:a} \cdot (A+0.3B) + \lambda_{w:a} \cdot (C+0.7B) \qquad (3)$$

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

3. Partitions coefficients of cancer-related VOCs

During the past decade, some 112 tentative cancer VOCs in exhaled breath have been reported [7, 30, 67, 109-121]. There were 36 hydrocarbons, 7 alcohols, 8 aldehydes, 2 acids, 12 ketones, 12 aromatic compounds, 2 heterocycles, 2 nitriles, 5 terpenes, 7 esters, 2 ethers, 1 sulfide, 2 halogenated compounds, and 15 compounds from other chemical classes. Examples of hydrocarbons among this list of 112 VOCs are 2-methyl-propane (CAS 75-28-5) or 5-methyl-tridecane (CAS 25117-31-1). Example of an alcohol is 1-octen-3-ol (CAS 3391-86-4). Examples of aldehydes are pentanal, hexanal, octanal and nonanal. An example for a ketone is 6-methyl-5-hepten-2-one (CAS 110-93-0). An example of an aromatic compound is benzophenone (CAS 119-61-9). An example of a terpene is "trans-caryophyllene" (CAS 87-44-5). In addition, also biomarkers in exhaled breath condensate have been published [122].

The 112 cancer VOCs in exhaled breath could be considered as being on an equal footing in exhaled breath. Nevertheless, the very different blood:air partition coefficients ($\lambda_{b:a}$) imply that the respective concentrations in blood are rather different, even if the concentration in breath would be the same. Each of the mentioned 112 compounds can be looked upon from many different viewpoints. Here are a few examples:

- 2-Methyl-propane (CAS 75-28-5) commonly appears in human breath, in smokers and non-smokers [123]. It is also released by *Streptococcus pneumonia* [31]. It has been suggested as volatile biomarker for breast cancer in exhaled breath [111].
- 5-Methyl-tridecane (CAS 25117-31-1) has been suggested to be a volatile biomarker of breast cancer [111] and belongs to the class of monomethylated hydrocarbons, which were

proposed to be used in the "breath methylated alkane contour" (BMAC) for detection of cancer and other diseases [124-130].

- Benzophenone (CAS 119-61-9) was observed in the context of feces,[131] axillary sweat [132] and saliva [133] and was suggested as a volatile biomarker for lung cancer [134].

- Trans-Caryophyllene (CAS 87-44-5) has been observed on the surface of juicy grapefruits [135], in feces [131], skin emanations [133], human breast milk and saliva [133, 136].

- 6-Methyl-5-hepten-2-one (CAS 110-93-0), is produced on skin by degradation of squalene (and, in particular so during the influence of ozone). This compound is, for example, observed when healthy volunteers sit in a chamber so that the skin emanations are collected and observed in the chamber indoor air. There are three volatiles, acetone, 6-methyl-5-hepten-2-one, and acetaldehyde, which exhibit especially high emission rates through skin exceeding 100 fmol×cm^{-2}×min^{-1} without ozone influence [137]. Interestingly, 6-methyl-5-hepten-2-one has recently be observed in exhaled breath of gastric cancer patients.[121]

Figure 2 presents the estimated values for the negative logarithm of the blood:air partition coefficients, *viz.* **$-\log(\lambda_{b:a})$**. These $\lambda_{b:a}$ are distributed over 12 orders of magnitude. Hence, if the concentrations of the cancer VOCs would be equal in exhaled breath – *e.g.*, at 1 ppb – their concentrations in blood would be very different and also be distributed over **12 orders of magnitude**. An analogous situation holds for the $\lambda_{f:b}$ (*see* Figure 3). Similarly as above, if the concentrations of the cancer VOCs would be equal in exhaled breath – e.g., at 1 ppb – their concentrations in fat would be very different and be distributed over **8 orders of magnitude**. For this reason when studying VOCs' concentrations in cancer and relating them to potential metabolic pathways one should estimate the blood/tissue concentration and by these validate the underline metabolic assumption. That is, elevated concentrations in the breath of a certain molecule will not necessary reflect the same magnitude of elevation in the tissue.

That volatile cancer biomarkers vary in concentration over many orders of magnitude is most surprising, and should change the way we look at these compounds. Typically, a high $\lambda_{f:b}$ (as, e.g., for 3-methyl-hexane) will lead to high concentration of the respective volatile compound in lipid membranes (e.g., in endothelial cells demarcating the blood vessels). High concentrations within the lipid membranes may change their permeability properties. It can be expected, that lipophilic volatiles bear considerable influence on pathophysiology of disease.

The fact that these cancer-related VOCs have very different physicochemical properties is expected to be an advantage. The different $\lambda_{b:a}$ and $\lambda_{f:b}$ imply that the concentrations in different compartments (blood, fat, muscle, etc.) are very different. As a consequence, the exhalation kinetics can be expected to be different. The release of these compounds from their "main storage" compartment depends on the blood flow through this compartment during sampling. Different sampling protocols (with or without exertion of an effort, or during increased blood flow through intestinal part of the body due to digestion processes after a meal, etc.) lead to different concentrations of volatiles. Measurements with different sampling protocols could be done for an arbitrary VOC and, in particular, for the 112 cancer-related VOCs mentioned above. In this context, lung and upper airways cancer cells hold a distinguished status as due to their anatomic location,

which allows them to release cancer-related VOCs directly into the exhaled air. Thus, the breath levels of these species can be enriched as compared to the levels expected from their blood concentrations. Consequently, the breath and blood concentrations of these volatile cancer markers cannot easily be related using e.g. the Farhi equation. Nevertheless, from the breath analysis point of view the location of these cancers is beneficial as their VOCs' fingerprints can be directly detected.

Detailed investigations and even *real-time* analysis should be possible for many of these 112 cancer VOCs in the foreseeable future. While all of these 112 compounds are the result of empirical studies, additional important aspects that would be needed to be addressed concern the connection of such compounds to real logical metabolic processes related to the disease. By doing so, one could biologically relate the concentrations emitted in breath, to concentrations in the different body compartments, and thus suggest indication for the disease development status. From a technological point of view it would be wanted to specify certain threshold concentrations of volatiles in breath to guide changes in the clinical treatment. This would result in a therapeutic monitoring guided by the exhaled concentrations of volatiles. By adapting the analytical and nano-technology in this direction one can achieve an on-line response by physicians.

4. Conclusions

The 112 volatile cancer biomarkers published during the last decade have very different physicochemical properties. Up to now, no advantage has been drawn from this fact. In this review, we showed that the blood:breath and blood:fat partition coefficients are very different for the 112 cancer-related VOCs. Even if the concentration of these 112 compounds would be identical in exhaled breath (*e.g.*, 1 ppb), the respective concentrations in blood and in the fat compartment would vary, respectively, over **12 and 8 orders of magnitude**. This means that different compounds may be stored (or exist in equilibrium) in different compartments of the body. To gain a more comprehensive understanding on the cancer-related VOCs, a combined information on appearance and concentration of these VOCs in breath and blood [1], or in breath and urine or saliva, is critical.[138]. Further information can be achieved by investigating exhaled breath *and* resected tumor tissue from the same patient [11, 30]. For a simple point of care screening tool, knowing the exhalation kinetics and optimization of the sampling procedure is important. For research and eventual pharmaceutical treatment also the understanding of blood and tissue concentration would be critical.

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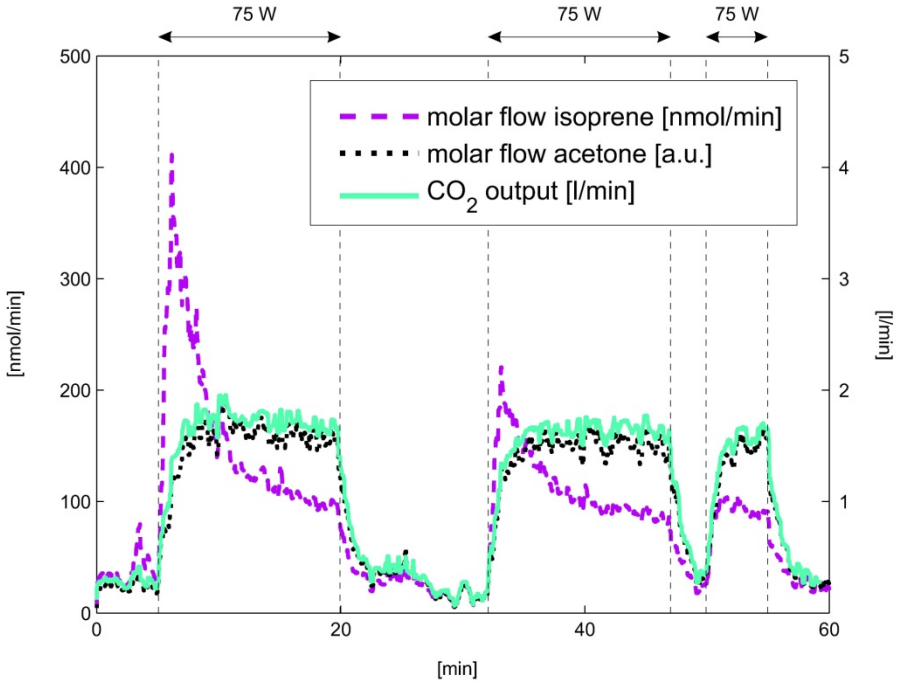

Figure 1: Output of isoprene (nmol/min), acetone (arbitrary units) and CO_2 (L/min) for a healthy volunteer during rest phases and exertion of an effort of 75W on a stationary bicycle. In case of CO_2, 15 exhalations per minute with 3 Liters of alveolar air with 4% of CO_2 correspond to 1.8 Liters/min of CO_2 (= 15*3*0.04 L/min). Isoprene output through exhaled breath may increase up to a factor ~10 during exertion of an effort, whereas the concentration in breath increases up to a factor ~5. In this experiment, two steady-states of isoprene exhalation appear, (A) at ~25 nmol/min (corresponding to production of isoprene in the liver) and (B) at ~100 nmol/L (corresponding to production of isoprene in the muscles). In a person with high cholesterol blood level, the production of isoprene in the liver would be decreased under the influence of statins [139]. Reproduced from Ref. [69], DOI 10.1088/1752-7155/3/2/027006,

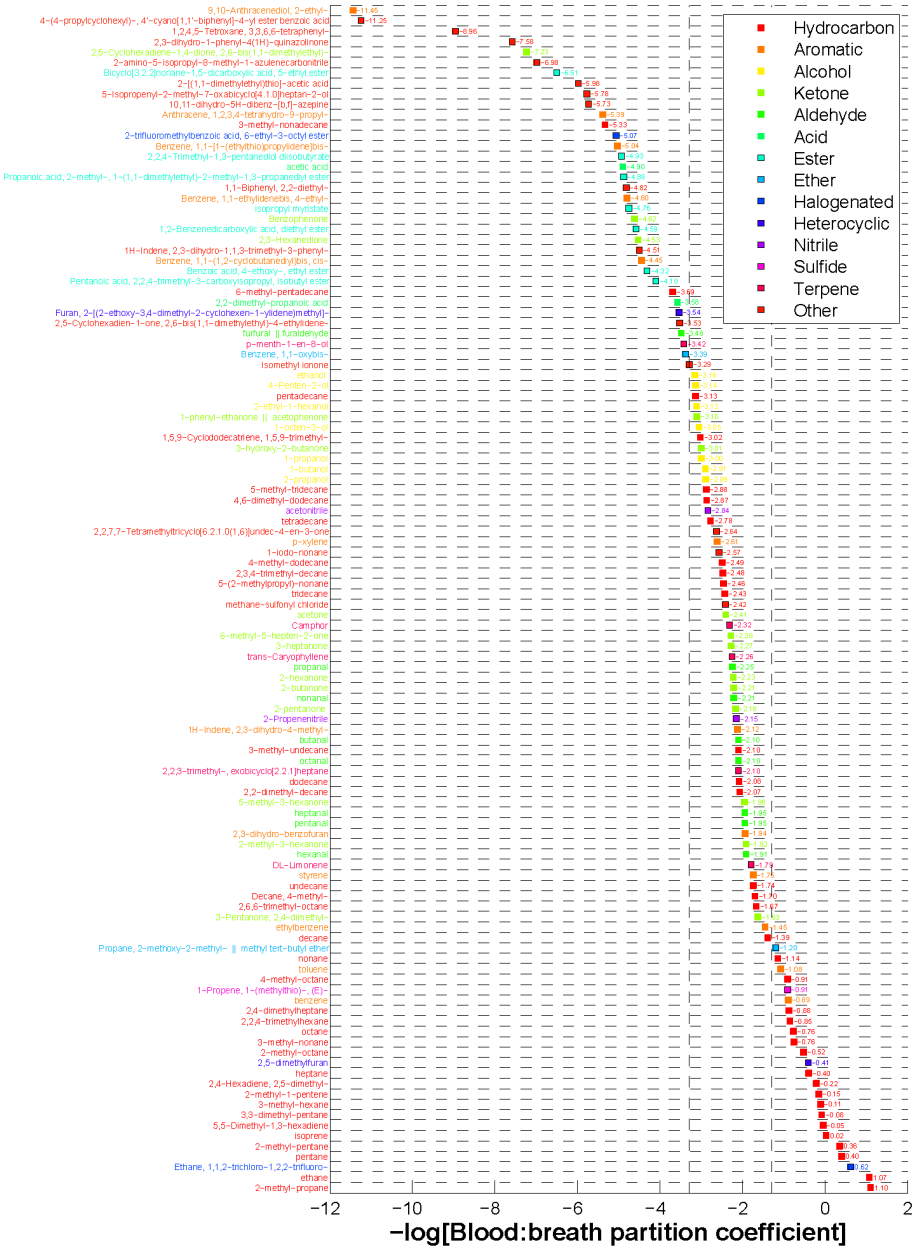

Figure 2: Partition coefficient, -log($\lambda_{b:a}$), for 112 volatile cancer biomarkers published during the last decade [7, 30, 67, 109-121], as well as acetone and 2-pentanone for comparison. The color for the different compound names is chosen according to the chemical class. Compounds with a higher log($\lambda_{b:a}$) will tend to be in the blood and vice versa.

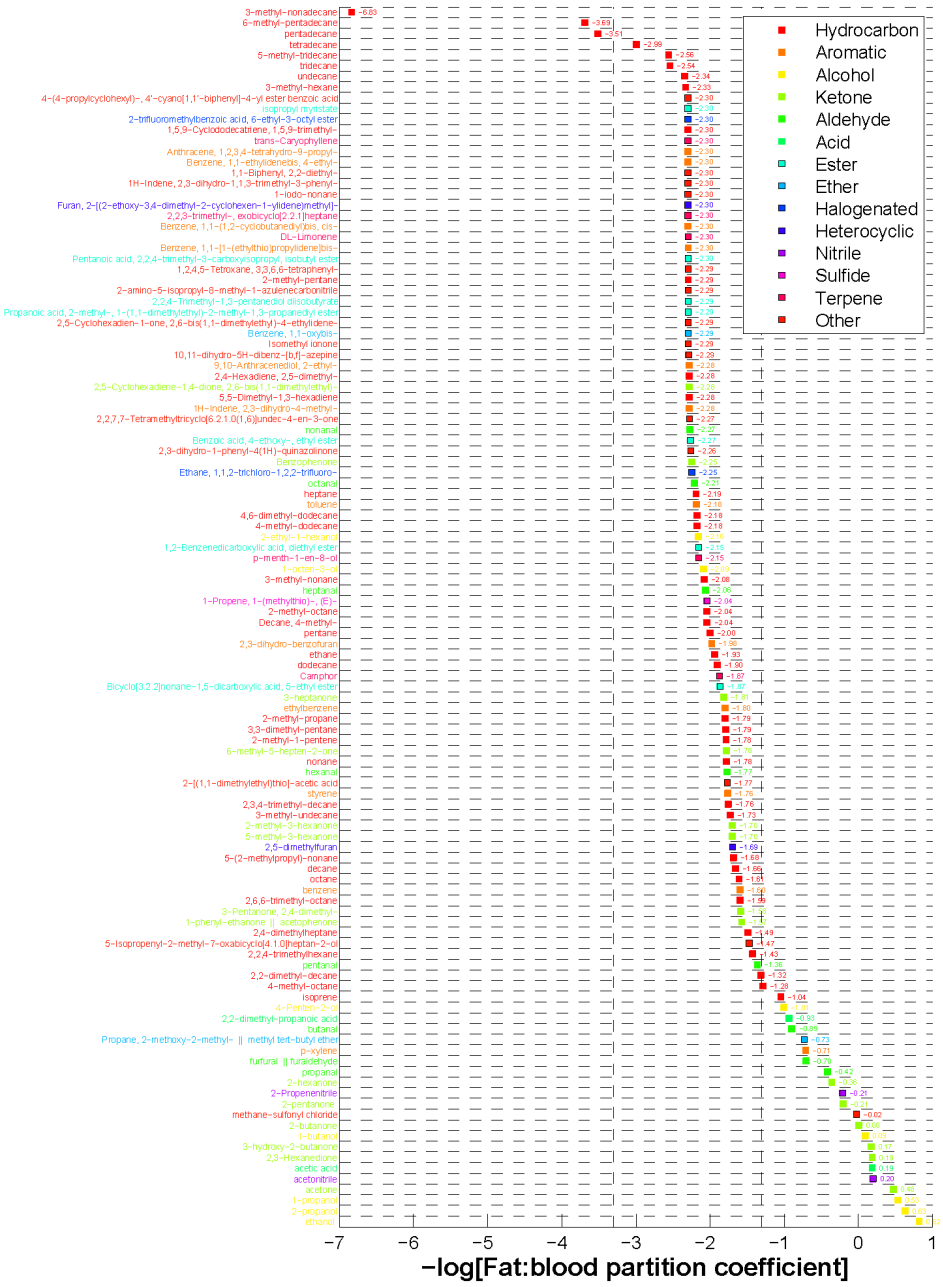

Figure 3: Partition coefficient, -log($\lambda_{f:b}$), for 112 volatile cancer biomarkers published during the last decade [7, 30, 67, 109-121], as well as acetone and 2-pentanone for comparison. The color for the different compound names is chosen according to the chemical class. Compounds with a higher log($\lambda_{f:b}$) will tend to be in the fat and vice versa.

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