Clinical breath analysis: Discriminating between human endogenous compounds and exogenous (environmental) chemical confounders

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ABSTRACT

Volatile organic compounds (VOCs) in exhaled breath originate from current or previous environmental exposures (exogenous compounds) and internal metabolic (anabolic and catabolic) production (endogenous compounds). The origins of certain VOCs in breath presumed to be endogenous have been proposed to be useful as preclinical biomarkers of various undiagnosed diseases including lung cancer, breast cancer, and cardio-pulmonary disease. The usual approach is to develop difference algorithms comparing VOCs profiles from nominally healthy controls to cohorts of patients presenting with a documented disease, and then to apply the resulting rules to breath profiles of subjects with unknown disease status. This approach to diagnosis has a progression of sophistication; at the most rudimentary level, all measurable VOCs are included in the model. The next level corrects exhaled VOCs concentrations for current inspired air concentrations. At the highest level, VOCs exhibiting discriminatory value also require a plausible biochemical pathway for their production before inclusion. Although these approaches have all shown some level of success, there is concern that pattern recognition is prone to error from environmental contamination and between-subject variance. In this article, we explore the underlying assumptions for the interpretation and assignment of endogenous compounds with probative value for assessing changes. Specifically, we investigate the influence of previous exposures, elimination mechanisms, and partitioning of exogenous compounds as confounders of true endogenous compounds. We provide specific examples based on a simple classical pharmacokinetic approach to identify potential misinterpretations of breath data and propose some remedies.

Introduction:

Current research for early diagnosis of human disease, status of oxidative stress, and monitoring of health status of critically ill patients has exploited exhaled breath analysis as a non-invasive tool (Risby and Sehnert 1999, Modak 2007, Miekisch et al. 2004, Buszewski et al. 2007, Spanel and Smith 2011). The working hypothesis of all these studies is that endogenously produced volatile organic compounds (VOCs) reflect deviations from control (nominally healthy) metabolic (anabolic or catabolic) patterns that can be interpreted as pre-clinical markers of adverse health events (Basanta et al. 2010, Poli et al. 2010, Fuchs et al. 2010). In practice, exhaled breath from a patient is analyzed for VOCs and the results are assessed using a diagnostic algorithm derived from control *versus* affected cohort analyses patterns (Phillips et al. 1999, Peng et al. 2010). Many candidate discriminatory compounds are straight-chain, branched, or aromatic hydrocarbons, so there is concern that exhaled VOCs are not necessarily endogenous, as from lipid peroxidation or tumor activity, but instead reflect previous or current uptake of environmental contaminants (Cao and Dunn 2006, Mukhopadhyay 2004). Furthermore, there could be overlap between true endogenous biomarkers (e.g. acetaldehyde, ethanol, acetic acid, phenol, and 2-propanol) and metabolic products of exogenous exposures or metabolism of foreign organisms (bacteria, viruses, fungi or yeasts) present in the gut (Kischkel et al. 2010, Ulanowska et al. 2010, Shestivska et al. 2012).

The value of breath biomarker measurements as a non-invasive tool for health assessment is generally accepted in the medical community (Amann and Smith 2005). There are, however, different schools of thought as to how much detailed knowledge is required to achieve defensible results (Risby 2008, Pleil 2010, Amann et al. 2010, Pleil 2011). Many clinicians regard empirical pattern recognition such as principal components analysis or sensor array patterns to be satisfactory as long as there is sufficient resolving power to categorize unknown patients into control or affected groups. Understanding the true origins of discriminating compounds, whether from endogenous or exogenous sources, is thought to be unnecessary (Moser et al. 2005, Fens et al. 2009). A second level of complexity involves the simultaneous measurement of exhaled and inhaled air. Here the concern is that the clinic, hospital, or ventilator air could confound the exhaled air constituents and incorrectly ascribe environmental compounds to endogenous sources. The standard approach is to subtract the inhaled concentration from the exhaled concentration. This is referred to as the "alveolar gradient" approach, which if positive, defines the compound as endogenous (Phillips et al. 1997, 2003). Finally, the most conservative level of assessing and defining compounds relevant to disease diagnosis articulates a well-defined metabolic pathway capable of producing the compounds of interest; otherwise, they are disregarded for analysis (Risby 2008,). The recognition that previous exposure history, whether from air, food, or dermal contact, affects breath pharmacokinetics is not new; in fact, recent review articles by Pleil (2008) and by Beauchamp (2011) effectively capture the research performed in this arena.

Each of the three approaches for dealing with co-exposures has merit. The first (empirical pattern) is to ignore the environment and is therefore simple to implement; it does not require the complexity of simultaneous air, water, food, etc. analyses. The

second (alveolar gradient) has an advantage in that obvious environmental influences can be quickly recognized and eliminated, and the third approach (metabolic pathway) not only provides the confirmation of an exposure effect, but also suggests possible damage mechanisms.

For this paper, we explore the potential pitfalls in the commonly implemented alveolar gradient approach and suggest some potential mathematical and empirical procedures to improve the confidence of the interpretation of the status of endogenously produced compounds. We have used as our model an extensively studied compound, trichloroethylene (TCE); we realize that TCE is definitely not endogenous, but it has mid-range kinetic parameters, well defined classical pharmacokinetic behavior, and sufficient volatility that make it suitable for representing a variety of common compounds of unknown origin (e.g. alcohols, alkanes, ketones, aldehydes, terpenes) that might be found in exhaled breath samples (Raymer et al. 1992, Pleil et al. 1997, Fisher et al. 1998, Pleil et al. 1998).

All data used in this article to build models and calculate PK parameters have been previously published and have Institutional Review Board (IRB) approvals from the respective performing organizations.

There are three steps to demonstrating the character of exogenous compounds that may mimic endogenous biomarkers and thus confound patterns for discerning health state.

1. Develop a robust kinetic model for an extensively studied compound in exhaled breath that can be used to ascertain time dependent responses of typical exogenous exposures.
2. Develop an understanding for basic parameters of compounds (breath partition, absorption rate, etc.) and how they could affect the temporal response of uptake and elimination.
3. Apply the model to a hypothetical compound (of unknown origin) and demonstrate how different exposure scenarios could affect the interpretation of whether it can be classified as endogenous or exogenous.

Methods:

The first step in assessing the environmental contributions to the exhaled breath is to understand the mechanisms and timing associated with the blood to air exchange of VOCs. We propose a conceptual classical pharmacokinetic (PK) model that allows for both an environmental and an endogenous metabolic source of compounds into the first compartment defined as the circulating blood with elimination of these compounds via exhaled breath and metabolism. Second and third compartments of distribution can be exploited to simulate the timing from highly-perfused and poorly-perfused tissues, respectively. Under the assumption of linear kinetics, we write a set of difference equations that can be used to incrementally calculate the absorption, distribution, metabolism, and elimination (ADME) behavior of such compounds. With examples drawn from existing empirical biomarker data, we demonstrate the effects of different exposure, environmental, and metabolic scenarios on exhaled breath concentrations. This methodology will allow us to compare different classification schemes to assess their relative accuracy *versus* increased calculation and data collection effort.

Conceptual model:

To illustrate classical PK behavior resulting in exhaled breath levels of VOCs, we construct the following model:

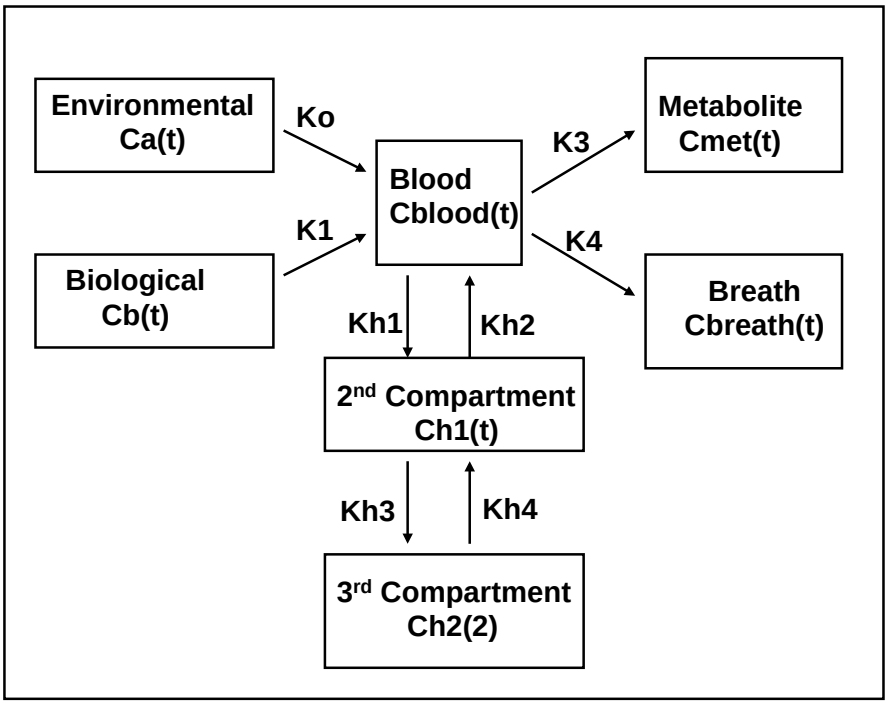

Figure 1: Conceptual ADME model for mixed environmental and metabolic inputs of VOCs to blood concentrations. Blood serves as the central compartment and exhaled breath is considered proportional to blood concentration. The "K's" serve as rate constants in units of 1/time for blood, tissues, and breath (Kh1, Kh2, Kh3, and Kh4 describing exchange among higher compartments and K3 and K4 describing losses from the central compartment), or in units of mass/time for environmental and biological inputs (Ko and K1). There are a series of simplifying assumptions that we make in the use of this empirical classical PK model:

1. Within-compartment mixing and gas exchange are rapid.
2. Time constants are independent of concentration (linear kinetics)
3. No energy is expended to maintain steady-state concentrations
4. Two or three theoretical compartments are sufficient to predict measurable behavior
5. All sources and losses are accounted for (mass balance)

We concede that human systems biology is much more complicated. Physiologically based pharmacokinetic (PBPK) models address these (and other) assumptions with additional compartments (for individual organs), more diffusion constants, calculations of induced and saturated metabolism, within and between-subject variance components and

other biological interactions derived from theory and animal studies (Kim et al. 2007, Clewell 1995, Furtaw 2001). However, we are only demonstrating the effects on exhaled breath from previous or concurrent exposures, so we assert that the classical PK model in Figure 1 is sufficient to approximate empirical ADME behavior without invoking more complex PBPK models for exhaled VOCs.

Difference equations:

Based on the model outlined in Fig. 1, we can write difference equations as:

$$\Delta Cblood(t)/\Delta t = Ko \times Ca(t) + K1 \times Cb(t) + Kh2 \times Ch1(t) - (Kh1 + K3 + K4) \times Cblood(t) \quad \text{eq. 1}$$

$$\Delta Ch1(t)/\Delta t = K'h1 \times Cblood(t) - K'h2 \times Ch1(t) + Kh4 \times Ch2(t) - Kh3 \times Ch1(t) \quad \text{eq. 2}$$

$$\Delta Ch2(t)/\Delta t = K'h3 \times Ch1(t) - K'h4 \times Ch2(t) \quad \text{eq. 3}$$

In addition, breath concentration is calculated as:

$$Cbreath(t) = K4 \times Cblood(t) \quad \text{eq. 4}$$

In equations 2 and 3, the **K'** notations for the rate constants indicate an empirical adjustment necessary to adjust for the differences in hypothetical compartment volume of distribution and to allow for some delay in mixing within these slower compartments.

We further note that the inspired concentration Ca(t) and the exhaled concentration Cbreath(t) are linked in this model as:

Cbreath(t)/Ca(t) = fr(t) and **fr(t = "large") = *f*** which is the *f*-value at steady state.

This "*f*-value" represents the fractional amount of the inspired concentration that is breathed back out because of the pulmonary steady-state established between the blood and breath; the value "1-*f*" therefore represents the steady state losses to metabolism and other elimination pathways (e.g. urinary excretion, trans-dermal respiration). Table 1 shows literature f-values measured empirically for a variety of exhaled VOCs. Note that these range from 0 to 1, depending on the compound.

Secondly, we consider the variability among compounds with respect to the partition of the analyte concentration between blood and breath. Although this parameter is generally referred to as the "blood/breath partition coefficient" and so in reality reflects the instantaneous exchange between alveolar blood and alveolar air, in practice, it is measured in the venous blood (usually from the antecubital vein in the arm) and in the bulk exhaled air and is labeled the "blood/breath ratio". These parameters can also be estimated *in vitro* as the "blood/air co-efficient". At steady state conditions, these parameters are essentially equal; therefore, for the purposes of the demonstrations here, we consider them equivalent. Table 1 presents literature values for this partition parameter for various compounds. Note that these have a wide range depending on the

solubility of the compound in blood and affinity to various tissues; the non-polar molecule, n-pentane, has a value of 0.38, whereas the very polar molecule, ethanol, has an accepted partition value of 2,100. Common environmental compounds are in the range 5 to 100, for example, chloroform ≈ 7, benzene and trichloroethylene ≈ 10, tetrachloroethylene ≈ 24, and o-xylene ≈ 70. Overall, the entries in Table 1 for both blood/breath partition and for f-value must be treated with some caution; we present them to show some typical values. We realize that within- and between-person variance components can be large, as can the differences among measurement techniques used by various groups of researchers.

This Table assembles the best available information from various peer-reviewed and internal government publications and so represents an overview of the current state-of-the –art for these parameters. Certainly, the differences in values from different studies represent unknown variability derived from the details of the research and human host factors including gender, age, ethnicity, workload, and body composition. These entries are meant to illustrate the differences among compounds that should be considered when correcting breath results at the individual study level, not as definitive correction factors.

Table 1: Parameter estimates for f-values and blood/breath partition.

Compound	f-value	bl/br or bl/air	reference
chloroform	0.06	-	Xu 2005
	-	7.4	Tan 2006
dichloromethane	0.23	12.0	Raymer 1993
carbon tetrachloride	0.26	-	Wallace 1996
1,1,1-trichloroethane	0.88	7.5	Raymer 1993
	0.21	23.0	Wallace 1996
1,1,1-trichloropropanone	0.03	-	Xu 2005
dichloropropanone	0.01	-	Xu 2005
trichloroethylene	0.23	-	Gage 1977
	0.28	5.4	Chiu 2007
	0.19	12.0	Wallace 1996
	0.23	9.7	Pleil 1998
	0.22	-	Raymer 1993
tetrachloroethylene	0.45	23.7	Chiu 2007
	0.75	10.0	Wallace 1996
m,p-dichlorobenzene	0.44	272.0	Wallace 1996
1,3-butadiene	0.80	1.9	Perbellini 2003
2,2-dimethylbutane	-	0.3	Perbellini 1985
2-methylpentane	-	0.4	Perbellini 1985
3-methylpentane	-	0.4	Perbellini 1985
3-methylhexane	-	1.3	Perbellini 1985
methylcyclopentane	-	0.9	Perbellini 1985
cyclohexane	-	1.3	Perbellini 1985

pentane	-	0.4	Perbellini 1985
hexane	0.35	-	Raymer 1993
	-	0.8	Perbellini 1985
heptane	-	1.9	Perbellini 1985
octane	-	3.1	Smith 2005 (rat)
	0.15	-	Wallace 1996
nonane	-	5.8	Smith 2005 (rat)
	0.14	-	Wallace 1996
decane	0.25	-	Wallace 1996
	0.10	-	Raymer 1993
	-	8.1	Smith 2005 (rat)
undecane	-	20.4	Smith 2005 (rat)
	0.23	-	Wallace 1995
dodecane	-	24.6	Smith 2005 (rat)
	0.25	-	Wallace 1996
benzene	0.17	11.0	Wallace 1996
	-	7.8	Sato 1979
	-	11.3	Perbellini 2003
	0.50	-	Pezzagno 1995
	0.31	-	Gage 1977
toluene	0.16	32.8	Raymer 1993
	-	15.6	Sato 1979
styrene	0.20	-	Wallace 1996
	-	51.9	Sato 1979
	0.50	73.7	Brugnone 1993
ethylbenzene	0.10	40.0	Wallace 1996
	0.08	51.9	Raymer 1993
	-	28.4	Sato 1979
m-xylene	-	26.4	Sato 1979
p-xylene	0.08	110.0	Raymer 1993
	-	37.6	Sato 1979
m,p-xylene	0.08	-	Wallace 1996
	-	26.4	Tardif 2004
o-xylene	0.08	-	Wallace 1996
	0.06	70.4	Raymer 1993
	-	31.1	Sato 1979
cumene	-	37.0	Sato 1979
methanol	0.23	-	Batterman 1998
acetone	-	245.0	Sato 1979
ethanol 101.7 ppmv	0.25	-	Tardif 2004
ethanol 25.9 ppmv	0.29	-	Tardif 2004
ethanol 990.8 ppmv	0.25	-	Tardif 2004
ethanol	-	2100.0	*legal def
MTBE	0.24	16.9	Pleil 2007
	0.33	23.5	Lee 2001
MEK	-	202.0	Sato 1979

MIBK	-	90.0	Sato 1979
2,5-dimethylfuran	-	9.5	Perbellini 2003
N2O	0.98	-	Yasuda 1991
Halothane	0.56	-	Yasuda 1991
Isoflurane	0.71	-	Yasuda 1991
	0.75	0.66	Landon 1993
Desflurane	0.90	-	Yasuda 1991
Sevoflurane	0.82	0.65	Yasuda 1991

** the blood/breath partition coefficient has been defined legally to be 2100/1 in the United States and other countries for evidentiary purposes. In reality, it can vary among individuals (Jones 1996)*

Incremental calculations:

For compounds with available kinetic data, one can develop estimates for the various rate constants and calculate the biological response for any combination of input functions (Pleil 2008). The incremental model is implemented for two compartments as follows:

$$\mathbf{Cblood(t+\Delta t) = Cblood(t) + [Ko \times Ca(t) + K1 \times Cb(t) + Kh2 \times Ch1(t) - (KH1 + K3 + K4) \times Cblood(t)] \times \Delta t} \quad \textbf{eq. 5}$$

$$\mathbf{Ch1(t+\Delta t) = Ch1(t) + [K'h1 \times Cblood(t) - K'h2 \times Ch1(t)] \times \Delta t} \quad \textbf{eq. 6}$$

For additional compartments, calculations are modified to reflect the structure of equations 2 and 3 above.

From any current value of **Cblood(t+Δt),** we estimate the concurrent exhaled breath level as:

$$\mathbf{Cbreath(t+\Delta t) = Cbreath(t) + [K4 \times Cblood(t)] \times \Delta t} \quad \textbf{eq. 7}$$

With pragmatic choices of **Δt** and initial conditions coupled with reasonable estimates of the "K's", these equations can be used to incrementally build the full behavior of the exhaled breath function over time depending on the input function Ca(t) and the calculated blood concentration Cb(t) as presented in Figure 1. Experience has shown that the half-times for the central compartment (blood) for typical volatile compounds is on the order of 2 minutes (Kh1+K3+K4 ~ 0.5/min), for the 2nd compartment on the order of 20 min (Kh2 ~ 0.05), and for the 3rd compartment on the order of hours (Kh3 ~ .005) (Pleil and Lindstrom, 1997 and 1998).

We note that there are generally no empirical measurements possible for the higher order compartments, so these parameters are empirically estimated to achieve a proper fit to blood or breath data. We further note that the compartments reflecting poorly perfused tissues could be approximated with a baseline constant as their half-times (hours to days) are much greater than any practical observational period in the clinic.

Model demonstration:

To demonstrate the behaviors of "typical" VOCs, we implement the conceptual and mathematical model with examples from the literature wherein concentrations and parameters have been published. Specifically, we use published data of trichloroethylene (TCE) (Raymer 1992) to develop 1, 2, and 3 compartment models, and then overlay the model onto multiple data sets (Raymer 1992, Pleil 1998).

Model Implementation:

The models are implemented to assess the effect of unknown (previous) exposures and an exposure period in the clinic where ambient measurements are made. We assume that all environmental and clinic exposures are through the inhalation pathway and that the subject/patient is at steady state with his environment before entering the clinic. We recognize that pulmonary and gut microbiota could also concurrently influence the exhaled breath, but for the purposes here, we treat these as stable contributions to the overall human (endogenous) metabolism. We also assume that breath samples are made accurately, the subject is at rest, that the samples reflect end-exhaled air without contribution from the tracheal dead-volume, and that our hypothetical patients breathe at a steady rate of 1,000 liters/hour.

Specifically, we consider the following scenarios:

Table 2: Hypothetical scenarios for demonstrating the environmental impact on estimating the concentrations of endogenous compounds in breath.

Scenario #	**Previous (environmental) exposure**		**Clinic exposure**		**Metabolic production**
1	10 ng/m3	*f* x 10 ng/hr	1,000 ng/m3	*f* x 1,000 ng/hr	0 ng/hour
2	1,000 ng/m3	*f* x 1,000 ng/hr	10 ng/m3	*f* x 10 ng/hr	0 ng/hour
3	1,000 ng/m3	*f* x 1,000 ng/hr	1,000 ng/m3	*f* x 1,000 ng/hr	0 ng/hour
4	10 ng/m3	*f* x 10 ng/hr	1,000 ng/m3	*f* x 1,000 ng/hr	200 ng/hr
5	1,000 ng/m3	*f* x 1,000 ng/hr	10 ng/m3	*f* x 10 ng/hr	200 ng/hr
6	10 ng/m3	*f* x 10 ng/hr	10 ng/m3	*f* x 10 ng/hr	200 ng/hr
4*	10 ng/m3	*f* x 10 ng/hr	1,000 ng/m3	*f* x 1,000 ng/hr	500 ng/hr
5*	1,000 ng/m3	*f* x 1,000 ng/hr	10 ng/m3	*f* x 10 ng/hr	500 ng/hr
6*	10 ng/m3	*f* x 10 ng/hr	10 ng/m3	*f* x 10 ng/hr	500 ng/hr

** indicates scenarios with a higher metabolism component making them "more obvious" compared to the hypothetical environmental levels.*

This table is a collection of different potential combinations of exposures at relatively high levels to use as examples. Basically, we propose that the scenarios cover the extremes of possible conditions that may result in difficulty assigning endogenous or exogenous status for measured compounds in exhaled breath. The scenarios marked with

"*" are intended to illustrate a very significant metabolic source in contrast to either ambient or clinic exposures.

We note that this table has mixed exposure units. From a practical perspective, inhalation air is measured as ng/m^3; to compare this to metabolic production rate, we convert to an uptake rate expressed in units of (*f* x ng/hr) at steady state assuming a stable ventilation rate of 1 m^3/hr. The steady state *f*-value depends on the particular compound and lies within the range from 0 to 1 (see Table 1). For implementing the kinetic models, we cannot use the *f*-value directly and so calculate the actual function *fr*(t) as described above. Furthermore, we use a finite value (i.e. 10 ng/m^3) for the "lowest" environmental exposures to reinforce the concept that all endogenous compounds exist in the environment at some non-zero level.

Endogenous status assignment

Given a pair of breath and environmental measurements of some compound made in the clinic, that is inspired air (A) and exhaled breath (B), there are a number of ways of combining these to assign a status of endogenous or exogenous source. The most common method is a simple subtraction: (B-A), which, if positive, is assigned endogenous status, and as discussed above, is referred to as the "alveolar gradient". Other possibilities are ratios such as (B/A), which, if greater than 1, is assigned endogenous status, and (B-A)/(B+A) which, if positive, indicates endogenous status and has the additional value of returning a bounded value between -1 and +1. One can also test the adjustment of "effective" environmental exposure as expressed in breath by using (f x A) in place of A. The practicality and accuracy of two different judgment methods is interpreted using the various scenarios of Table 2 for a hypothetical compound with *f*-value and ADME behavior of TCE.

Results:

To demonstrate the incremental model approach, we use available time dependent data for TCE as presented and/or described in the literature (Pleil et al. 1998, Raymer et al. 1992, Wallace et al. 1997). We caution that TCE is not considered an endogenous compound, but serves well as an example here as we have developed a robust PK model based on empirical blood and breath measurements from EPA studies. First we employ the conceptual models as outlined above and construct an empirical fit to a long term (10 hr) chamber exposure at 2750 ng/liter using 1, 2, and 3 compartment models overlaid onto the data for five subjects (Raymer 1992). Figure 2 demonstrates the effect of adding higher theoretical compartments.

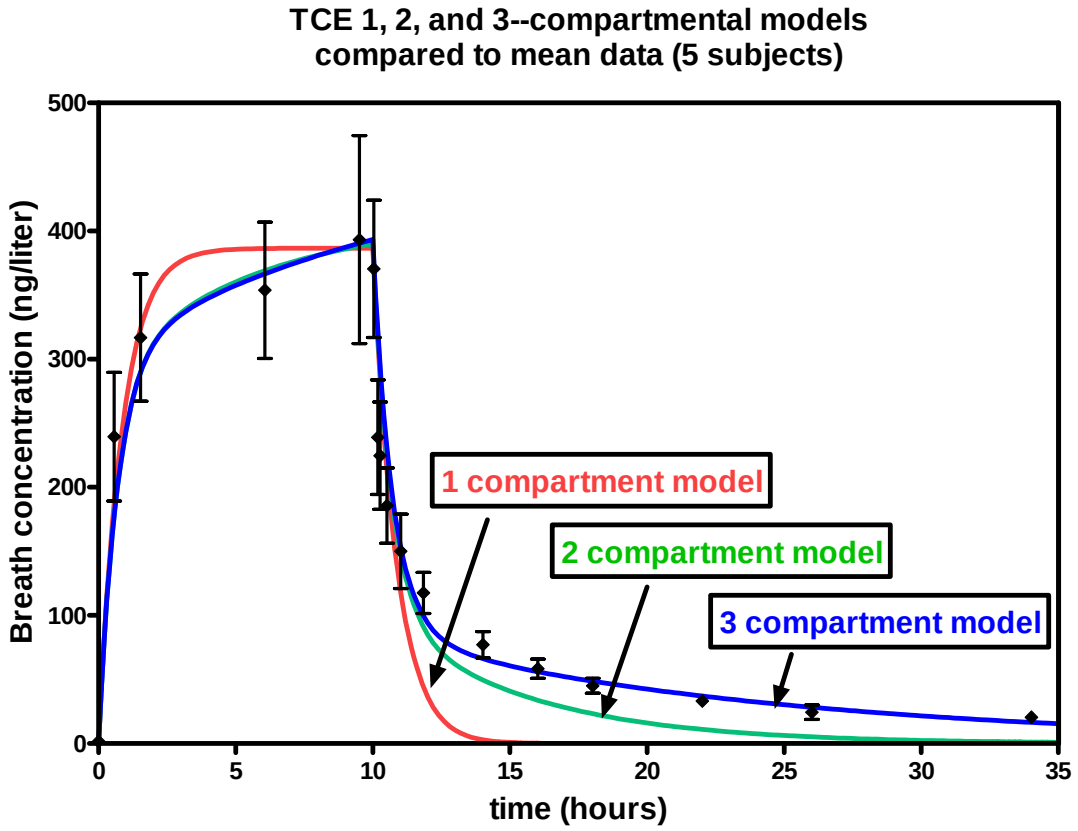

Figure 2. Comparison of 1, 2, and 3-compartmental models with empirical data. All three models capture uptake and steady-state, but only the 3-compartment model properly captures the long-term elimination data. Error bars represent the 95% confidence interval for 5 independent subjects (data from Raymer, 1992).

As seen in Fig. 2, both the 1 and 2-compartment models capture the dynamic behavior of the uptake and elimination phases reasonably well, especially for the crucial steady-state time span where the instantaneous change in the blood level is zero. From eq. 1 above, we can set $\Delta C_{blood}(t)/\Delta t = 0$, and further realize that the net transfer between the

central compartment and the 2nd compartment is essentially zero as well, leaving the reduced approximation:

Ko x Ca(t) + K1 x Cb(t) ≈ (K3 + K4) x Cblood(t) **eq. 8**

which helps estimate the elimination terms as the left side of the equation are known inputs. The later breakdown occurs because the slower 3rd compartment (poorly perfused tissues) that contributes to the delayed low-level elimination is not yet included. Pragmatically, 2-compartments are usually acceptable as patients will not likely be under observation for such extended periods of time, however the 3-compartment model is clearly more useful for assessing long-term elimination.

So far, Figure 2 only demonstrates a "bootstrap" confirmation; that is, we have shown as a first step that the character of the model assumptions is likely correct in that we could fit our model onto the empirical data from which it was created. The next step is to apply the exact same model to TCE data-sets that were not used to construct the model. Figure 4 (semi-log presentation) shows the 3-compartment model applied to the original data-set (10 hr exposure, 2750 ng/l), and to a 2-hour exposure at 1900 ng/l (Raymer 1992). We also include a data-set from a study where 6-subjects were exposed for 4-hrs at level about 250 times higher at 600,000 ng/l (Pleil 1998).

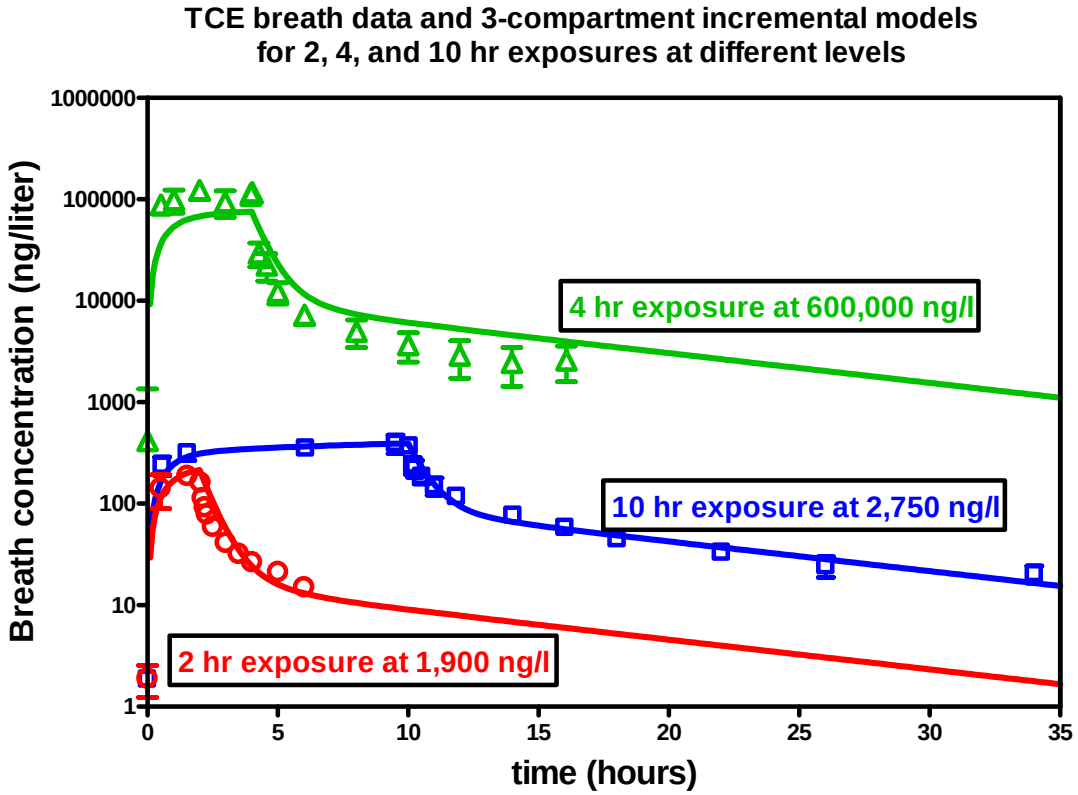

Figure 3. Application of incremental 3-compartment TCE model to three different time-dependent data-sets (Pleil et al, 1998, Raymer et al. 1992a, Raymer et al. 1992b, Wallace et al. 1997)

The comparisons in Figure 3 show that the model is relatively robust across a wide range of exposure times and exposure levels. The high exposure at 600,000 ng/l (about 100 ppmv) displays non-linearity of uptake; that is, the subjects are actually absorbing more than the model expects during the uptake phase and therefore the measured exhaled concentrations are higher than predicted. Subsequently, the model overcompensates during the elimination phase and predicts higher concentrations than measured. In fairness, we note that such high exposures are only possible in confined spaces under special conditions (typically in occupational scenarios) and so should not be considered for further discussion regarding incidental (unknown) previous exposures.

We demonstrate the effects of the scenarios outlined in Table 2 as groups of curves relating exhaled breath concentration vs. time. Recall that we assume that the patient is at steady state with his or her environment when he arrives at the clinic and that we only have access to objective data while the subject is physically present in the clinic. We also assume that the clinic atmosphere has stable concentrations for all scenarios. Figure 4 shows the family of curves for TCE that has estimated *f*-value of 0.37 and blood/air partition co-efficient of 9.7, respectively (from Table 1). The time scale is set to reflect time in hours and assumes that the patient arrives at the clinic at 9 am. Note that for modeling and graphing purposes we used military time; in the tables and graphs, "16" is 4 pm.

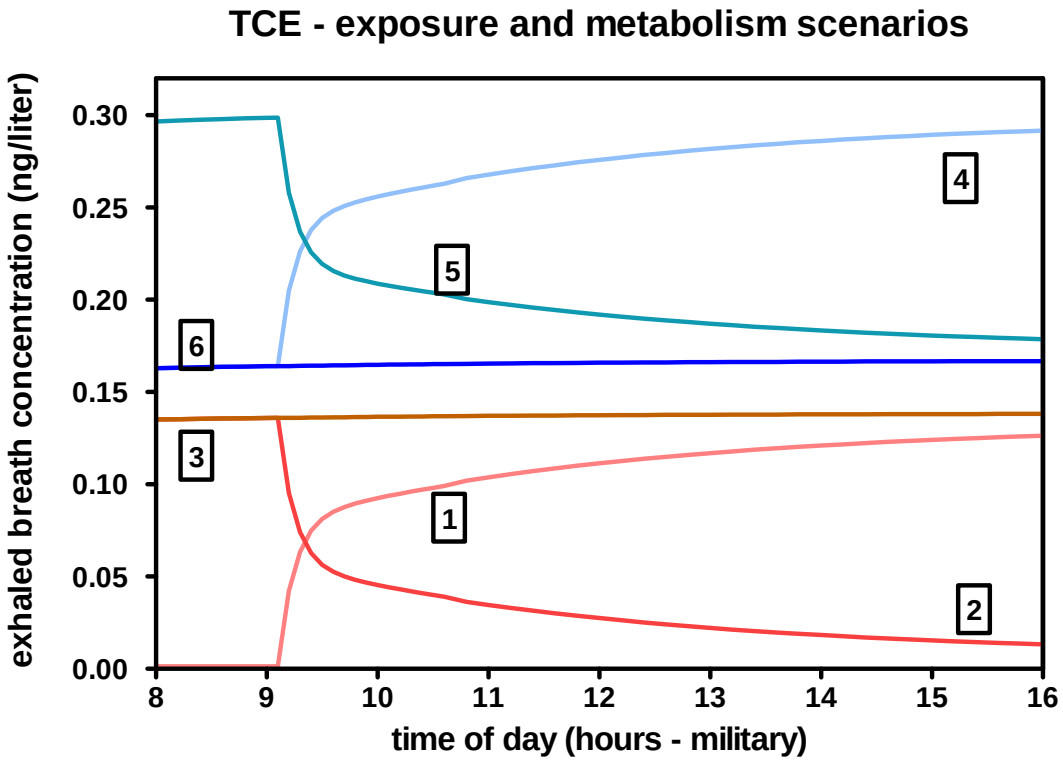

Figure 4. Calculated time dependent exhaled breath concentrations subject to six hypothetical exposure and metabolism scenarios as described in Table 2.

Under the assumption that the hypothetical patient arrives at 9 am, and that we measure his exhaled breath at 10 am, 12 pm, and 4 pm (16 hrs), we construct a table for deciding the endogenous nature of the exhaled compound. Note that TCE is not considered an endogenous compound, but for the purposes of this example, we will treat it as an unknown compound that may or may not be endogenous as defined by the scenarios in Table 2.

Table 3. Hypothetical scenarios for previous exposure, clinic exposure, and metabolic production of hypothetical compounded calculated for alveolar gradient and modified alveolar gradient

Scenario	metabolite produced (ng/hr)	previous air exposure (ng/m3)	clinic air exposure (ng/m3)	expected metabolite? (yes/no)	B-A 10:00 AM assigned metabolite? (right/inc/wrong)	B-A 12:00 AM assigned metabolite? (right/inc/wrong)	B-A 4:00 PM assigned metabolite? (right/inc/wrong)	B-fA 10:00 AM assigned metabolite? (right/inc/wrong)	B-fA 12:00 AM assigned metabolite? (right/inc/wrong)	B-fA 4:00 PM assigned metabolite? (right/inc/wrong)
1	0	10	1000	no	right	right	right	right	right	right
2	0	1000	10	no	*wrong*	*wrong*	inc	*wrong*	inc	inc
3	0	1000	1000	no	right	right	right	right	right	right
4	200	10	1000	yes	*wrong*	*wrong*	*wrong*	*wrong*	inc	right
5	200	1000	10	yes	right	right	right	right	right	right
6	200	10	10	yes	right	right	right	right	right	right
4*	500	10	1000	yes	*wrong*	*wrong*	*wrong*	right	right	right
5*	500	1000	10	yes	right	right	right	right	right	right
6*	500	10	10	yes	right	right	right	right	right	right

From this exercise, we see that a simple subtraction of ambient concentration from breath concentration (B-A) is actually a reasonable approach for most scenarios for assessing the endogenous nature of the compound. The method breaks down for subjects with high (but unknown) previous exposures that do not produce a metabolite, and for subjects producing metabolites in conjunction with high concurrent clinic exposures. In some cases the method is inconclusive ("inc" designation in Table 3) in that the subtraction gives a value indistinguishable from zero.

If we, however, interpret the data using the "*f*-value" concept, these incorrect assignments are improved to some extent. Although it is still difficult to correct for unknown previous exposures, a time delay of a few hours during which the subject can approach a steady-state condition with the clinic exposures does improve the assignment of metabolite status.

Conclusions:

The demonstrations and calculations in article alert the breath measurement community to some potential pitfalls and recovery methods for interpreting the exhaled breath to estimate environmental exposure and make clinical assessments. We conclude that the analysis of breath is a powerful tool for a variety of clinical and environmental diagnostic investigations; however, it is of the utmost importance to understand what the exhaled breath measurements actually mean. We show that there are two underlying physiological parameters that can affect the measurement of trace compounds in breath: 1. "*f*-value" describing how the human absorbs and then re-emits compounds from the air, and 2. blood/breath ratio that describes how much of a blood-borne compound is actually emitted into the breath. Although they may be related to some extent, these two parameters are very different in how they are used to interpret breath data. The "*f*-value" is primarily an indicator to gauge uptake (time dependent dose) of exogenous compounds, whereas the blood/breath ratio is a modeling tool for interpreting current circulating blood levels of any volatile chemical, metabolic or exogenous, based on a simple breath measurement. Both parameters can vary depending on a compound's chemical properties such volatility, polarity, and solubility in blood, as well as human parameters such as age, gender, body mass index, and health state. Table 1 shows interpreted literature values for these parameters as a guide.

A second consideration is identification and quantitation of human metabolic processes as expressed in exhaled breath. We have shown herein that exogenous and endogenous biomarkers are easily confused, not because of inattention or error by the investigators, but because exhaled breath was just previously inhaled from the surrounding microenvironment. We conclude that the days of ignoring the immediate environment's influence on exhaled breath constituents have been over since Michael Phillips began developing the concept of the alveolar gradient. Additionally, we conclude that a simple subtraction of inspired concentration may not go far enough as it does not account for previous exposures before the patient or subject arrives at the clinic. Finally, the kinetics of uptake and elimination may further complicate data interpretation in that the physiological parameters of blood/breath partition and the inspired/exhaled ratio are dependent on compound type, and only constant at steady state. Before exhaled compounds are assigned to a particular meaning, we need to be aware of confounding temporal, exposure, or other parametric influences contributing to the analyses.

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