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Exhaled breath aerosol (EBA): the simplest non-invasive medium for public health and occupational exposure biomonitoring

JD Pleil¹, MAG Wallace¹, and MC Madden²

¹National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, United States of America

²National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, United States of America

Overview

Blood, breath and urine represent the three primary diagnostic fluids for assessing environmental exposures and human health state. Although there are overlaps in biological media choice for many applications, each have their individual strategic and analytical advantages: blood is representative of the overall human exposome and metabolism in that it contacts all living cells in the body; breath represents the gas exchange between the blood and the ambient air as well as pulmonary function; and urine represents the elimination of wastes from environment and metabolic processes. As such, given a specific diagnostic need, the first step is generally to choose the most appropriate sampling approach. Often, this choice is not only driven by the chemistry, but also by the practical aspects of sample collection.

Exhaled breath collection has long been recognized as requiring the least invasive methods, and so is preferred for environmental and public health studies. In contrast to blood and urine, breath sampling does not require trained medical personnel or privacy, does not create potentially infectious wastes, and can be done essentially anywhere in any time frame. In the medical/clinical communities, blood and urine are still considered the gold standard; however, breath analysis is coming into favor for monitoring health states of compromised patients on ventilators, monitoring anesthesia during operations, and for tracking asthma status in children where more invasive techniques are impractical.

This commentary begins with the premise that breath biomonitoring is generally preferred for certain logistical reasons in public health and occupational biomonitoring. Three different fractions of exhaled breath are discussed: gas-phase, liquid condensate, and aerosols. The ultimate focus is on the biological constituents of deposited aerosols found on mask and filter surfaces wherein the gas and liquid phases are not collected. For now, this methodology is relegated to qualitative assessments; ongoing research explores relative quantitation from wipe and extractive samples.

Gas-phase breath collection

Originally, breath was considered only for gas-phase monitoring and was collected into bags or rigid containers for subsequent laboratory analysis. In addition, some clinical applications were developed wherein the patients' breath is directly analyzed by specialized gas monitors. Such monitoring is especially useful for assessing recent environmental exposures to volatile organic compounds (VOCs), and for patterns of endogenous metabolites for health diagnostic applications. Collecting the gas-phase requires strict quality assurance regarding leaks and additional attention to shipping and handling as the samples tend to be bulky (Wallace et al 1986, Lindstrom and Pleil 2002).

Condensed phase breath collection

Over the past 25 years, the concept of collecting exhaled breath condensate (EBC) was developed wherein the subject breathes through a chilled tube for 10 min and only those analytes captured in the resulting ice/water sample are analyzed. Although the EBC format discriminates against most non-polar VOCs, it has the advantage of collecting polar compounds and heavier analytes including semi- and non-volatile organics, cytokines, proteins, cellular fragments, DNA, and bacteria (Peralbo-Molina et al 2016, Xu et al 2012). Field sampling requires dry ice or another coolant, and access to a laboratory-like space to transfer samples into vials. Ultimately, the samples are very small, typically 1–2 ml of liquid, but they need to be shipped on ice to maintain integrity (Mutlu et al 2001, Grob et al 2008). Furthermore, standardization of the sampling process is still undergoing research, especially for controlling breathing rates and volumes (Winters et al 2017).

Exhaled breath aerosol collection

The newest form of breath sampling collects only the exhaled breath aerosol (EBA) fraction. In addition to the expected gases and water vapor, the exhaled breath also contains tiny aerosols (including both liquid and solid particles) that are created by surface film disruption at the alveolar level and by upper airway turbulence. These aerosols give mobility to materials that are otherwise relegated to the liquid layers within the lung and, as such, are that part of the EBC which contributes the non-volatile analytes (Schwarz et al 2010, Johnson and Morawska 2009). EBA impacts surfaces forming a layer; only the residues are subsequently harvested using various wiping or extraction techniques. Sampling is simplified as the subject only wears a mask for some period of time. This could be either a surgical-style mask for public health monitoring, or a standard mask used for occupational respiratory protection (figure 1). The elegance of the technique is that only dried residues are transported to the lab; there are no special requirements for shipping.

Focus of the commentary

This commentary proposes selected applications for EBA biomonitoring wherein the sampling logistics dictate ultimate simplicity, such as for public health and occupational biomonitoring with the express purpose of avoiding interference with normal activities.

EBA rationale

The rationale for exploiting non- or low-volatility compounds rather than conventional VOCs approaches for environmental, metabolomic, health state, and odor-based diagnostics was developed in response to observations and specific needs from the biomonitoring community. (Note, we adhere to the definition of ‘low-volatility’ as representing mass loss less than 5% by evaporation under ambient conditions at 6 months.)

Organics detection by dogs

The initial concept for EBA analysis arose from the puzzle: how can dogs track animals or humans through the woods long after any residual VOCs would likely have dissipated? Could the probative chemicals be much less volatile substances that are ‘rattled-off’ surfaces by the wind, the dog’s own activities, and high velocity sniffing? This hypothesis was bolstered by the contention from certain organizations that trained dogs can diagnose cancer based on simple surgical masks worn by subjects for 10 min and then sent through the mail (www.cancerdogs.ca, Hackner and Pleil 2017). Presumably VOCs are not captured initially on the paper mask, and any possible residual VOCs must long be gone from the shipping/handling procedures. As such, the probative signals are most likely coming from residual adsorbed low volatile organics or dried aerosols captured on the mask filter material.

Organics detection by laboratory instruments

The analytical biochemistry community has long used liquid chromatography, immunochemistry, and other specialized instrumentation to detect complex organic chemicals in liquid matrices, especially blood and urine. As indicated above, condensed phase breath (EBC) has now successfully joined the ranks of liquid samples for such analyses. In particular, a series of cytokines have been documented in all three media for assessing inflammatory response from human inhalation exposures (Stiegel et al 2015). Recently, we have documented various human fatty acids recovered from surgical masks (Pleil and Giese 2017). In ongoing research, we are now exploring cytokine recovery from presumed dried aerosols on other respirator and mask materials. As these endogenous compounds can only come from human sources and are proximate to large volumes of exhaled breath, the rationale for invoking the EBA sample pathway is reasonable. We note that analytical sensitivity is not a concern: EBA extractions and wipes are similar in relative concentrations to EBC for probative compounds.

EBA-proposed applications

The exploitation of EBA as the simplest of the biological media has merit for situations wherein avoiding interference with normal activities is paramount.

Public health

The most important consideration for public health and environmental health applications is to gather samples from as many and diverse subjects as possible. Statistically speaking, if we want to contrast parameters such as sex, age, socioeconomic status, ethnicity, body mass

index, etc., we will need hundreds of subjects to assure that all combinations are represented from sufficient numbers of individuals. This goal is thwarted by recruitment efficiency, sampling effort and cost.

We want to make the collection of samples as easy as possible to encourage participation; consider that asking the public for venous blood draws is less likely to gain volunteers than asking them to wear a paper mask for 10 min. Secondly, we want to streamline the sampling logistics; it is difficult to bring vials, waste bags, canisters, syringes, pipettes, solvents, cooled shipping containers, etc., into the field. Speaking for the research staff, bringing a few boxes of surgical masks and some shipping envelopes is much preferred. Finally, there is the cost of the study. The laboratory costs are generally fixed; however, the greatest limitation for collecting enough samples is often the cost involved with the field sampling procedures, which require travel, shipping of equipment, and purchase of expendable supplies. For example, to collect blood samples, one needs to provide trained medical personnel, a clinical setting, a variety of containers and expendable supplies, a mechanism for disposing infectious wastes, and specialized shipping (on ice). Sending a laboratory technician with surgical masks and mailing envelopes is certainly less costly. If we target specific endogenous and exogenous biomarkers, the simplicity and higher numbers of EBA samples can easily outweigh the additional probative value of more detailed fluids analyses. Furthermore, there is an added value in such sampling in that the inhaled air is filtered by the mask material; exogenous aerosol and particulate constituents could thus also be targeted for complementary analyses.

Occupational studies

For example, let us consider sampling firefighters for external exposures and health state (Pleil et al 2014, Fent et al 2014). Certainly, we cannot sample them during their work. However, post-activity swabs from the interior of their respirator gear are preferable to post-work collection of blood and urine; even minimally invasive breath sampling for 10 or 20 min could be considered bothersome after high-risk fire suppression activity. For example, if we consider that certain low-volatility compounds such as polycyclic aromatic hydrocarbons (PAHs) are markers of combustion products, then their presence as aerosol in the breathing zone can confirm the level of exposure independently of interaction with the subject. Furthermore, ongoing assessment of inflammatory cytokines and other probative messenger molecules could become a standard procedure for monitoring stress and health state from occupational activities. As such, implementing EBA collection directly from used respirators is an excellent non-invasive complement to standard environmental and health-based screening. The one caveat for replacing aerosol-only for total EBC sampling is that quantitation of analytes common to both methods is currently more of a challenge as deposition and recovery rates are dependent on surface adhesion properties.

Summary of EBA implementation

The proposed applications for EBA are, as yet, in their infancy. The concept, however, seems sound. In fact, there are distinct advantages to adding EBA methods to the overall biomonitoring and diagnostics arsenal. Direct, non-invasive access to low-volatility

pulmonary markers, even after some elapsed time, is a valuable feature as one does not have to plan ahead. For example, we have recently embarked on a series of experiments wherein we have sampled retrospectively from respirators used hours and days prior to collection. A second application is to use surgical-style masks for dual purposes; the exhaled aerosols from the subject are just as likely represented as those that have been collected from the ambient air during the inhalation phase of breathing. With judicious analytical targeting, both exogenous compounds and endogenous compounds could be monitored in the same sample format. Finally, the example of dog-based cancer diagnostics implies that low-volatility compounds found in EBA may provide an additional vector to standard VOCs pattern recognition for pre-clinical diseases.

For now, we are confident that we can measure a variety of non-volatile endogenous compounds from breath samples adhered or dried on surfaces; we expect to develop the additional capability for assessing different exogenous biomarkers in the near future. However, the quantitation aspects are as yet unknown, primarily because it is difficult to assess the collection efficiency of EBA onto different surfaces. Furthermore, the extraction efficiency, specific methods for collecting and processing samples for analysis, and other pragmatic laboratory procedures still need to be rigorously developed, and ultimately developed into a standardized protocol. In all, there is much work to be done to bring EBA to the forefront of breath-based diagnostics. In summary, we encourage the breath community to further explore EBA as a biological medium and hope to publish such new work in the *Journal of Breath Research*.

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References

- Fent KW, Eisenberg J, Snawder J, Sammons D, Pleil JD, Stiegel MA, Mueller C, Horn GP and Dalton J 2014 Systemic exposure to PAHs and benzene in firefighters suppressing controlled structure fires *Annals of Occupational Hygiene* 58 830–45 <https://academic.oup.com/annweh/article/58/7/830/157036> [PubMed: 24906357]
- Grob NM, Aytekin M and Dweik RA 2008 Biomarkers in exhaled breath condensate: a review of collection, processing and analysis *J. Breath Res.* 2 037004 [PubMed: 21386165]
- Hackner K and Pleil JD 2017 Canine olfaction as an alternative to analytical instruments for disease diagnosis: understanding ‘dog personality’ to achieve reproducible results *J. Breath Res* 11 012001 [PubMed: 28068294]
- Johnson GR and Morawska L 2009 The mechanism of breath aerosol formation *J. Aerosol Med. Pulm. Drug Delivery* 22 229–37
- Lindstrom AB and Pleil J 2002 A review of the USEPA’s single breath canister (SBC) method for exhaled volatile organic biomarkers *Biomarkers* 7 189–208 [PubMed: 12141064]
- Mutlu GM, Garey KW, Robbins RA, Danziger LH and Rubinstein I 2001 Collection and analysis of exhaled breath condensate in humans *Am. J. Respir. Crit. Care Med.* 164 731–7 [PubMed: 11549524]
- Peralbo-Molina A, Calderón-Santiago M, Priego-Capote F, Jurado-Gámez B and de Castro ML 2016 Metabolomics analysis of exhaled breath condensate for discrimination between lung cancer patients and risk factor individuals *J. Breath Res.* 10 016011 [PubMed: 26866403]

- Pleil JD and Giese R 2017 Integrating exhaled breath diagnostics by disease-sniffing dogs with instrumental laboratory analysis *Journal of Breath Analysis* 3 031001
- Pleil JD, Stiegel MA and Fent KW 2014 Exploratory breath analyses for assessing toxic dermal exposures of firefighters during suppression of structural burns *J. Breath Res* 8 037107 [PubMed: 25190461]
- Schwarz K, Biller H, Windt H, Koch W and Hohlfeld JM 2010 Characterization of exhaled particles from the healthy human lung—a systematic analysis in relation to pulmonary function variables *J. Aerosol Med. Pulm. Drug Delivery* 23 371–9
- Stiegel MA, Pleil JD, Sobus JR, Morgan MK and Madden MC 2015 Analysis of inflammatory cytokines in human blood, breath condensate, and urine using a multiplex immunoassay platform *Biomarkers* 20 35–46 [PubMed: 25495125]
- Wallace LA, Pellizzari ED, Hartwell TD, Whitmore R, Sparacino C and Zelon H 1986 Total exposure assessment methodology (TEAM) study: personal exposures, indoor-outdoor relationships, and breath levels of volatile organic compounds in New Jersey *Environment International* 12 369–87
- Winters BR, Pleil JD, Angrish MM, Stiegel MA, Risby TH and Madden MC 2017 Standardization of exhaled breath condensate (EBC) and exhaled breath aerosol (EBA) collection using a feedback regulated sampling device *J. Breath Res.* 11 047107 [PubMed: 28894051]
- Xu Z, Shen F, Li X, Wu Y, Chen Q, Jie X and Yao M 2012 Molecular and microscopic analysis of bacteria and viruses in exhaled breath collected using a simple impaction and condensing method *PloS One* 7 e41137 [PubMed: 22848436]

Figure 1.

Collage of different situations where EBA sampling could be implemented for environmental exposure testing and for occupational health state assessment. (Photos from official U.S. Government sources, US EPA, CDC, Air Force, and FEMA.)