Improved chemical and molecular analytical techniques allow measuring contaminants and/or early biological changes related to contaminant exposure directly in human biological fluids and tissues. It is a promising technique to estimate internal doses in the human body bridging the gap of knowledge between external environmental exposure and health effects. The joint workshop of the Belspo project ANIMO and the EU project COPHES addresses the potential of obtaining valuable information from human samples collected by non-invasive sampling techniques. This technology opens possibilities to include vulnerable population groups such as children in human biomonitoring studies for environmental health research. Challenges and opportunities will be discussed at the workshop.

A joint initiative of

EU COPHES project

(Consortium to perform human biomonitoring on a European scale www.eu-hbm.info)

and

the Belgian ANIMO project:


9.00 hr Registration with coffee, Poster setup

Morning session- Chair: A. Castano, A. Bernard

9.30 hr Welcome Belgian Federal Science Policy (Frank Monteny, FOD)

9.45 hr COPHES: Needs for biomarkers (L. E. Knudsen, University of Copenhagen, DK)

10.00 hr Non-invasive matrices in human biomonitoring – based on review (R. Smolders, VITO, BE)

10.25 hr A metabolomic approach by GC-MS to identify volatile organic compounds in exhaled air as indicators of disease (F. Van Schooten, Univ Maastricht, NL)
10.50 hr  Development and application of non-invasive biomarkers in exhaled breath  
(K. Bloemen, VITO, BE)

11.15 hr  Coffee break

11.30 hr  Indoor risk factors for childhood respiratory diseases: application of non-invasive 
biomarkers in a prospective study with schoolchildren  
(C. Voisin, UCL, BE)
11.55 hr  Metabonomics on analysis of biofluids (EBC and urine) of asthmatic children 
(C. Guillou, F. Reniero, European Commission, Joint Research Center, IT)
12.20 hr  NO, from chemical compound to non-invasive biomarker of airway inflammation 
(E. Peirsman, UA, BE)

12.45 hr  Lunch

**Afternoon session - chair: L. E. Knudsen, V. Nelen**

13.45 hr  State of the art in biomonitoring of non-invasive samples. Use of urinary samples 
(J. Angerer, H. Koch, IPA-DGUV, DE)
14.15 hr  Wheezing in preschool children is associated with increased levels of inflammatoty 
markers in exhaled breath condensate (KDG van de Kant, Maastricht University, NL)
14.30 hr  Attogram level measurement of POPs in human: From adipose tissue to dried-blood 
spots (J.-F. Focant, University of Liege, BE)
14.45 hr  Investigation of the use of salivary lead as a non-invasive marker of exposure to lead 
(Kate Jones, Health Protection Agency, UK)

15.00 hr  Coffee break + posters

15.30 hr  Sampling and analyses of exhaled particles- a new non-invasive method to monitor 
distal airways (Anna-Carin Olin, Sahlgrenska, SE)
15.45 hr  Non-invasive biomonitoring of metals using hair 
(M. Esteban, National Centre for Environmental Health, ES)
16.15 hr  Applicability of hair in human biomonitoring of organic chemicals 
(A. Covaci, UA, BE)
16.45 hr  Use of placenta and umbilical cord blood for HBM  
(L. E. Knudsen, University of Copenhagen, DK)
17.00 hr  Evaluation of non-invasive biomarkers of low-to-medium mercury exposure in 
humans (Janja S. Tratnik, Joseph Stefan Institute, Slovenia)
17.15 hr  Wrap up – future developments, views from the ethical/medical and environmental 
perspective  
(L. Casteleyn, University of Leuven, BE)
17.30 hr  Closure  
(G. Schoeters, VITO, BE)
Abstracts

TITLE:
COPHES: Needs for biomarkers

AUTHOR:
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ABSTRACT:
The use of biomarkers in environmental health is increasing due to increasing demands on information about health risks from unfavourable exposures and biomarkers provide information about individual load. The use of biomarkers as integrated measures of exposures and/or effects may assist in exposure source identification and demands of more integrated data for risk assessment. Urine and blood have been preferred media but more focus is on non-invasive tissue with examples shown in table from Neri et al (2006).

Non-Invasive

<table>
<thead>
<tr>
<th>Type of tissue</th>
<th>Mode of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccal epithelia</td>
<td>Swab of inner lining of cheek with tongue depressor or cytobrush</td>
</tr>
<tr>
<td>Saliva</td>
<td>Sterile plastic pipette or specially prepared cotton swab</td>
</tr>
<tr>
<td>Urine and urothelial cells</td>
<td>Separated by centrifugation</td>
</tr>
<tr>
<td>Nasal epithelia</td>
<td>Swab of inner lining of the nose with cytobrush or cotton swab</td>
</tr>
<tr>
<td>Cord blood</td>
<td>Drained into sterile container from the cord after delivery</td>
</tr>
<tr>
<td>Expired air</td>
<td>Spirometer attachment</td>
</tr>
<tr>
<td>Hair</td>
<td>In container after cut or fallen out</td>
</tr>
<tr>
<td>Finger nails</td>
<td>Clippings in sterile container</td>
</tr>
<tr>
<td>Extracted teeth</td>
<td>Collected in sterile container after loss</td>
</tr>
</tbody>
</table>

A number of ethical issues arise from use of biomarkers with predictive value aiming at respecting the autonomy of the study person in participation (only upon written informed consent and with obligations of withdrawal at any time), access to personal information (right to know and right not to know the study result) and securing proper data management (data protection to avoid misuse in employment, insurance, loaning and learning opportunities).

REFERENCES:
TITLE:
Applicability of non-invasively collected matrices for human biomonitoring

AUTHOR:
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ABSTRACT:
With its inclusion under Action 3 in the Environment and Health Action Plan 2004-2010 of the European Commission, human biomonitoring is currently receiving an increasing amount of attention from the scientific community as a tool to better quantify human exposure to, and health effects of, environmental stressors. Despite the policy support, however, there are still several issues that restrict the routine application of human biomonitoring data in risk assessment, one of the most restricting issues being the need to routinely collect human samples for large-scale surveys. Particularly for small children and babies, the collection of invasive samples suffers from ethical and practical limitations which hamper the implementation of human biomonitoring as an instrument for risk assessment and management. Unfortunately, these are the very populations most susceptible to the influence of environmental stressors, and for whom invasive sampling should be minimised. Children, pregnant women, elderly, or chronically-ill people are among those that would benefit the most from non-invasive, repeated or routine sampling. Because of these concerns, the use of non-invasively collected matrices for human biomonitoring should as much as possible be promoted as an ethically appropriate, cost-efficient and toxicologically relevant alternative for many biomarkers that are currently determined in invasive matrices. The presentation provides an overview of the most applicable non-invasively collected matrices that are currently utilised in human biomonitoring. For several non-invasively collected matrices, an overview of existing biomarkers of exposure and effect is presented, which are compared to biomarkers in other matrices. The main aim is to provide readers with an insight into the current state-of-the-art on the use of non-invasively collected matrices for human biomonitoring, and to illustrate how they could be further developed as matrices in environment and health research.
A metabolomic approach by GC-MS to identify volatile organic compounds in exhaled air as indicators of disease

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Exhaled air is a mixture of nitrogen, oxygen, carbon dioxide, water, inert gases and traces of volatile organic compounds (VOCs). In human breath thousands of different VOCs are exhaled and the idea behind the diagnostic value of VOCs is based on oxidative stress that occurs due to inflammatory processes accompanying disease. In the process of lipid-peroxidation polyunsaturated fatty acids in cell membranes are oxidized by the reactive oxygen species and a wide variety of VOCs as breakdown products are produced. The thus formed volatile organic chemicals demonstrate a very low solubility in blood and consequently as transported through the body by the bloodstream these VOCs will pass the blood lung barrier easily and become available in the gas phase of exhaled air. It has considerable advantages to analyse the gas fraction of exhaled breath and recently we have developed a new methodology to analyse the entire range of VOCs present in breath based on GC-MS analysis. We focussed on finding sets or profiles of VOCs related to disease instead of single biomarker evaluation in order to increase performance and robustness of our methodology. To determine which compounds are of interest regarding the classification of diseased versus healthy persons, we applied support vector machines (SVMs). We choose to use SVMs because of its ability to select those compounds that provide the best performance as implemented into a classifier, and its ability to construct predictive models with large generalization power even in the case of large dimensionality of the data or when the number of observations available for training is low. We performed studies on smokers, patients with inflammatory bowel disease (IBD) and inflammatory lung diseases (COPD, CF and asthma). Generally we could select a limited number of VOCs, that were able to correctly classify disease form healthy controls. Mostly sets of 6-8 VOCs showed sensitivities and specificities above 90%. From this it can be concluded that the analysis of a profile of VOCs in exhaled air provides an accurate, non-invasive, easy to perform diagnostic tool in the diagnosis of inflammatory diseased patients.

The project was financial supported by the province of Limburg, The Netherlands. We thank the patients and volunteers for sample donation.
TITLE:
Development and application of non-invasive biomarkers in exhaled breath

AUTHOR:
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ABSTRACT:
The ANIMO project addresses children’s respiratory health by developing non-invasive indicators which are easily applicable in children, which may enable to detect adverse effects in an early stage allowing preventive measures to be taken before disease outbreak, and which can be used in environmental research. Exhaled breath has been proposed as an interesting matrix for biomarker discovery since it can be easily collected in a wide range of individuals including children. In a pilot study, we focused on the measurement of exhaled nitric oxide (eNO) and the analysis of exhaled gasses and molecules in exhaled breath condensate (EBC) in astmatic and healthy children aged 6-12 years, for the development of a biomarker for asthma. Protocols for the analysis of exhaled VOC patterns and protein patterns in EBC were developed during the project. An improved procedure for sample preparation and detection of gasses and proteins was developed, together with a statistical method for comparison of the VOC / protein profiles between two groups. This approach enabled to study the exhaled VOCs and exhaled breath proteome for biomarker analysis, resulting in profiles to discriminate between the healthy and astmatic groups. Although the exact role in the disease development or physiological state of the airways of the gasses and proteins described in the presented potential biomarker patterns is not clear at this moment, this is an important step in the search for exhaled biomarkers for asthma. Additionally, specific exhaled markers such as leukotriene B4, 8-isoprostane and EBC pH were analysed in this pilot study. Predictivity of the selected profiles was studied in a follow-up of a birth cohort study. This study demonstrates the potential and feasibility of VOC and protein profiling to identify biomarkers for airway toxicity. The work performed in this study is a first step towards the development of non-invasive biomarkers that can be applied in environmental health biomonitoring studies and in clinical practice.

ACKNOWLEDGEMENTS:
This study was supported by the Belgian Science Policy (Contract number SD/HE/05A: ANIMO project). We thank the patients and volunteers for sample donation.
Indoor risk factors for childhood respiratory diseases: application of non-invasive biomarkers in a prospective study with schoolchildren

Catherine Voisin
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Children’s respiratory health is among the priorities of environmental health programs. There is a clear evidence that children are more susceptible to some stressors in the environment. Respiratory diseases are a major cause of illness in children in developed countries and asthma and allergies are increasing even up to 30% in certain age groups. Environmental factors are thought to affect a child’s likelihood to develop these diseases but are still largely unknown.

The ANIMO project addresses children’s respiratory health by developing non-invasive Biomarkers easily applicable in children that enable us to detect adverse effects in an early stage allowing preventive measures to be taken before disease outbreak, and which can be used in environmental research. We conducted an epidemiological study in 5 years-old children since September 2007 to May 2010. At 5 and 7 years old, a consentment and a questionnaire - inquiring about health, family history, environment and life style- were filled by the parents. Children were examined and tested twice with the following measurements: collection of exhaled breath condensate, spirometric tests, measurement of weight and length and of exhaled NO, collection of urine sample and of a nasal lavage, screening of sensitization to the most prevalent aeroallergens using the rhinosticks. We have recruited and examined a cohort of 394 young children originated from schools located in urban and rural areas. The protocol of the study could be applied successfully to almost all children at the exception of the rhinostick test that a few children (less than 10%) refused to perform. The exhaled NO, nasal lavage and EBC test did not pose any problem. Almost all children could also provide a sample or urine.

If the results obtained with EBC were not conclusive, exhaled NO values, rhinosticks and measurement of CC16 in urine and in the nasal lavage provide interesting information about the environmental exposure and the screening of some respiratory diseases or symptoms.

This study was supported by the Belgian Science Policy (Contract number SD/HE/05A: ANIMO project). We thank the patients and volunteers for sample donation.
TITLE:
Metabonomics on analysis of biofluids (Exhaled Breath Condensate and urine) of asthmatic children

AUTHOR:
Claude Guillou¹, Fabiano Reniero¹, Elia Mattaruchi¹ Guiseppe Giordano¹² and Eyugenio Baraldi²
¹European Commission, Joint Research Center, Unit of systems toxicology, Ispra, Italy and ²Department of Pediatrics, University of Padova, Italy
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ABSTRACT:
Omics technology is an approach generating comprehensive data sets of genes (genomics), mRNA (transcriptomics), proteins (proteomics), and metabolites of physiological substrates (metabonomics) or xenobiotics (metabolomics). Genomics and transcriptomics have been extensively studied and proven to be very useful for instance for the characterization of tumour cells. Specificity is limited by the difficulty in differentiating between adaptive response and adverse effects of a treatment. Cell type-specific responses and changes with time after exposure are additional problems that have not so far been solved for these techniques to be used for large-scale human biomonitoring or environmental exposure. Metabonomics is based on the analysis of Nuclear Magnetic Resonance (NMR) and/or Mass Spectrometry (MS) spectra for changes in basal metabolites excreted in urine has great potential, with the aim of pinpointing the best predictor variables for a given response variable (individual trait or exposure or dose). The method has early been recognized for its potential to identify novel biomarkers in preclinical testing of renal and hepatic toxicity and provide insight into mechanisms of action or for drug-induced vascular injury. The recent development of high throughput systems for Metabonomics application makes this approach very attractive for the reproducible analysis of large number of biofluid samples as required for Human Biomonitoring studies. Through scientific collaborations with hospitals, the Joint Research Centre has tested Metabonomics approach on small scale studies allowing some evaluation of the potential and current limitations of this approach. This approach has first been tested with NMR analysis of exhaled breath condensate (EBC) of asthmatic and healthy children¹. Further results are currently under evaluation using mass spectrometry on EBC and urine.

REFERENCES:
Non-invasive sampling techniques for human biomonitoring

10 December 2010

TITLE:
NO, from chemical compound to non-invasive biomarker of airway inflammation

AUTHOR:
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ABSTRACT:

Background: Fractional exhaled nitric oxide (FeNO) is a well-known biomarker of airway inflammation and can be measured using non-invasive techniques. FeNO is increasingly used in the diagnosis and management of childhood asthma.

Objective: The aim of the FeNO trial was to investigate the potential yield of incorporating FeNO measurements in the monitoring of paediatric asthma.

Methods: 99 children aged 5 to 14 years with a documented history of mild to severe persistent allergic asthma were included in a multicentre, randomized, parallel group effectiveness study. One strategy involved the determination of asthma control and treatment based on symptoms and spirometry according to GINA guidelines. In the second strategy supplementary FeNO measurements were performed to guide the decision-making concerning asthma treatment.

Results: Health outcomes were evaluated over a one-year timeframe. Asthma control, evaluated by a doctor every 3 months was similar for the two strategy groups. Determining asthma treatment with FeNO resulted in less asthma exacerbations and unscheduled asthma related contacts.

Conclusion: The implementation of FeNO measurement in clinical practice features a promising tool in the monitoring of childhood asthma, reducing the number of asthma exacerbations and unscheduled asthma related contacts.

ACKNOWLEDGEMENTS:

Participating physicians FeNO trial: Prof. Dr. Kristine Desager, Dr. Thierry Carvelli, Dr. Pierre Hage, Dr. Laurence Hanssens, Dr. Luc Pattyn, Dr. Marc Raes, Dr. Kate Sauer and Dr. Françoise Vermeulen.

Protocol author FeNO trial: Prof. Dr. Kristine Desager

Research (FeNO trial) was supported in part by a research grant from the Investigator Initiated Studies Program of Merck &Co., Inc. Aerocrine provided the NO analyzers (NIOX MINO) used in the study. Independent analysis was performed.

Medical students: Mathias Michiels, Nicolaas Vanderhoydonck, Ellen Vancamp, Karen Van der Gucht and Jolien Herbruggen.

All participating children.
TITLE:
State of the art in biomonitoring of non-invasive samples. Use of urinary samples

AUTHOR:
B. Schindler, J. Angerer & H.M. Koch
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ABSTRACT:
A huge number of state of the art human biomonitoring methods for analysing biomarkers in urine exists today. Most of these biomarkers in urine reflect exposures to non persistent environmental or occupational toxicants. The assessment and interpretation of urinary biomarkers of non persistent chemicals is fundamentally different compared to persistent chemicals (that are mainly analysed in blood). Cotinine, creatinine, phthalates and cadmium are only some of those established biomarkers which have been used in a large number of examinations on several populations.
Nevertheless there is still some uncertainty concerning urinary biomarkers of non persistent chemicals in terms of variable dilution of samples, interindividual and age-dependent variations in metabolism and spot urines used as a snap shot of a long term exposure scenario. Possible pitfalls will be identified and strategies to circumnavigate them will be provided.
Examples of our daily routine on phthalates and other biomarkers will help to illustrate the suitability of urine as a matrix for human biomonitoring of non persistent chemicals.
Wheezing in preschool children is associated with increased levels of inflammatory markers in exhaled breath condensate

Abstract:

Background

Wheeze is a common symptom among preschool children. About 30% of these children have persistent wheeze. The rest of the children have transient wheeze. So far it is not possible to differentiate between different wheezing phenotypes. In the ADEM study (Asthma DEtection and Monitoring study) wheezing patterns in young children are studied using non-invasive measurements in exhaled breath condensate (EBC). The aim of the study is to examine differences in inflammatory markers in EBC in preschool children with and without recurrent wheeze.

Methods

We recruited 250 children (aged 2-4 years) with (n=200) and without (n=50) recurrent wheeze. EBC was collected in an efficient condenser with a glass coating and a breath recirculation unit (patent:07102586). Various inflammatory markers (IL-1α, IL-2, IL-4, IL-5, IL-8, IL-10, IL-13, TNF-α, sICAM, CCL5, CCL11) were measured in EBC using xMAP® technology.

Results

The success rate of EBC collection was 95%. Elevated levels of IL-1α, IL-2, IL-4, IL-5, IL-8, IL-10, IL-13 and sICAM were found in EBC of children with recurrent wheeze compared to non wheezing controls (p<0.05, Mann-Whitney U test). No differences were found in levels of TNF-α, CCL5, CCL11 (p>0.05, Mann-Whitney U test).

Conclusions

EBC collection is possible in preschool children using an efficient condenser system. Various inflammatory markers in EBC are elevated in children with recurrent wheeze. This indicates increased airway inflammation in children with wheezing symptoms. Whether EBC is of use for discrimination between wheezing phenotypes remains to be determined during the longitudinal follow-up.
TITLE:
Attogram level measurement of POPs in human: From adipose tissue to dried-blood spots

AUTHOR:
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ABSTRACT:
For the last 25 years, background levels of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and related persistent organic pollutants (POPs) in humans have declined to the point that their measurement has become increasingly difficult. Improving sample preparation procedures to accommodate larger sample sizes has been carried out to a certain extent at the beginning of the last decade but these methods became of limited use because of the increasing demand to reduce the size of the specimen collected for human biomonitoring. Additionally, moving from an invasive adipose tissue collection to a more readily available blood (serum) matrix also contributed to reducing the quantity of the lipophilic toxicants available for measurement due to the reduced lipid content of serum. For this reason, more and more compounds are present at levels below the limits of detection (LODs) established using quality assurance/quality control (QA/QC) criteria. Therefore, increasing the sensitivity of the state-of-the-art gas chromatography coupled to isotope dilution magnetic sector high resolution mass spectrometry (GC-IDHRMS) measurement method is an area of considerable interest not only for analysts to report valuable congener-specific data but also for toxicologist who need a full picture to assess human exposure and the health impacts of these POPs. A liquid nitrogen jet-cooled thermal modulator has thus been coupled to GC-IDHRMS. The data acquisition parameters (scan rates and multiple ion descriptors (MIDs)) of the HRMS instrument have been optimized to accommodate the description of the narrow modulated GC peaks. The target sensitivity enhancement due to cryogenic zone compression (CZC) was maximized and resulted in the ability to detect low attogram (ag) amounts of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) (313 ag gives a S/N of 400:1), a level that could not yet been attained using classical GC-IDHRMS. Calibration spanned the range of 500 ag/µL to 35,000 ag/µL (R² = 0.9953). Analyses of a natural human reference serum-matrix NIST SRM 1589a showed 223 ag of 1,2,3,7,8-pentachlorodibenzo-p-dioxin (1,2,3,7,8-PeCDD) with a S/N of 188:1. Measurement of 2,2-Bis (4-chlorophenyl)-1,1,1-trichloroethane (DDE) and 2,2’,4,4’,5,5’-hexabromobiphenyl (BB-153) in human dried-blood spot (DBS) samples is also reported to illustrate the capacity of such a sensitive technique to move towards non(less)-invasive human biomonitoring. Some of the challenges related to sample preparation, blank levels, and to the fact of measuring of such a limited number of molecules (less than 600,000 TCDD molecules) are also discussed. The use of such a technique would be extremely valuable for considering biomonitoring studies in which babies and child are targeted.

REFERENCES:
TITLE:

Investigation of the use of salivary lead as a non-invasive marker of exposure to inorganic lead

AUTHOR:

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ABSTRACT:

Although environmental blood lead levels have dropped dramatically there are still concerns that lead exposure is a contributor to intellectual impairment in children and a contributing factor for other diseases. Blood is the preferred matrix for inorganic lead exposure however the invasive and traumatic nature of blood sampling from children and the elderly makes this matrix less acceptable for these age groups. Furthermore the need for trained phlebotomists and many other factors including ethical approval issues make blood far from ideal for human biomonitoring. Salivary lead measurements have been reported in the literature and compared with blood lead levels with varying success; differences in the methods of collecting saliva may have contributed to this.

This study collected 89 paired samples of blood and saliva from workers occupationally exposed to inorganic lead. Haemoglobin (in blood) and albumin (in saliva) were also measured. A collection device that collected a fixed volume of saliva was used and the sample was desorbed into buffer before analysis. The average sample volume after use was 2.3 ml (range 0.9 to 3.7 ml) with a coefficient of variation of 18.9%. Blood lead levels ranged from 2 to 54 µg/dl (i.e. all workers were below the current UK suspension limit of 60 µg/dl). Thirty one workers (35%) had blood lead levels less than 10 µg/dl, a benchmark which has previously been seen as an acceptable level for environmental exposures. All saliva samples contained measurable levels of lead (range 0.4 to 182.1 µg/l).

There was a linear correlation between log-transformed blood lead and salivary lead levels (correlation coefficient 0.65, p<0.001). When considering blood lead levels less than 10 µg/dl, the correlation dropped to 0.31 (p = 0.089), this correlation could be improved to 0.53 (p=0.002) by applying a linear regression model to ‘protein-adjusted’ results (blood lead adjusted for haemoglobin, µg/g and salivary lead adjusted for albumin, µg/mg, not log-transformed). Bland-Altman plots suggest that there is a reasonable agreement between the blood lead and salivary lead methods when looking at log-transformed data but that there may be a concentration-related correlation when using the ‘protein-adjusted’ results.

This pilot study gives encouraging evidence that blood lead and salivary lead are correlated (statistically significant) across the occupational exposure range. Further data are required at environmental exposure levels to further define the relationship but initial results are promising. The use of a standardised collection device is probably significant in obtaining consistent results.

ACKNOWLEDGEMENTS:

The project was sponsored by the Health Protection Agency Strategic R&D Fund.
Sampling and analyses of exhaled particles- a new non-invasive method to monitor distal airways

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ABSTRACT:
Background
We have developed a new method to sample non-volatile material in exhaled breath. The method is based on collection of endogenous particles in exhaled air (PEx) by impaction. PEx have been shown to contain phospholipids and phosphatidylglycerol in proportions resembling surfactant (1). We have also shown that the majority of PEx are formed during airway re-opening, i.e. in the terminal bronchiolii (2).

Aims:
1. To determine the protein composition of PEx in healthy subjects using a global proteomic approach
2. To examine the feasibility of analyzing Surfactant-protein A (Sp-A) in PEx, and to compare the repeatability of these analyses using PEx and EBC

Method
PEx were collected using an in-house built sampling device (1). All subjects breathed particle-free air three minutes prior to sampling.
1. Pooled samples from 6 respectively 8 healthy subjects were analyzed (total exhaled volumes of 3000L and 4400L, respectively). Extracted proteins were separated by polyacrylamide gel electrophoresis and subjected to in-gel trypsinolysis and analyzed by liquid chromatography coupled to mass spectrometry (LC-MS). The detected peptides were used to identify protein composition of PEx.
2. Nine healthy subjects were exhaling 100 L for PEx analyses, using a breathing maneuver allowing airway opening, and 100 L for EBC using tidal breathing, at two sampling-sessions within one week. Sp-A and Albumin in PEx and EBC were analyzed using ELISA.

Results
1. In total 133 proteins were identified from the two pooled samples, of which 100 are known from broncho-alveolar lavage. The majority of the proteins were either extracellular or membrane-associated. Apart from albumin the major protein was Sp- A, but also Sp-B andSp- C, CC16 and immunoglobulins were detected. No amylase was detected, indicating absence of saliva contamination.
2. SpA was detected well above the detection limit in all PEx-samples, but only in 5/18 EBC samples. The mean concentration (SD) of Sp-A in PEx was 2.6 (0.7) and Sp-A in EBC 0.2 (0.4), reported as arbitrary units. The number of PEx was significantly correlated to amount of Sp-A in PEx (rs=0.86 , p<0.01). The intra-individual CV for Sp-A in PEx was 13% and in EBC 76%.

Conclusions
Identification of surfactant proteins in PEx confirms their origin to RTLF. Sp-A in PEx is analyzed with good reproducibility. It seems important to register number of PEx for
quantitative analyses. PEx have a high potential as biological matrix to monitor pathophysiological changes in the distal airways.

ACKNOWLEDGEMENTS:
Swedish Heart and Lung Foundation
Swedish Research Council Formas

REFERENCES:
TITLE:  
Non-invasive biomonitoring of metals using hair

AUTHOR:  
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ABSTRACT:  
Human hair has been frequently employed in human biomonitoring (HBM) programs of environmental exposures. This biological matrix presents numerous advantages such as easy collection, low cost, easy transport and storage. Hair samples may be considered preferable over other matrices since they are less invasive under certain situations (e.g. pediatric exposures). Hair is a tough and highly stable structure built by sulfur-rich proteins. These proteins have the capacity to bind and trap many types of drugs, metabolites and environmental contaminants. Hair grows in cycles, with periods of growth and quiescence, with some differences depending on the body location. The growth rate of scalp hair is on average 12 centimeters per year, which makes segmental analysis (i.e., looking at concentration trends along the length of the hair) possible, in order to assess exposure over time (e.g. identification of a high-dose acute exposure). Although attractive, there are many sources of error and this should only be considered on a subject-, substance-, and situation-specific basis. Hair analysis may be useful for retrospective purposes when blood and urine are no longer expected to contain a particular contaminant or for historical specimens. Hair can be used in HBM of different types of substances, from drugs to metals or organic pollutants. Methyl mercury and to some extent arsenic (e.g., segmental analysis for forensic analysis) are the only contaminants, for which hair concentrations have been connected to measurable health outcomes. One difficulty in hair analysis is to distinguish endogenous (internal) exposure from exogenous (external) contamination. Therefore, it was suggested that identifying metabolites (or other unique markers of internal exposure) for substances of interest, where possible, could be a way out in distinguishing internal from external contamination. Hair is a nonvascular tissue (separate from liquid phase transfer kinetics), consequently, understanding the rate of uptake in hair for substances and how they are incorporated is of critical importance, however, scientific information on these issues is still scarce. Neither kinetic models nor metabolite data are available and many basic mechanisms on how chemicals are incorporated in hair are not fully understood. Much more basic research is needed before hair analysis can be fully applied in HBM for a larger variety of environmental chemicals.

It is important to establish Standard Operating Procedures (SOPs) for sampling, sample preparation and analysis in order to achieve reliable and comparable results. We will illustrate this with the extended use of scalp hair in HBM mercury exposure.

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REFERENCES:  
TITLE:
Applicability of hair in human biomonitoring of organic chemicals

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ABSTRACT:
Although known since more than 40 years for the possibility to detect (toxic) metals in hair, it is only in the last decade that hair has been introduced as non-invasive biomonitoring matrix for organic contaminants.
The literature regarding human biomonitoring with hair for different classes of organic contaminants will be reviewed, with emphasis on persistent organic pollutants, such as pesticides, polychlorinated biphenyls (PCBs), dioxins and polybrominated diphenyl ethers (PBDEs). The distribution of various pharmaceuticals and illicit drugs in hair will also be highlighted together with the forensic and judicial implications. Animal studies which have investigated the distribution of various organic compounds, e.g. pesticides or pharmaceuticals, between hair and blood (or other relevant matrices for estimation of internal dose) will be discussed. Advantages and shortcomings of the use of hair as non-invasive biomonitoring matrix for organic compounds will be elaborated and critically presented. Finally, the usefulness of other keratinous matrices, such as bird feathers for wildlife biomonitoring or nails for humans, will be discussed.

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TITLE:
Placenta and umbilical cord blood samples for human biomonitoring studies – non invasive sampling

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ABSTRACT:
Recruitment of study persons for biological sampling in mother/child cohorts requires intensive information about the purpose, logistics, management, data privacy and access to results. In studies of fetal exposures, samples from the umbilical cord and placenta as non invasive unique samples from newborns provide valuable knowledge reflecting the accumulated fetal exposure. Obtaining questionnaire information about lifestyle etc. and parallel samples from the mother at the time of birth, provide important media for studies of placental transfer.

Our experiences are very positive in recruiting mothers for donation of samples and questionnaire information. We have analysed a number of biomarkers of exposure and early effects in paired samples and investigated substance transport in our ex vivo placental transfer system, demonstrating comparability between the results.

The ex vivo placental perfusion system was used to test transport of substances across the human placenta. After birth or cecarian section, the maternal and fetal circulations of a single cotyledon are reestablished by cannulation. Test substances are added to the maternal circulation and samples are taken at specific timepoint during the perfusion, which lasts between 2.5 and 6 hours depending on the substance of investigation.

Studies on BFRs in maternal and fetal blood samples show a clear correlation between the levels in the mother and her child indicating transplacental transport of the compounds. This was also found in the ex vivo placental perfusion system with BDE-47 and BPE-99.

Comparative studies of biomarkers in maternal blood and the respective umbilical cord blood in DK showed significantly higher levels of maternal and fetal bulky DNA adduct levels and increased cord blood micronuclei frequency in pairs living in homes located in traffic-dense areas.

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REFERENCES:
TITLE:
Evaluation of non-invasive biomarkers of low-to-medium mercury exposure in humans

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ABSTRACT:
Results obtained in two studies, preliminary and large-scale study (PHIME), were evaluated to examine the suitability of non-invasive biomarkers of low-to-moderate exposure to mercury (Hg).
In the preliminary study, MeHg (methyl mercury) and THg (total mercury) were determined in maternal blood, umbilical cord blood, placenta, meconium, maternal scalp and pubic hair collected from 28 mother-child pairs. In the following study, THg and MeHg were determined in scalp hair, maternal blood, umbilical cord blood and breast milk from 618 mother-child pairs.
Preliminary study showed variable proportion of Hg as MeHg in cord blood (12 to 100 %) and scalp hair (18 to 100 %), and highly variable inter-individual hair-to-blood MeHg ratio (108 to 760). Maternal scalp hair MeHg was associated more strongly with blood than pubic hair MeHg (r=0.621, p=0.008 and r=0.468, p=0.058, respectively). The significance of placenta as an indicator of MeHg or inorganic Hg exposure is questionable, while meconium was found to indicate MeHg exposure to a certain extent, although the negligible proportion of Hg present as MeHg in meconium indicated possible demethylation of MeHg in the foetus. The large-scale study confirmed the observed high-variability in proportion of Hg as MeHg in maternal and cord blood (14 to 100 %) and hair (9 to 100 %) and highly variable hair-to-blood MeHg ratio (67 to 398). The median values for proportion of Hg as MeHg in cord blood and hair were 97 and 99 %, respectively, but the proportion did not increase with increasing THg in cord blood (r=-0.001, p=0.992) or hair (r=-0.219, p<0.001). In contrast to blood and hair, very weak association between THg and MeHg was observed in breast milk (r=0.138, p=0.025), while the proportion of Hg as MeHg was negatively associated with THg (r=-0.305, p<0.001). In all conducted studies, Hg in blood and hair was positively and significantly associated with frequency of fish consumption, while THg in urine was positively and significantly associated with the number of dental amalgam fillings. Hg in milk was not associated with the frequency of fish consumption, or the number of amalgam fillings.
Although Hg in blood is the most suitable biomarker of inorganic Hg or MeHg exposure, when speciation analysis is performed, THg in hair was found to be sufficient as an approximate indicator of exposure to MeHg resulting from fish consumption and THg in urine as a good indicator of exposure to inorganic Hg from dental amalgam fillings. Breast milk THg was observed to indicate mainly inorganic Hg exposure.

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TITLE:

Estimation of human exposure to PAHs via indoor and biomarker measurements

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ABSTRACT:

To assess background PAH indoor concentrations in Flanders (Belgium), a study was set up by the Flemish Government. In 25 non-smoker houses PAHs samples were collected during winter and summer 2010. The indoor concentrations in the combined gas phase and suspended matter were measured during 24h, using cartridges connected to low-flow pumps (100 mL/min). PAHs in sedimented particles of house dust (diameter <100 µm) were sampled over a 3 weeks period with vacuum cleaners. The particle fraction was analyzed. In both collected samples the 16 EPA-PAHs were measured. Exposure to traffic during the 2 seasons was assessed using different methods: participants wore a personal dosimeter for NO₂ (IVL, Sweden) during 8 days before medical examination. NO₂, PM₁₀ and ozone concentrations at the home address were calculated by interpolation of measured concentrations at stationary stations of the Flemish Environmental Agency (VMM-IRCEL). Also, GIS was used to calculate the distance from the home address to the nearest regional road and nearest highway. Furthermore, non-invasive urine and exhaled breath samples were collected in both seasons, from two inhabitants per residence (N=49 per season). Urinary PAH metabolites were measured as biomarkers of exposure. Air pollution (including PAHs) can induce oxidative stress and inflammation reactions after inhalation. For this reason, exhaled breath NO was assessed as a biomarker of airway inflammation, by means of a portable Niox Mino (Aerocrine) device.

The study population consisted of 25 men and 24 women, with a median age of 38,6 (IQR 31,5-45,5) years. Median values for eNO were 20,3 (IQR 17,0-26,5) ppb during winter and 19,5 (IQR 14,0-30,6) ppb in summer. eNO values in winter and summer were significantly correlated. In both seasons significantly higher eNO values were observed in men compared to women. During the winter season a correlation between eNO and interpolated concentrations of NO₂ and PM₁₀ at the home address was observed (summer not yet analyzed).

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