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INCREASED PRECISION IN EXPOSURE AND RISK ASSESSMENT BY INTEGRATION OF BIOMONITORING: THE CASES OF BISPHENOL A AND PHTHALATES

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Exposure assessment is an essential part of risk characterization. In contrast to the detailed requirements regarding toxicity studies used for hazard assessment, only limited guidance for exposure assessment is available. Therefore, many exposure assessments still rely on conservative assumptions of human behavior. Modern analytical chemistry is able to quantify trace amounts of chemicals in human body fluids and thus permits a precise assessment of exposures from all routes. In order to translate determined concentrations of chemicals in human body fluids to a received dose, knowledge of human toxicokinetics and biotransformation for the monitored chemical is essential. Integration of well defined toxicokinetic data have enabled to precisely define human exposures from different sources to many widely used chemicals such as phthalate esters used as plastisizers and bisphenol A used in polycarbonate plastics and epoxy resins in food contact materials. The available large databases on the excretion of these chemicals through the measurement of their relevant metabolites in urine demonstrate that actual human exposures assessed by biomonitoring are significantly lower as compared to estimated exposures based on release data and assumed human behaviors. Biomonitoring data therefore play an important part in the risk characterization process and should be used in such activities whenever reliable data are available.

BIOMONITORING IN THE WORKPLACE: RECENT EXPERIENCE FROM REACH APPLICATIONS FOR AUTHORISATION

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Part 1: Authorisation of substances of very high concern under REACH

Substances of very high concern (SVHC) are listed on Annex XIV of REACH for authorisation of their uses in the EU. This part of the regulation is intended to allow continued use while progressive substitution takes place. Authorisation is the last part of the REACH Regulation to be implemented, starting in 2013/14; in the meantime ECHA's Risk Assessment Committee - RAC - has evaluated 115 applications and issued close to 200 opinions to advise the European Commission. SVHC may only be used if an authorisation has been granted or an application has been submitted before the substance's latest application date and its evaluation by ECHA or the Commission is pending. Applicants submit to ECHA a chemical safety report (risk assessment), an analysis of alternatives and may submit a socio-economic analysis demonstrating that the benefits outweigh the risks of continued use; this latter applies primarily to non-threshold substances, e.g. some carcinogens. This part of REACH operates in parallel with (and without prejudice to) worker protection legislation.This part of the presentation explains the authorisation process and reports on some of its beneficial aspects which enhance understanding of workplace exposure and contribute to the protection of workers from hazardous chemicals.

Part 2: Use of biomonitoring in workplace risk characterisation: recent experience from REACH applications for authorisation

The chemical safety report in an application for authorisation should contain a full assessment of the exposure and the risks to human health both directly and indirectly (via the environment) of the continued use of the substance. This requires a detailed description of the industrial process, the activities carried out by the workforce, the risk management measures (RMM) in place and the resulting emissions and exposure of workers to the hazardous chemical. In close to 200 evaluations between 2013 and 2017 of uses of about 20 substances listed on Annex XIV of REACH, ECHA's Risk Assessment Committee, RAC has reviewed a wide range of exposure data. This varies from extensive datasets of static and personal air samples plus biomonitoring of workers to less developed approaches based purely on modelling. This body of evidence provides a clear cross-section of RMM and exposure monitoring activities in some major industries, e.g. chrome plating but also sectors such as pharmaceuticals manufacture.

In all cases RAC and SEAC have recommended to the Commission that authorisation be granted but has also recommended technical conditions related to improved risk management measures and a particular focus on monitoring. The ECHA Committee for Socio-Economic Analysis (SEAC) assesses whether the benefits outweigh the risks and then recommends a review period to the Commission after which the applicant – should they wish to continue using the substance - has to resubmit (4 years = short, 7 years = normal and 12 years = long).

Biomonitoring data has featured in about 5% of applications and during it's evaluation RAC has occasionally asked applicants (with some success) to supplement their risk assessments with this type of data. This paper looks at 4 case studies (covering uses of diarsenic trioxide, chromium trioxide, MOCA and technical MDA) in which biomonitoring played a substantial role in the exposure assessment and risk characterisation. In one of the cases, the Commission has already granted the application with a condition that biomonitoring should be continued as part of the routine exposure monitoring obligation for the applicant.

IMPROVING RISK ASSESSMENT OF CHEMICALS BY THE USE OF HUMAN BIOMONITORING – HBM4EU PROJECT ACTIVITIES

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The default approach in the risk assessment (RA) of chemicals is to assess external exposure by combining different sources and routes of exposure. This kind of approach contains various uncertainties and may overestimate exposure, since conservative estimates are needed due to the limited data on, for example, the absorption of the chemical and interspecies and intraspecies differences. Human biomonitoring (HBM) can help improve RA by providing measured data on combined exposures. In some cases, biomonitoring data can even provide a direct link to health effects. In some cases, biomonitoring allows to link exposure to specific contexts such as occupational settings. Although recent years have seen good examples of the use of biomonitoring in the risk assessment of chemicals, much work is still needed to improve its use in regulatory RA and human impact assessment (HIA).

The European Human Biomonitoring Initiative (HBM4EU) was recently launched for fulfilling the gap between the exposure to hazardous chemical agents and their impact on human health. One of the aims of the HBM4EU project is to enhance the use of HBM data in RA and HIA of chemicals in different regulatory contexts including legislations on chemicals, plant protection products and biocides, as well as legislation on cosmetics, food safety and occupational safety. RA models for mixtures are also considered. Firstly, current RA practices are evaluated: is the use of biomonitoring integrated in the available RA guidance, and do given RA schemes have good examples of the advanced use of biomonitoring? A survey is also conducted to gather information from national regulatory risk assessors (in the EU, but also in non-EU countries) on their risk assessment practices, the use of HBM, and the obstacles and challenges related to its use. The challenges of the use of HBM data in RA may include a lack of guidance in the use of biomonitoring, a lack of knowledge regarding the interpretation of biomonitoring results, or the inability to link biomonitoring data to different exposure sources. Using a selected group of priority chemicals as example, we can determine whether these challenges can be overcome by including the recent HBM data, collected during the HBM4EU project, in the existing RA schemes. Finally, proposals will be made for the better use of HBM in RA and HIA in different policy domains.

THE IMPORTANCE OF TOXICOKINETICS IN THE INTERPRETATION OF HUMAN BIOMONITORING DATA

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Accurate estimation of exposure following an accident is often very difficult unless human biomonitoring can be applied. When the time of the potential exposure is exactly or at least reasonably well known, the actual exposure that has occurred can reliably be determined provided the toxicokinetics of the substance are known.

A good example of the importance of toxicokinetics for the interpretation of human biomonitoring was found with 1,3-butadiene (BD). A number of years ago, we investigated the metabolism of BD in mouse and rats[1] and the application of two of its mercapturates, monohydroxy-3-butenyl mercapturic acid (MHBMA) and 1,2-dihydroxybutyl mercapturic acid (DHBMA) as biomarkers for human exposure[2]. However, in the routine application of these mercapturates in our operations, we found results that were difficult to interpret. In 1315 samples from operators with potential exposure to BD we measured simultaneously MHBMA and DHBMA. These values were used to calculate the corresponding airborne concentrations of BD. However, we found a huge discrepancy in the calculated airborne levels based on MHBMA compared to DHBMA. In 180 cases data were available from the same worker from an end-of-shift sample and the next day pre-shift sample, which allowed to estimate the apparent urinary half-life of both MHBMA (25.7 ± 2.8 h) and DHBMA (4.7 ± 1.6 h). When a simple toxicokinetics model was used to correct the data for the apparent urinary half-life, the discrepancy disappeared and a very good correlation between metabolite level and corresponding airborne correlation was found. The toxicokinetics did not only explain the observations, but also helps to identify the most suitable biomarker: MHBMA is the preferred biomarker to pick up any exposure, since the longer half-life makes it less likely an exposure is missed, whilst DHBMA is more suitable to link certain activities to BD exposure as the short half-life limits the time window.

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BIOLOGICAL MONITORING (BM) OF WORKERS EXPOSED TO INORGANIC MOLYBDENUM (MO) COMPOUNDS AND ITS USE IN RISK ASSESSMENT

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Background: Mo is a biologically essential element and also one of significant industrial importance, but little published reliable occupational exposure or BM data exists.

Study Aim: Firstly, a BM programme assessed occupational exposure to Mo and compounds using urinary and serum concentrations in a number of representative Mo and Mo compound-producing and processing industries using a standardised sampling and analytical protocol. Secondly, serum Mo concentrations in rats exposed orally in toxicological studies were starting points for calculating BM-based derived no-effect levels (DNELs), as per EU REACH Regulation methodology. These DNELs were compared in a risk characterisation exercise with serum Mo concentrations from the BM study in workers.

Methodology: Occupational health providers used a comprehensive BM sampling and analytical programme developed by the International Molybdenum Association (IMOA). Results were analysed, and "typical" and "worst case" (50th & 95th percentile) blood serum Mo values derived for exposed and non-exposed workers.

Results: Mo in blood serum of exposed workers (n=546): Typical: 8.2 µg/L & Worst case: 53.6 µg/L. Unexposed controls (n=50) values: Typical: 0.89 µg/L & Worst case: 2.06 µg/L. Good linear correlation between Mo in serum and Mo in urine (corrected for creatinine) with R^2=0.756, meaning Mo serum concentrations could be estimated from urinary values.

Biomonitoring based DNELs were derived from a sub-chronic (90-day) repeated dose toxicity study (OECD TG 408), and a prenatal developmental toxicity study (OECD TG 414) where rats were exposed orally to the highly bioavailable test item sodium molybdate dihydrate. DNELs were derived based on those study NOAELs, applying appropriate assessment factors.

Key DNEL derived for workers: 1440 µg Mo/L. This is the concentration of Mo in human blood serum, up to which no systemic effects due to Mo exposure are expected.

Discussion and Conclusions: Results show that even the most exposed workers (95th percentile = 53.6 µg/L Mo in blood serum) are many times below the derived DNEL (1440 µg/L). This gives a high degree of confidence to industry and regulators that adequate control of Mo exposure is being achieved. The significant value of a reliable BM programme and its use in risk characterisation and assessment is demonstrated.

BIO-MONITORING OF OCCUPATIONAL EXPOSURES TO CARBON DISULPHIDE: A SCOPING REVIEW

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Background: Occupational exposure to carbon disulfide (CS_2) has been cause for health concerns and has been generally acknowledged in the past few decades. Exposure to CS_2 has been associated to numerous adverse health effects, predominantly neurological and cardiovascular diseases.

Aim of the study: The objective of the study was to identify in a standardized, systematic and more objective way the most recent developments in bio-monitoring of occupational exposures to CS_2, capturing the state of the art by applying and further adapting available scoping review approaches to the needs of occupational risk assessment procedures.

Methods: A systematic scoping review was carried out to assess the updating needs of the currently available recommendations of Biological Limit Values (BLVs) on CS_2 specifically looking at developments in the area of bio-monitoring of CS_2, over the period 2008-2015. A general framework containing five main stages was followed (1) the identification of research question; (2) database search throughout stipulated search phrases; (3) selection of relevant studies and exclusion of non-significant studies; (4) data extraction and assessment of the included articles; (5) assembling, summarizing and reporting of the results in a scoping review.

Results: Following the application of the scoping review approach, using the PubMed database, 12 studies were finally considered eligible for further consideration in the eventual updating of current BLVs. The analysis of these studies highlighted that TTCA is the biomarker of choice at higher doses although it showed low sensibility and specificity in biological monitoring when environmental concentrations are below 0.73 mg m^{-3}. Among these studies, Jiang et al. (2012) described a cohort analysis and demonstrated a directional relationship between TTCA and CS_2. Consequently the authors suggest a lower national exposure level, in accordance with 1.2 mg TTCA/g Cr, which corresponds with 1.66 ppm (5.15 mg/m^3) CS_2, calculated from the regression equation of the article. The biological exposure index of CS_2 in China might be revised for 1.2 mg/g Cr from the current 2.2 mg/g Cr level. Moreover, several relevant candidates to be flagged as 'biomarkers of effect' have been suggested including the use of serum mi-RNA and telomere length shortening.

Discussion/conclusions: Overall, the scoping review demonstrated to be a useful tool since it allowed to screen the available literature on the bio-monitoring of CS_2. in an efficient and transparent way, that can be reported in a standardized format. As a consequence, the observed correlation between TTCA and CS_2 exposures in occupational settings based on data published after 2008, will eventually contribute to the establishment of a novel BLV value in the region of exposures to 1-3 ppm, as opposed to the existing recommendations, which only cover occupational exposures above 4 ppm.

ASSESSING EXPOSURE TO METALS USING BIOMONITORING. ACHIEVEMENTS AND CHALLENGES

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A large body of evidence has demonstrated the advantages of using biomonitoring for quantifying occupational or environmental exposures to metallic agents, especially the "heavy metals" (lead, cadmium, mercury) and the metalloid arsenic. In this presentation, we will address various issues and questions that have arisen from our experience during the conduct of various surveys of metal exposures in populations outside Europe.

An important technical achievement of the past decades has been the availability of ICP-MS, allowing the measurement of multiple elements in a single (small) sample. This has undoubtedly expanded our knowledge of the exposure and toxicokinetics of metallic agents, including lesser known elements. However, the production of large amounts of highly correlated data has also led to statistical challenges. Because urine is easy to obtain, spot samples of urine have been the most frequently used medium for biomonitoring studies. External contamination of urine by dirty hands or clothing may be an issue in field studies. Correction for urine dilution remains problematic. The most frequently used method, based on the concentration of creatinine, is not adequate for some elements and in certain circumstances, since the daily urinary output of creatinine depends on age, sex, physiological and nutritional factors, thus complicating comparisons between individuals and between groups. Venous blood samples are more difficult to obtain, especially in children; moreover, the concentrations of many elements are often below their limits of quantification in whole blood. Other media [exhaled air, bronchoalveolar lavage, sputum, saliva, faeces, semen, hair, nails, teeth, milk, placenta, meconium] have been used, with varying success, for assessing exposure to metals or other agents. These alternative media have their theoretical and practical advantages, but also problems, such as accessibility, external contamination, sample preparation, analytical sensitivity, standardization and expression of results.

The interpretation of biomonitoring results often relies on comparisons with "normal" values and upper (or lower) limit values. Most reference values have been determined in populations living in industrially developed countries and these may not be valid for people living in other environments. However, the latter statistical consideration poses the ethical challenge of what constitutes an acceptable exposure.

BIOMARKERS OF METAL EXPOSURE AND TOXICITY IN URINE: NEW OPPORTUNITIES AND NEW CHALLENGES

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Because of their non-invasiveness, urinary biomarkers are commonly utilized to characterize exposure to metals and to detect their toxic effects in particular on the kidneys. If urinary biomarkers have proved their usefulness in monitoring metal exposure in industrial workers with high inhalation exposures, their application to the general population exposed mainly via food faces several challenges. The first challenge is that with a few exceptions, the urinary concentrations of most metals mainly reflect the recent exposure to the metal, which complicates the study of the long term effects of metals and their associations with chronic diseases. Another challenge is the risk of confounding by renal physiology and by the nutritional status. If at high exposure levels, deficiencies in essential elements are potential effect modifiers, at low exposure levels they may act as confounders by upregulating the uptake of the metal, generating spurious associations with some chronic diseases. Regarding renal physiology, the major challenge is to avoid confounding by the hydration status or the urine dilution. The adjustment with creatinine as systematically done for decades actually abolishes the influence of diuresis only when the urinary concentrations of the biomarker and creatinine correlate with a beta coefficient close to one. Unfortunately, this is rarely the case and it appears now that this incorrect adjustment is a source of misclassification, which may lead to spurious associations (e.g. cadmium and protein HC in urine). Despite these difficulties, urinary biomarkers hold many promises especially when no blood samples can be collected (e.g. very young children). Particularly promising are the epigenetic biomarkers and the lung biomarkers (e.g. club cell protein) that are currently investigated.

EFFECTS OF ENVIRONMENTAL AND OCCUPATIONAL EXPOSURE TO LEAD AND OTHER XENOBIOTICS ON TELOMERE LENGTH

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Background: Recently, researchers are interested in telomere length as a biomarker of pathological states, including lifestyle diseases (diseases of civilization, including diabetes, metabolic syndrome, cardiovascular diseases, cancers) on one hand, and occupational and environmental exposure to xenobiotics (heavy metals, polycyclic aromatic hydrocarbons, particulate matters and others) on the other.

Aim of the study: The aim of the study was to assess the effect of lead exposure on telomere length.

Material and methods: Occupational exposure was examined in a group of 334 male workers employed in lead and zinc smelter (aged 19-62) and 60 age-matched control ones without occupational exposure to lead. Environmental exposure was studied in a group of 99 children aged 8, living in the industrialized area. A questionnaire on life style and health status was filled. The relative telomere length was studied using relative telomere length assay according to Cawthon (2002). Blood lead levels (B-Pb) were determined using atomic absorption spectroscopy.

Results: Occupational exposure: In the lead exposed workers the mean B-Pb was 330 microg/l (geometric mean GM, range 23-611 microg/l), and in control group 22 microg/l (GM, range 16-87 microg/l). Lead exposed workers had significantly shorter telomeres comparing to control ones and higher oxidative stress.
Environmental exposure: The mean (GM) B-Pb in children was 32,8 microg/l (range 9-142 microg/l). There was a negative correlation between B-Pb and telomere length. There was also a negative effect of prenatal tobacco smoke exposure on telomeres. There was no association between B-Pb and oxidative stress in studied children.

Discussion: Changes in telomere length are not only related to aging. They may result from toxic effects of xenobiotics. For instance exposure to tobacco smoke, both in smokers as well as in children with prenatal tobacco exposure (when their mothers smoked during pregnancy) was associated with shorter telomeres. Effects of other xenobiotic exposure on telomere length are currently widely studied.

Conclusions: Our project showed that both occupational and environmental exposure to lead results in telomere shortening. Also prenatal exposure to tobacco smoke was a risk factor of shorter telomeres in studied children.

The study was supported by National Science Center, Poland, UMO-2011/03/D/NZ7/05018.

BLOOD LEAD LEVELS FOLLOWING CONSUMPTION OF GAME MEAT IN ITALY

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Background: An increase of exposure to lead in consumers of meet gamed with lead bullets has been reported.

Aim of the study: To evaluate the impact of game meat consumption on the level of lead in blood (Pb-blood) in Italian consumers, taking into account possible other sources of lead exposure such as hunting activity, dietary habits, and occupational or leisure activities.

Methods: A spot blood sample was obtained from 95 subjects. A questionnaire was used to collect general information and data on game meat consumption, hunting, wine drinking and other possible sources of lead exposure. Pb-blood was measured by inductively coupled plasma-mass spectrometry.

Results: Pb-blood was not influenced by age, gender, residence in urban or rural area, consumption of game meat, tobacco smoking and hobbies associated with potential exposure to lead, and was generally low, mostly below the reference value of our laboratory. A multiple linear regression analysis (containing the covariates gender, age, hunting, wine drinking, game meat consumption, tobacco smoking, shooting range, and occupational exposure) found an association with hunting (Pb-blood almost two-fold higher in hunters) and wine drinking (40% higher in wine drinkers) but not with consumption of meat game or other parameters. Whether the increase was due to inhalation of lead fumes during shooting with lead ammunition, to manipulation of lead ammunition or both could not be ascertained.

Conclusions: Game meet intake in Italian consumers was not associated with an increase of exposure to Pb.

URINARY MANGANESE RELATED TO EXPOSURE AMONG MANGANESE ALLOY PRODUCTION WORKERS

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Background: Manganese (Mn) is neurotoxic, and there is a need to provide tools for improving surveillance of Mn exposed workers.

Aim: To examine if monitoring of urinary Mn (U-Mn) can be used as a tool for surveillance of Mn alloy production workers.

Methods: Forty tappers were recruited from three Mn alloy producing plants and compared with 20 referents. The tappers (and referents) delivered urine samples on two successive workdays in the morning, post-shift and in the evening. Urine was also collected the evening before and the morning after the two workdays. Air samples in the respirable aerosol fraction were collected by personal sampling both work-days. Manganese was determined by inductive coupled plasma mass spectrometry.

Results: The mean age of the tappers was 44.1 years vs 44.0 among the referents. Their respective B-Mn were 11.4 vs 8.2 µg/L. The mean concentration of air-Mn was 48 $\mu g/m^3$, of which 21 was soluble and 24 was non-soluble in the lung lining fluid simulant Hatch solution. The mean U-Mn concentrations increased post-shift on both days to 1.2 and 1.6 µg/g creatinine (cr.) from 0.2 and 0.3 µg/g cr. in the respective morning samples and returned to 0.3 and 0.3 µg/g cr. in the respective evening samples. The median U-Mn concentrations were below 0.1 µg/g cr. at all eight sampling occasions among the referents.

Discussion: The bioaccessibility of Mn in the workroom air of tappers appears to be higher than what we have previously observed among Mn exposed welders. There is a significant increase in post-shift U-Mn from first voided morning urines among the workers. However, these enhanced concentrations return to their background levels in the evening, suggesting a relatively short half-life of U-Mn.

Conclusions: Due to the apparently short half-life of U-Mn, it may be difficult to use urine as medium for surveillance of Mn alloy production workers.

BIOLOGICAL MONITORING OF INORGANIC MERCURY – CAN THE KIDNEY BURDEN BE ESTIMATED?

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Background: For a long time blood and urinary mercury (B-Hg and U-Hg) have been used for monitoring of mercury (Hg) exposure, and sometimes also mercury in plasma (P-Hg). Based on animal studies it has been assumed that B-Hg and U-Hg also can reflect the body burden of mercury, a large part of which is in the kidneys. This has, however, never been tested in humans with low-level exposure.

Methods: We had the opportunity to examine if U-Hg (24 h urine as well as an overnight spot sample), B-Hg and P-Hg could be used to estimate kidney Hg in 109 living healthy kidney donors, in whom mercury was also analyzed in kidney biopsies.

Results: There were strong associations between kidney Hg and all measures of U-Hg (Pearson r=0.73–0.84), and P-Hg (r=0.65) while the association with B-Hg was weaker (r=0.29), albeit statistically significant. After adjustment for creatinine, correlations were almost as strong for overnight urine (r= 0.79) as for 24 hour urine (r=0.83). Using a regression model including only U-Hg in first morning urine + gender, 64% of variability in kidney Hg could be explained with a high correlation (r=0.80) between estimated and measured kidney Hg. The mean ratio between kidney Hg (in µg/g) and U-Hg/24h (in µg) was 0.22.

Discussion: It is to be expected that U-Hg and P-Hg in the general population have a closer association with kidney Hg than B-Hg since almost all mercury in the kidney is inorganic. B-Hg is affected also by methyl Hg in the erythrocytes. Moreover, at varying exposure, B-Hg fluctuates more than U-Hg since the half-life is shorter after cessation of exposure.

Conclusion: The kidney Hg burden can be estimated from overnight creatinine-adjusted mercury in urine.

HEALTH-BASED GUIDANCE VALUES FOR BLOOD LEAD AND URINARY CADMIUM – DO THEY PROTECT US?

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Background: There are health-based guidance values (HBGV) for blood lead (B-Pb) and urinary cadmium (U-Cd) from several international bodies, e.g. WHO/JECFA, ATSDR/US EPA and EFSA (European Food Safety Authority). EFSA (2010) derived the HBGV for lead on benchmark modelling and arrived at B-Pb 12 µg/L ($BMDL_{01}$) for developmental neurotoxicity in children, 36 µg/L for blood pressure in adults ($BMDL_{01}$), and 15 µg/L ($BMDL_{10}$) for chronic kidney disease. The HBGV for cadmium was U-Cd 1 µg/g creatinine based on a $BMDL_{05}$ of 4 µg/g for increased beta-2-microglobulin in urine and an factor of 4 for inter-individual variability (EFSA 2009).

Discussion: These HBGVs were based on epidemiological studies in humans (while most other HBGVs are based on animal tox data). Since 2009 many hundred new human studies have been published on health effects related to B-Pb and/or U-Cd.
The overall epidemiological evidence for slight effects on children's behavior and cognition at low lead exposure levels were strong already in 2009, and a number of additional studies after 2009 have confirmed this. The evidence on increased blood pressure/hypertension and chronic kidney disease at low-level exposure (today's levels of B-Pb in Europe) was limited (few studies, mostly cross-sectional) in 2009, but provided some additional support for an association.
For cadmium the scientific basis has changed since 2009. Increased excretion of low molecular weight proteins is still likely at U-Cd levels of 4 µg/g creatinine, but new studies have shown that associations found at low levels (0.5 – 1 µg/g creatinine) which are common in the general population are probably not causal (Akerstrom 2013, Bernard 2016). Instead increased risk of osteoporosis/fractures (Engstrom 2012, Akesson 2014, Wallin 2016), certain types of cancer (Akesson 2008, IARC 2012) , and cardiovascular disease (Tellez-Plaza 2013, Fagerberg 2015, Barregard 2016) should be considered in the risk assessment (Akesson 2014).

Conclusion: The strict health-based guidance values for B-Pb seem valid and should be protective enough. The EFSA and WHO/JECFA health-based guidance values for U-Cd should protect us from adverse kidney effects, but it is likely that they are not protective versus other health effects.

HUMAN BIOLOGICAL MONITORING FOLLOWING CHEMICAL INCIDENTS – EXPERIENCE WITH A GUIDELINE IN THE NETHERLANDS

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Background: In the Netherlands response to chemical incidents is organized on a regional level. Twenty five public health advisors hazmat (GAGS) are trained and work in teams in 24/7 duty shifts to support 25 safety regions. These experts perform rapid risk assessments to support public health authorities. They consider the potential value of human biological monitoring (HBM) in exposure assessment during chemical incidents. End-users with a GAGS background as well as physicians, toxicologists and epidemiologists contributed to the preparation of a national guideline that was published in 2012.

Aim of the study: To review the experience with a new guideline for decision-making regarding the use of HBM in the context of chemical incidents.

Methods: Chemical incidents were analyzed to find out how a decision concerning the use of HBM was taken and how it influenced evolvement of these incidents.

Results: Over the past three years HBM was considered in 12 chemical incidents. In five cases experts recommended not to use HBM to resolve the questions related to exposure and health effects. Of the cases that were followed up, in three cases workers had been exposed to industrial chemicals during an incident (fumigants, metals and organic solvents) and in one case first responders had direct contact with hazardous substances (related to illicit drugs laboratory). In the three remaining cases residents were presumably exposed (mercury, polyurethane and metals) and in two of these incidents young children were involved. The strength of the HBM guideline is that it highlights potential pitfalls and provides a framework to take a decision in an early stage of an incident. In all of these cases this decision was made after consultation of a toxicologist in the safety region or from the national poison center. An inherent weakness of applying HBM in this setting is that HBM is often not perceived as a priority in an early stage of an incident and some of the requests for support were too late to be able to respond to, adequately.

Short discussion/conclusions: HBM was employed in about half of the incidents and contributed to resolving questions concerning exposure. In most cases HBM was used in an assessment that could be used for reassurance of the public. In March 2017, five GAGS from the Netherlands were appointed by DG SANTE as members of an international panel of experts and will now use their experience of HBM to support response to transboundary chemical incidents.

HUMAN BIOMONITORING (HBM) AFTER CHEMICAL INCIDENTS – CONCEPTS, EXAMPLES AND LESSONS LEARNED

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Human Biomonitoring (HBM) is a well-established approach for exposure analysis and assessment, derived decades ago mainly for the examination of workers' exposure in industry. In the meantime, HBM has been developed significantly beyond this application and it is nowadays applied, e.g., in environmental surveys of the general population, and in the investigation of short-term or accidental contact to hazardous chemicals [1,2]. For this purpose, a broad spectrum of sensitive and reliable analytical methods has been optimized. However, there are fundamental and practical differences that require consideration if HBM is intended to be applied for incident monitoring.

This presentation summarizes the core aspects of incident HBM such as biomarker selection, sampling procedures including sampling times, and aspects of biomarker half-life and stability. Examples of successful incident HBM programs inside and outside industry will illustrate the strengths and weaknesses of recently published concepts. A particular focus will be set on HBM for firefighters and emergency response forces. In this presentation, the results of a standardized HBM program for firefighters of a large chemical site are summarized.

Due to the complex chemical processes in fires and pyrolysis reactions, the selection of appropriate and meaningful biomarkers of exposure is a special challenge, even if the identity of the burned material is known. Based on a review of existing reports on typical fire by-products, a new tiered fingerprint approach for key biomarkers of simple and polycyclic aromatics, chlorinated substances and irritant vapors will be presented. Other decisive aspects are preparedness (concise pre-information of emergency responders, sampling material, sampling protocols, information network, expert contact persons) and the collection of reliable add-on information about the incident and the potential exposure, e.g., by questionnaire.

In conclusion, HBM after chemical incidents is an appropriate and meaningful approach for exposure analysis and assessment. The interpretation and communication of HBM results after chemical incidents is, however, still one major challenge which should be addressed in a multidisciplinary approach.

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HUMAN BIOMONITORING AS A TOOL OF OBJECTIVE EXPOSURE ASSESSMENT: A CASE-STUDY OF A MAJOR TRAIN ACCIDENT WITH ACRYLONITRILE IN BELGIUM

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Background: Following a train derailment, several tons of acrylonitrile (ACN) exploded, inflamed and part of the ACN ended up in the sewage system of the village of Wetteren (Belgium).

Aim of the study: The objectives of the present study were: 1) To assess the human exposure to ACN in the populations with the highest suspected exposure, i.e. the local population and the emergency responders; 2) To investigate potential determinants of exposure to ACN; and 3) To explore the association between a biomarker of exposure and self-reported short-term health effects in the local population.

Methods: 242 residents and 841 emergency responders participated in the study. *N*-2-cyanoethylvaline (CEV), a highly specific biomarker for ACN exposure, was measured in blood. To account for potential influence of smoking, cotinine was determined in the urine. Participants also filled in a questionnaire including reporting of short-term health effects.

Results: In the evacuated zone, 37.3% of the non-smokers and 40.0% of the smokers had CEV concentrations above the reference values of 10 and 200 pmol/g globin, respectively, at the time of the train accident. Spatial mapping of the CEV concentrations depending on the residential address showed a distribution pattern following the sewage system. The most frequently reported symptoms were local symptoms of irritation. In the non-smokers, a dose-response relation was observed between the CEV concentrations and the reporting of short-term health effects. Overall, the value of self-reported symptoms to assess exposure was limited, with the exception of some local symptoms known to be prominent for the specific chemical exposure studied. Even then, consistent symptom reporting was observed only in case of exposures that resulted in CEV values exceeding 10 times the reference value. For the lower exposure ranges, there was no clear relationship between symptom reporting and exposure. In the emergency responders, 26% of the non-smokers exceeded the CEV reference value. ACN exposure among the non-smokers was predicted by (1) the distance to the accident, (2) the duration of exposure, and (3) the occupational function. In contrast with the local population, CEV concentrations in the emergency responders remained relatively moderate and were comparable with background levels for a smoking population.

Short discussion/conclusions: The present study is one of the first to relate accidental exposure to short-term health effects. The results of this study confirm that a critical view should be taken when considering self-reported health complaints and that ideally biomarkers are monitored to allow an objective assessment of exposure.

HIGH PFAS IN SERUM IN SWEDISH POPULATIONS EXPOSED TO FIRE FIGHTING FOAM CONTAMINATED DRINKING WATER

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Background and aim of the study: Exposure to perfluorinated compounds (PFAS) through drinking water has emerged as a potential health hazard in local populations living near firefighting training ranges, especially at military and large commercial airfields. We here report PFAS serum levels in two exposed populations, and a reference population with uncontamined drinking water.

Material and methods: In Ronneby, a municipality in southern Sweden with 27 000 inhabitants, 1/3 of the population had for decades contaminated municipal drinking water. Between 2014 and 2016 3418 persons participated in exposure monitoring. In 2016 an external control group of 242 persons from Karlshamn, a nearby municipality, was also enrolled. In Lulnäset in northern Sweden around 175 persons with summer houses near a military airfield used contaminated drinking water for a few months per year at the most; here a representative sample of 20 persons were invited for serum sampling. Chemical analysis was performed using LC-MS/MS.

Results: In Ronneby, the median serum levels were PFOS 180 (10, 90 percentile 24, 560) ng/ml, PFHxS 150 (18, 540) ng/ml and PFOA 10 (2.2, 29) ng/ml. One drinking water sample from the municipal waterworks in Dec 2013 showed PFOS 8000 ng/L, PFHxS 1700 ng/L, PFOA 100 ng/L. In Lulnäset the median serum levels among 20 persons sampled were PFOS 17 ng/ml, PFHxS 42 ng/ml and PFOA 4 ng/ml. One drinking water sample from the common well showed PFOS 434 ng/L, PFHxS 680 ng/L, and PFOA 55 ng/L. In the control population the serum levels were PFOS 4.2(2.1, 9.9) ng/ml, PFHxS 0.84 (0.37, 2.6) ng/ml and PFOA 1.6 (0.95, 2.9) ng/ml.

Short discussion/conclusions: Firefighting foam PFAS exposure was dominated by PFOS and PFHxS. Drinking water (but not serum) concentrations are lower for PFHXs than PFOS in Ronneby, indicating that population uptake and retention of PFHxS is relatively much higher than PFOS at these concentrations, or that the historical exposure profile had been different. For PFOA the relationship between average serum and average water concentration is similar to that reported in the C8 study in the US. The firefighting foam exposure profile contrasts with the C8 study population, where local PFAS exposure was dominantly by PFOA. Thus, the Ronneby study offers a unique opportunity to assess whether health links with PFOA reported in the C8 studies, or any other health impacts, are evident in the population with substantial exposure to PFHxS and PFOS.

INCIDENT PREPAREDNESS – IDENTIFICATION OF CHEMICALS SUITABLE FOR HUMAN BIOMONITORING (HBM)

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A group of experts in Sweden has recently produced a report[1] where they identify the most important industrial chemicals of concern for risk and vulnerability analyses, as well as emergency preparedness and enhancement measures by emergency services, healthcare professionals and other actors dealing with emergency preparedness for chemicals. The report identifies chemicals that could, in the event of a discharge to air, be a burden on society's rescue organizations and health care system. About 90 substances are listed in the report, these were selected based on a combined analysis of volume (tonnes per year in Sweden 2012-2014), chemical-physical properties (volatility/vapor pressure/boiling point, reactivity with water resulting in toxic products), and nature and severity of toxicity. The possibilities to monitor exposure by air measurements or by human biomonitoring (HBM) are not addressed. Furthermore, contamination of the ground or ground water is not considered, neither are substances that are expected to cause an immediate strain on the rescue organizations and health care system even if the might be highly persistent, bioaccumulating and cause long-term health effects. Still, the list may help identify priority areas for HBM preparedness in accidents and disasters. As a first step, the chemicals may be split in three categories: (1) useful HBM method available, (2) no useful HBM method identified, and (3) HBM not applicable or not likely applicable. Based on the list of German MAK BAT[2] and ACGIH BEI[3] values, examples of chemicals that would be belong in the first category are: acrylamide, acrylonitrile, benzene, butadiene, cadmium oxide, carbon disulfide, carbon monoxide, chlorobenzene, chloroform, dichloromethane, dimethylformamide, ethylbenzene, ethylene oxide, fluorine, hydrogen fluoride, phenol, styrene, tetrachloroethylene, toluene, toluene diisocyanate, trichloroethylene, and xylene. For such chemicals, action can be taken to improve the emergency preparedness, including e.g. descriptions on sampling, sample treatment and analytical methods and where to send samples for analysis. For category 2, the possibility to develop suitable HBM should be investigated. This presentation will present case studies with substances from the Swedish list falling in the different categories.

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BIOMONITORING OF SOME COMMON NON-PERSISTENT PESTICIDES AND DIETARY DETERMINANTS IN SWEDISH POPULATIONS

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Background: Conventional agriculture employs extensive use of pesticides to increase agricultural yields. The southern part of Sweden is intensively cultivated but the contribution of pesticide residues in Swedish grown crops is much lower than what is found in commodities imported from countries outside the EU. Although compounds used nowadays often have short excretion half-lives in humans, the constant low level exposure to the general population may be of concern. Diet is suggested to be the dominating route for pesticide exposure in general populations.

Aim of the study: We aim to examine the exposure to a selected group of pesticides in Swedish populations by analyzing residues in urine. Further, we briefly address the influence of characteristics and diet, on these urinary levels.

Methods: The study population of 413 participants consisted of sections of the general population in southern Sweden. Written informed consent for participation was obtained. Morning void samples and filled-in questionnaires including socio-demographic and health-lifestyle related questions and a diet diary were collected.
A total of 17 pesticide residues were determined in urine. The analysis included determination of residues of common insecticides, fungicides, herbicides and plant growth regulators. All samples were analyzed using liquid chromatography coupled with mass spectrometry.
We used non-parametric tests to evaluate the influence of characteristics and dietary differences on the measured residues.

Results: Almost all the urine samples (99.8%) had detectable levels of at least one of the residues. The 95th percentiles of the concentrations of 3,5-DCA (10 µg/L), MQ (8.4 µg/L), CCC (15 µg/L) and TCPy (11 µg/L) were higher than the percentiles of the other compounds. Females, vegetarians and immigrants had significantly higher residue levels than the rest of the population. Further, participants consuming more grain based foods had higher CCC levels than the others and higher coffee consumption showed higher MQ levels. Higher intake of fruits and wine was associated with higher insecticide and fungicide levels and intake of salad leaves showed higher urinary pyrethroid residues.

Discussion/Conclusions: Parts of the general population in southern Sweden are exposed to several pesticides, probably mainly via diet, not only to pesticides used in the production of domestic products but also to pesticides banned for use in Sweden but used in imported foods.

ASSESSMENT OF THE ENVIRONMENTAL LEVELS AND PREDICTORS OF EXPOSURE TO SOME ENDOCRINE DISRUPTORS IN A BELGIAN ADULT POPULATION: FOCUS ON MERCURY, CADMIUM, ORGANOCHLORINE PESTICIDES AND PCBS

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Aims: A human biomonitoring study focused on endocrine disruptors was carried out in 2015 on 252 individuals from the general population living in the Province of Liege (Belgium). The urinary levels of cadmium and mercury, and the serum concentrations of some organochlorine pesticides (OC) and polychlorinated biphenyls (PCBs) were determined, and their relations to demographic characteristics, lifestyle behaviors and dietary habits were assessed. Although these chemicals were not considered as emergent compounds, the aim was to try to assess a temporal trend in Belgium, and to highlight potential sources of exposure within the daily habits of the general population.

Methods: The participants were aged from 18 to 76 years old, provided urine and blood samples, and answered to a questionnaire during a face-to-face meeting. The urinary mercury and cadmium analyses were preformed by Flow Injection Mercury system and graphite furnace atomic absorption respectively, whereas 15 OC pesticides or metabolites and 3 PCBs were measured in serum by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS).

Results: The median levels measured in the urine were 0.81 µg/l and <0.5µg/l for mercury and cadmium respectively. The mercury level was higher for people having at least 3 dental amalgams, and for those who reported to consume sea fish more than once a week. The cadmium concentrations measured increased for people older than 60 years old, and were significantly for current smokers compared to former or never smokers, and for passively exposed vs non-exposed people. Median serum levels observed for PCB-153 and -180 were 53.8 and 41.1 ng/g lipid respectively, the PCB-138 being below the limit of quantification (0.15 µg/l) in 49% of the samples. Among the OC pesticides, 4,4'-DDE, beta-hexachlorohexane and hexachlorobenzene were the most frequently positively detected, in respectively 48%, 49% and 37% of the samples. Age, body mass index, and breastfeeding duration were the most predictive determinants of pesticide or PCB serum concentration, whereas dietary habits seemed to not significantly affected these pollutant levels.

Conclusions: This study provides data on the exposure level of a general Belgian population living in the Province of Liege to some several endocrine disruptors, and highlighted some predictors of exposure demonstrating the impact of some already implemented legislations for some chemicals, or the potential need of regulation for others.

EXPOSURE TO ENVIRONMENTAL CHEMICALS IN ADOLESCENTS IN FLANDERS: GEOGRAPHICAL AND TEMPORAL VARIABILITY

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Purpose: In 2002 the Flemish Government started a human biomonitoring programme to study the exposure to environmental chemicals in the Flemish population. Reference values for the general population including geographical variability and time trends had to be determined. In the first FLEHS campaign (2002-2006), 8 areas differing in pollution pressure were chosen: 2 urban areas, an industrial harbor, a chemical industry zone, a nonferrous industry zone, the immediate surroundings of waste incinerators, an area with intensive fruit cultivation and a rural area. To follow time trends in exposure to environmental chemicals, three successive FLEHS campaigns were organized between 2002 and 2015.

Methods: Adolescents (14- 15 years) were recruited via schools, selected by probability sampling in predefined geographical areas. They donated a blood and urine sample and filled in a Dutch questionnaire. Differences in area or in time were tested using multiple linear regression of the exposure biomarker concentrations including relevant covariates to adjust for differences in population characteristics between the campaigns

Results: Compared to a reference mean, internal exposure in adolescents was significantly higher or lower in some of the areas: e.g. Cd and Pb (Antwerp agglomeration), PCBs, DDE and HCB (Ghent harbor), Cd, PCBs, DDE and HCB (rural area), DDE (non-ferrous and chemical industry areas). Adolescents living in an area with intensive fruit cultivation showed the lowest values. The area of residence and sex explained part of the variation in internal exposure among the adolescents. For POPs also BMI was an important factor.
The concentration of serum biomarkers for persistent organic pollutants (POPs), such as PCBs, DDE and HCB, decreased significantly with time. The serum levels of DDE and those of PCBs were almost halved in a time period of ten years, while HCB levels were reduced by a factor of 4. In the period 2013 compared to 2007, mean metabolite levels of di-2-ethylhexyl phthalate (DEHP) and of di-nbutylhthalate (DBP) decreased significantly in urine samples of adolescents with sharper declines for DEHP than for DBP. Cadmium and lead levels in adolescent blood samples were significantly lower in the recent campaigns than 10 years before. Also concentrations of t,t'-muconic acid, a marker of benzene exposure, showed clearly reduced levels. Such favorable trends were not observed for polycyclic aromatic hydrocarbons (PAHs): the levels in adolescent urine in 2013 were not lower than in 2003.

Conclusions: The FLEHS biomonitoring programme 2002-2015 shows that concentrations of well-regulated chemicals especially traditional POPs and cadmium and lead are decreasing in the population of Flanders. However, geographical differences as well as sex, have to be considered.

BIOMONITORING OF ORGANOPHOSPHORUS FLAME RETARDANTS IN A SWEDISH POPULATION – RESULTS FROM FOUR INVESTIGATIONS BETWEEN YEARS 2000 – 2013

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Background: Flame retardants are chemical substances used in furniture, plastics, building materials, household products and consumer goods to reduce their flammability. Their widespread use has resulted in measurable concentrations of the compounds or their residues in the environment, biota and human biological samples. After the brominated flame retardants were phased out due to their persistent, bioaccumulative and potentially neurotoxic properties the use of organophosphorus compounds have increased. The exposure levels, chemical properties and health impacts of the organophosphorus flame retardants have not been studied to the same extent, but according to results from animal studies these compounds and their metabolites have short half-lives.

Aim of the study: The aim of this study was to biomonitor residues from the organophosphorus flame retardants tributyl phosphate (TBP), triphenyl phosphate (TPP), tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) and tris(2-butoxyethyl) phosphate (TBOEP) in urine samples from a Swedish population of young men and women not occupationally exposed.

Methods: The urine samples were collected through the enrolment for military service year 2000, 2004, 2009 and in upper secondary school year 2013 (n = 801). All samples were analysed for the targeted metabolites DBP, DPP, BBOEP and BDCIPP with LC-MS/MS. The statistical analysis focused on temporal trends of exposure levels.

Results: The biomarkers DBP, DPP and BDCIPP were found in concentrations above LOD (0.03 – 0.1 ng/ml) in the majority of the samples. DPP were found in all samples and had the highest concentrations with median concentrations between 1.2 – 2.6 ng/ml each year of measurement. BBOEP were only found above LOD in some samples and in very low concentrations with median concentrations below LOD. The median concentrations for DBP were between 0.21 – 0.31 ng/ml with a statistically significant decreasing trend over time ($\beta = 0.038$, $p < 0.05$).

Discussion and conclusions: The population seems to be constantly exposed since the biomarkers could be found in the majority of urine samples from the individuals in this study. In general, the concentrations found in this study are consistent with findings in other biomonitoring studies.

The study was supported by grants from the Swedish Environmental Protection Agency.

CONTAMINANT AND NUTRIENT BIOMONITORING IN THE NORTHWEST TERRITORIES, CANADA: SHEDDING LIGHT ON THE RISKS AND BENEFITS FROM FOOD CHOICES

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Background: Northern Indigenous communities in Canada sometimes face higher contaminant exposures than the general Canadian population because of elevated contaminant levels in local, wild-harvested foods (also called "country foods"). For example, contaminant concerns in particular types of country foods led to a series of consumption notices from the Government of the Northwest Territories. But, because remote First Nations communities are not included within the Canadian Health Measures Survey (CHMS), it is not yet known if the elevated contaminant levels noted in some country foods have led to higher exposures in the region.

Aim: A 5-year contaminant biomonitoring project, funded by the Northern Contaminants Program, is currently underway in the Dehcho and Sahtú regions of the Northwest Territories. This project is: 1) providing an exposure baseline for participating communities and 2) helping promote country food reliance in ways that maximize nutrient benefits while limiting contaminant exposure.

Methods: To date, over 300 participants, (including children, adults, and elders) have been recruited from 6 communities in the Dehcho and Sahtú Regions. Each participant provided hair, blood, and/or urine samples for the measurements of metals, persistent organic pollutants, and lipid biomarkers. Dietary patterns were assessed with two food surveys while risk perceptions and communication preferences of participants were evaluated with an electronic questionnaire.

Results: Preliminary results (from Year 1 participants) show that country foods represented on average 8% of participants' caloric intake on the day prior to sample collection. Generally, contaminant levels among participants were similar to those seen in other biomonitoring studies in Canada. However, the levels of uranium and some micronutrients appeared to be higher than usually observed. All samples fell below the established health guidelines for mercury, cadmium, and lead.

Conclusions: Despite high levels of contaminants in particularly country foods, preliminary results suggest that current food usage patterns have not led to elevated exposures in participating communities. These early results reinforce messages that the benefits of current country foods generally outweigh contaminant risks. However, additional analyses are underway to help clarify how generalizable these results are across the region. Follow-up work is underway to evaluate whether uranium exposures have remained high.

THE ALBERTA BIOMONITORING PROGRAM PHASE THREE: ENVIRONMENTAL CHEMICALS IN POOLED MATERNAL AND CORD SERUM SAMPLES

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Background: The Alberta Biomonitoring Program was developed to examine exposure to environmental chemicals in the population of the province of Alberta, Canada. The program has focused thus far on vulnerable populations. During pregnancy, some chemicals can be transferred across the placenta while postpartum transfer can occur through breastfeeding. The first phase of the program investigated exposures in pregnant women and the second phase examined exposures in children.

Aim of the study: The objective of the third phase was to examine exposure to environmental chemicals in paired, pooled, pregnant women and cord blood serum. The chemicals of interest included: cotinine, metals, perfluoroalkyl substances (PFASs), methylmercury, dioxins and furans, phytoestrogens, polychlorinated biphenyls, organochlorine pesticides, polybrominated diphenyl ethers (PBDEs), phenols, parabens, and phthalates.

Methods: Pregnant women were actively recruited in seven regions using an informed consent procedure. Maternal samples were collected during the third trimester, at delivery, or post-delivery and the cord samples were collected post-delivery. Samples were placed in matched maternal and cord pools and stratified based on age and geography.

Results: Preliminary results from three sites showed levels of PFASs were higher in the maternal serum than in the cord serum. Comparison to previous biomonitoring results from pregnant women in Alberta highlighted a significant decrease in levels of perfluorooctane sulfonate (PFOS), from 7.41 (± 0.50) ng/mL in 2005/06 to 1.80 (±0.65) ng/mL in 2013/16. Cord to maternal concentration ratios for the PFASs ranged from 0.4 – 0.8, with correlations of r=0.81 or higher. All of the PBDE congeners detected showed a significant decrease in maternal concentration from 2005/06 to 2013/16, except for BDE 209. Cord to maternal concentration ratios for the PBDEs ranged from 0.4 – 0.8, with good correlation observed for the lower brominated PBDEs (r=0.70-0.90).

Discussion/conclusions: The concentrations of the chemicals detected were similar to levels detected in other Canadian and US biomonitoring studies. The detection rates of the PBDEs were significantly higher than other Canadian studies using individual samples, which highlights the strength of the pooling approach. Cord to maternal concentration ratios compared well with other studies and temporal trends in concentration could be tracked against previous Alberta biomonitoring data.

EXHALED BREATH CONDENSATE: A NOVEL MATRIX FOR BIOLOGICAL MONITORING TO ASSESS OCCUPATIONAL EXPOSURE TO RESPIRABLE CRYSTALLINE SILICA

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Introduction: Biological monitoring is a useful way of determining overall exposures to chemical substances; however in the case of respirable crystalline silica (RCS) this has not been analytically feasible in conventional biological matrices. The aim of this study was to investigate the utility of exhaled breath condensate (EBC) as a potential biological matrix in which to determine exposure to RCS.

Method: Firstly it was established that RCS particles could be qualitatively determined using single particle inductively coupled plasma mass spectrometry (spICP-MS). After this a small pilot study was undertaken collecting EBC from quarry workers, foundry workers and occupationally unexposed persons. The samples were then analysed using both spICP-MS and transmission electron microscopy (TEM).

Results: The results showed that EBC obtained from the occupationally unexposed persons exhibited low background levels of dissolved silica but that silica particles of various sizes were present in samples from quarry workers and the foundry workers.

Discussion: This is the first study to report EBC as a potential biological matrix that allows differentiation of RCS concentrations between samples from workers and occupationally unexposed controls. The results obtained confirmed the presence of RCS in EBC by both spICP-MS and TEM. However, there are difficult analytical challenges still to be overcome before this can be used as a biological monitoring method to determine workplace exposure, these are currently being investigated.

OCCUPATIONAL EXPOSURE TO BTEX IN AN OIL REFINERY ASSESSED BY URINE ANALYSIS: COMPARISON BETWEEN STANDARD WORK AND SPECIAL CLEAN-UP

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Background: Mixed exposures to chemicals are a topical issue for occupational health. A method to evaluate occupational exposures to VOCs through the analysis of unmetabolized VOCs in urine was developed and validated in our laboratory.

Aim of the study: This method was applied in an oil refinery to assess exposure to benzene, toluene, ethylbenzene and xylenes (BTEX) through 4 field campaigns divided into two functioning modes: two during standard weeks of work and two during major clean-up weeks, when activities were expected to be more exposing.

Methods: Dynamic headspace system linked to a mass spectrometer is a sensitive method for BTEX analysis (LOQ: 10 ng/L). During 5 days, urinary samples were collected before and after shift (about 30 workers plus controls).

Results: 886 urinary samples from exposed workers and 356 from controls were collected. Workers were classified into 7 (clean-up weeks) or 9 (standard weeks) Homogenous Exposure Groups (HEGs). Smoking effect was statistically significant in both functioning modes. BTEX excretions of smokers were always significantly higher than non-smokers'ones, for controls or exposed workers. Indeed, the tobacco smoke contains a lot of aromatic hydrocarbons, most especially benzene and toluene. Different profiles of BTEX excretions also appeared for the two functioning modes, depending on exposition and on the moment of urine collection (pre- or post-shift). During the standard weeks, only a few HEGs led to BTEX excretions significantly higher after work-shifts than before work-shifts, especially to benzene. These differences were greater during the clean-up weeks and the number of HEGs concerned was higher.

Discussion/conclusions: This study showed that, for control workers (even non-smokers) and low-exposed workers, urinary excretions after work-shifts were significantly lower than before work-shifts, probably due to an efficient air-purification system inside the buildings of the plant, which were also non-smoking areas. Indeed, the comparison of post-shift urinary concentrations of exposed workers to controls'ones seems more relevant than the usual comparison of pre- and post-shift urinary concentrations.

USING TWO DIFFERENT URINARY BIOMARKERS OF BENZO(A)PYRENE TO ASSESS OCCUPATIONAL EXPOSURE AND INDIVIDUAL CANCER SUSCEPTIBILITY

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Background: Polycyclic aromatic hydrocarbons (PAHs) are commonly found in occupational environments. Among them, Benzo(a)pyrene (BaP) is the only PAHs classified as human carcinogens. To exert its carcinogenic effects, metabolic activation is required but many studies demonstrate large interindividual differences in the metabolism of PAHs which can be critical in assessing individual cancer susceptibility. Urinary 3-hydroxyBaP has been proposed to estimate BaP occupational exposure but this metabolite is produced by the BaP detoxification pathway. We have recently developed the urinary analysis of BaP-7,8,9,10-tetraol (BaPT) which is a practical biomarker of BaP metabolic activation by the diol epoxide pathway.

Aim of the study: To assess BaP occupational exposure and individual cancer susceptibility, we would like to compare 3OHBaP and BaPT levels measured in the same samples collected at different time among several workers.

Methods: Urine samples of seven workers involved in an electrode production plant were collected at the beginning and at the end of the shift every day of the working week. Furthermore, subjects collected all urinations in their entirety during the weekend. After hydrolysis and solid-phase extraction, 3OHBaP was quantified by high pressure liquid chromatography coupled with fluorescence detection while BaPT was quantified by gas chromatography–tandem mass spectrometry in negative chemical ionization mode after derivatization. The limits of quantification (LOQ) for 3OHBaP and BaPT were 0,06 ng/L and 0,02 ng/L, respectively. After Log-transforming values of urinary metabolites concentrations, Pearson coefficient and Student T test were performed.

Results: Among 138 urine samples, 11 concentrations were below the LOQ for 3OHBaP and none for BaPT. There was a good correlation between Log3OHBaP and logBaPT for 6 subjects. For the subject where no correlation was found, 3OHBaP levels were very low. While the median of BaPT/3OHBaP ratio was equal to 5.8, 5.1 and 4.3 for 3 subjects, it was equal 2.9, 2.6 and 2.1 for the others. The ratio of BaPT/3OHBaP cumulated amounts eliminated during the weekend was 7.4, 7.1 and 4.0 for the first three and was 3.0, 2.3 and 2.0 for the last three.

Short discussion/conclusions: Similar differences between the subjects were found that the BaPT/3OHBaP ratio calculated from spot urine samples or from cumulated amounts eliminated during the weekend was used. Those people who have a high BaPT/3OHBaP ratio metabolically activate BaP more extensively and should be at higher risk for cancer.

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OCCUPATIONAL EXPOSURE OF CASHIERS TO BISPHENOL S, ALTERNATIVE OF BISPHENOL A IN THERMAL PAPER

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Background: Bisphenol A (BPA) is widely used in the production of diverse industrial and consumer products. BPA also has been used in the paper industry as color developer in thermal paper. Human exposure to BPA is widespread and has been associated with an array of adverse health outcomes. However, controversial discussions around the effects of BPA are still ongoing. Given the societal pressure and the regulation put forward to limit its applications, BPA is gradually being replaced by alternative chemicals to produce "BPA-free" products. In thermal paper, Bisphenol S (BPS) is the primarily replacement for BPA. Limited information on the hazards of BPS is available. The question of occupational exposure to BPA in particular during thermal paper handling was in the foreground these last years. In this study, we investigated the urinary BPA levels as well as the urinary BPS levels of cashiers and controls from several workplaces.

Methods: BPA and BPS were quantified in urine in their free (unconjugated) and total (unconjugated and conjugated) forms using liquid chromatography tandem mass spectrometry (LC-MS/MS). Spot urine samples, including pre-shift and post-sift samples and first morning void were collected from each participant. In addition, thermal paper receipts were analyzed for the presence of BPA or BPS.

Results: Urines samples were collected from 90 cashiers and 44 controls for BPA and from 17 cashiers and 15 controls for BPS. Free and total BPA were detected in all samples. A significant increase in urinary total BPA concentration for cashiers was observed when compared to controls. The median urinary total BPA concentration was 3.54 µg/L for controls and 8.92 µg/L for cashiers.
Total BPS was detected in 95 % of samples in the control group and a significant increase was found in urinary total BPS concentration for cashiers when compared to controls. The median urinary total BPS concentration was 0.67 µg/L for controls and 2.53 µg/L for cashiers.

Conclusion: The detectable levels of BPS in urine of controls would suggest the exposure of general population to BPS. In addition, frequent contact with thermal paper receipts is associated with an increase in urinary total concentration for both BPS and BPA, for cashiers.

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EVALUATION OF THE EXPOSURE TO SOLVENTS IN WORKERS FROM A THERMOPLASTIC PANELS FACTORY: AIR, DERMAL AND BIO-MONITORING

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Background: Biomonitoring the exposure to chemicals in the workplaces is an important component of exposure assessment and prevention of adverse health effects. Generally, exposure is highlighted by the determination of pollutants in environmental matrices (e.g. air samples) and the subsequent assessment of the transfer to human. Additionally, the assessment of dermal exposure is becoming increasingly important in occupational settings, but its contribution to the overall body burden is not yet well established.

Aim of the study: Thus, in this study was evaluated the contribution of dermal exposure to solvents (e.g. styrene, acetone and toluene) to the total body burden as reflected by human biomonitoring, together with respiratory and dermal exposure assessment.

Methods: Systemic exposure was assessed by quantification of mandelic acid, acetone and hyppuric acid in urine, as biomarkers for styrene, acetone and toluene, respectively. Inhalation (using organic vapor monitors, OVMs) and dermal exposure (using activated charcoal cloth, ACC patches) were measured simultaneously in 40 subjects performing different jobs: hydraulic press (HP) workers, sheet molding composite (SMC) workers and office workers.

Results: The urinary concentration of mandelic acid (549±85 µg/mL) was higher in workers, that were simultaneously exposed to styrene via inhalation (58 ±11 mg/m^3) and dermal (1490 ±125 µg/cm^2), like the HP workers. The workers, exposed to similar respiratory levels (60 ±8 mg/m^3) without any dermal exposure had lower urinary concentrations of mandelic acid (331 ±21 µg/mL), like the SMC workers. Also, a good correlation between urinary, air and dermal levels of acetone have been found. Unlike for styrene and acetone, the levels of air concentration observed for toluene were less than 1% of TLV, regardless of the different jobs performed by the workers. No relevant dermal or inhalation exposure were found for office workers, for which the urinary concentrations of the target metabolites were near or lower than the limits of quantification.

Short discussion/Conclusions: These results have shown that urinary concentration of mandelic acid and acetone is able to reflect the contribution of dermal exposure to the overall exposure. Thus, one could further conclude that an increased urinary concentration of solvents metabolites, without important changes in the respiratory exposure, might reflect the contribution of dermal exposure to the overall body burden.

BIOMONITORING OF THE HERBICIDE GLYPHOSATE IN A POPULATION FROM ZARCERO, COSTA RICA

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Background: Glyphosate (GLY), N-(phosphonomethyl) glycine, is the most widely used herbicide in the world. It is a broad-spectrum herbicide, also used in crop desiccation. General populations may be exposed to GLY, mainly via diet and agricultural personnel may be exposed occupationally. GLY is considered to pose relatively low environmental risks to non-target species but some studies suggest GLY to exhibit adverse health effects. GLY was recently classified by WHO IARC (International agency for Research on Cancer) as "probably carcinogenic to humans'', but EFSA, EPA and also ECHA have rejected this conclusion.
In Costa Rica, the Zarcero province is an area with extensive agriculture, ranging from small-scale conventional farming to organic farming. The use of a broad range of hazardous pesticides has been reported.

Aim of the study: We aim to study kinetics of GLY by measuring parent GLY and its metabolite aminomethylphosphonic acid (AMPA) in urine obtained in an exposure experiment. We also aim to examine GLY exposure in an agricultural population from Costa Rica.

Methods: LC-MS/MS analysis of the samples was performed with minor modifications of a published method by P. K. Jensen et al. 2016.
In the exposure experiment, two healthy volunteers received an oral dose of GLY. The dose corresponded to around 25% of the ADI and was approved by the ethics committee. Samples were collected up to 100 h.
Urine samples were collected in a cross-sectional survey of agricultural workers from conventional and organic farms in Zarcero with the objective to maximize exposure contrast. Of the 600 samples, 95 were randomly selected for analysis.

Results: In the exposure experiment, urinary excretion of GLY seemed to follow first-order kinetics and a two-phase excretion. The excretion half-life of GLY (creatinine-adjusted) in the rapid phase was around 17 h and 4 h in the female and male, respectively. The total dose recovered as GLY in urine was around 1% and 0.4% in the female and male volunteer, respectively. The amount of AMPA was negligible.
Around 70% of the samples from the workers had detectable levels of GLY. The median urinary GLY and AMPA levels were both 0.3 µg/L and the 95th percentiles were 1.8 µg/L and 0.9 µg/L, respectively.

Discussion/Conclusions: Preliminary data of the exposure experiment suggests short excretion half-life. The results suggest exposure to GLY among the farm workers. However, the levels of GLY were low and the fate of GLY should be studied further.

BIOLOGICAL MONITORING OF WORKERS EXPOSED TO ENGINEERED NANOMATERIALS

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Background: Nanoparticles can affect the lung, cardiovascular and most organ systems. From air pollution epidemiology it is known that particles in the nanoscale size range are responsible for excess respiratory and cardiovascular mortality. Recently, various nanoscale particles in air pollution and some in animal studies have been shown to be related to adverse neurological changes. Also, nanoparticles in welding fumes are known to cause toxicological and carcinogenic effects. It is too early to identify the exact burden of engineered nanomaterials (ENM) to workers but it is anticipated that exposure to engineered nanoparticles can be similar to ultrafine air pollution or other dusts and fumes that cause pulmonary and cardiovascular effects. Therefore, it is likely the pneumoconiosis and COPD as well as other dust-related conditions could arise. ENMs can enter the circulation and all organs and tissues by blood flow. However the toxicity of engineered nanomaterials and the risks to workers is not well established but it seems clear that ENMs are generally more potent than larger size particles of the same composition. There is a need to consider what biological monitoring might be useful as part of a risk management plan for workers exposed to ENM.

Aim of the study: The focus of this study is to identify the use of “omic” and “non-omic” biomarkers in addressing potential adverse effects of engineered nanomaterials in workers.

Methods: A 9x6 matrix has been developed to organize the results of a search of published scientific literature. The matrix rows consist of different types of biomarkers: Non-omic: functional, homeostatic and pathologic and omic: genomic, epigenomic, proteomic, transcriptome metabolomic, adductomic. The columns consist of: animal and cell toxicology, epidemiology, engineering control/exposure assessment, medical surveillance/biomonitoring, risk assessment/guidance, and pre-market testing. The matrix row and column labels are the search terms for the period 2000-2017.

Results: Initial search of the literature showed a limited but growing understanding of biomarkers that relate to ENM exposure, effect or susceptibility. Various toxicogenomic biomarkers have been shown to be indicative of exposure, toxicity or activation of adverse outcome pathways. Biomarkers of susceptibility to the effect of ENM exposure have also been identified.

Short discussion/conclusions: The critical need for all types of biomarkers is that they be validated for the purpose for which they will be used. Validation is lacking behind discovery and there is a need to promote validation research. Since there are so many different types of ENMs it may be necessary to develop standard panels of biomarkers for various categories of ENMs.

RISK ASSESSMENT AND MANAGEMENT OF ENGINEERED NANOMATERIALS: THE RELEVANCE OF SUSCEPTIBILITY BIOMONITORING

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Background: Recent advances in nanotechnology are expected to lead increasing occupational exposure to nanomaterials (NMs) whose health impacts are still not understood. Therefore, there is the need to achieve a suitable NM risk assessment to guide the adoption of appropriate workplace preventive and protective measures. In this context, biological monitoring (BM) may be helpful, not only to assess NM exposure and early biological effects (Iavicoli et al. 2014), but also to identify susceptible populations that may be at increased risk of adverse effects from NMs. In this way, advanced risk assessment and management models may be tailored on inter-individual/subpopulation variability in NM response.

Aim of the study: To provide an update overview on susceptibility aspects potentially affecting heterogeneous responses to NMs, to identify possible susceptibility indicators for BM programs.

Methods: PubMed, ISI web of Science and Scopus scientific databases were searched for *in vitro* and *in vivo* studies addressing susceptibility issues potentially influencing responses to NMs.

Results: Information on heritable genome alterations able to affect individual susceptibility to NM effects is still lacking. NM induced alterations in gene expression of enzymes responsible for xenobiotic metabolisms (i.e. cytochrome P450, glutathione transferase), and of some DNA repairing pathways, together with epigenetic modifications, may all act as possible determinants of different reactions to NMs. Individual conditions, i.e. age, gender and health status could predispose to a different NM susceptibility.

Conclusions: Our preliminary results pointed out some interesting data concerning NM modes of action useful to guide future research to identify potentially sensitive, specific and ethically acceptable susceptibility biomarkers to be explored and validated in workplaces. The role of NM physicochemical properties in affecting susceptibility profiles, and the pathways primarily involved in NM toxicokinetic/dynamic behaviour whose inherited or acquired alterations may determine different effects should be deeply addressed. Overall, to invest into the "nano-susceptibility" field may provide key information for adequate risk assessment and management strategies to support healthy NM working conditions also for susceptible individuals.

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BIOMONITORING OF OXIDATIVE STRESS AND INFLAMMATION IN NANOCOMPOSITES PRODUCTION WORKERS

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Background: Human studies in nanomaterials exposed workers are extremely rare.

Aim of the study: Our previous studies in workers producing (nano)TiO2 and nanoFe oxides found elevated markers of oxidative stress. This study focused on nanocomposites research workers.

Methods: 20 employees (41.8±11.4 years), working in nanocomposites producing plant for average 6.5±3.9 years were examined pre-shift and post-shift; in addition to 21 control subjects (42.7±11.5 years). Markers of oxidative stress, malondialdehyde (MDA), 4-hydroxy-trans-hexenale (HHE), 4-hydroxy-trans-nonenale (HNE), 8-isoProstaglandin F2α (8-isoprostane), in addition to markers of nucleic acid oxidation: 8-hydroxy-2-deoxyguanosine (8-OHdG), 8-hydroxyguanosine (8-OHG), 5-hydroxymethyl uracil (5-OHMeU), proteins: o-tyrosine (o-Tyr), 3-chlorotyrosine (3-ClTyr), and 3-nitrotyrosine (3-NOTyr); markers of inflammation - leukotrienes and tumor necrosis factor (TNF) were analyzed in exhaled breath condensate (EBC) by LC-ESI-MS/MS. Aerosol exposure in two workplaces was measured using offline and online aerosol instruments: Berner Low-Pressure Impactor, Scanning Mobility Particle Sizer, Aerodynamic Particle Sizer, Condensation Particle Counter and Optical Particle Sizer.

Results: Total mass concentrations ranged from iron casting (120 $\mu g/m^3$), machining (804 $\mu g/m^3$) to welding (1840 $\mu g/m^3$). The number percentage of the particles in the nano-size was the highest at casting (97%), lower at machining (60%) and the lowest at welding (37%). Markers of oxidative stress in workers were elevated, including pre-shift and post-shift MDA, HNE, 8-OHdG, 8-OHG, 5-OHMeU, o-Tyr, 3-ClTyr, 3-NOTyr; and post-shift 8-isoprostane. LTB4, LTE4 and TNF appeared most important among inflammation markers.

Discussion: Elevated markers confirm oxidative stress and inflammation in the lungs. The results support our earlier findings in workers exposed to TiO_2 and Fe oxides nanoparticles and may point to a similar pathogenesis.

Conclusions: These markers may be helpful for monitoring biological effect of nanoparticles in exposed workers. Spirometry and FeNO are not sensitive enough. The level of oxidative stress was lower comparing to findings in (nano)TiO2 and (nano)Fe oxides-exposed workers. The results support the hypothesis of lipids, nucleic acids and proteins damage in the lungs of nanoparticles-exposed workers, which may result in long-term changes.

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THE ROLE OF BIOLOGICAL MONITORING IN NANOTECHNOLOGY HAZARD AND RISK ASSESSMENT

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Although no overt health effects in humans caused by nanomaterials (NM) have been reported yet, ensuring the safety of workers is mandatory for the responsible development and the long-term sustainability of nanotechnology-enabled industry. To improve the safety assessment of NM, many pragmatic approaches have been proposed, including non testing strategies (reading-across and categorization), whereas our current testing strategy is actualized by using simplified models, different dosing regimens and exposure routes which represent a compromise to understand what could happen in humans. These settings are not truly representative of nano-bio-interactions occurring in a real-life scenario, which requires more caution in the interpretation of findings with consideration of the weight of evidence. As a result, insufficiency and ambiguity of the existing *in vitro* and *in vivo* toxicological data on NM hamper a consistent risk assessment (RA) required for scientifically sound regulatory and policy decision-making.

Research of biomarkers and their validation in biomonitoring (BM) programs have been considered a complementary approach to decrease the uncertainty in estimating exposures by biologically relevant measures and to determine whether individuals or a population are at increased risk of adverse health effects. Within the hierarchy of biomarker development, a pragmatic approach is to draw exploratory and candidate biomarkers from other fields of particle and metal toxicology, to find similarities and differences in molecular pathways of toxicity. Comparative proteomic studies have shown strong similarities in the pulmonary response to different NM with known hazardous particles and fibers. Achievements in this field may make more consistent also the regulatory approach to hazard based on dynamic adverse outcome pathway (AOP) models.

Since BM has different meanings in the research and practice, candidate biomarkers, which are potentially useful for occupational health surveillance, epidemiology and environmental sustainability should meet validity criteria, and thus undergo field validation and assessment of their predictive value towards relevant health outcomes and, ultimately, potential risks.

BM represents a valuable component of an integrated strategy and a pro-active approach to RA and management, an opportunity for companies committed with the responsible development of nanotechnology, and also an ethical obligation towards all workers population.

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FIRST RESULTS OF THE NANO LONG-TERM INHALATION STUDY WITH TWO NANOMATERIALS, CERIA AND BARIUM SULPHATE

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The the chronic effects of two poorly soluble, nanoparticles of low toxicity, Ceria (CeO_2) and Barium Sulphate ($BaSO_4$), in the low concentration range in order to assess the risks for the health of humans and to deliver data for the derivation of limit values. The study was designed based on results of prior in vitro and *in vivo* short-term studies on the kinetic and effect [1, 2, 3, 4]. The test protocol of this long-term study followed the OECD test guideline no. 453 with additional examinations [5]: 100 Wistar rats per concentration inhaled nanoparticles aerosol concentrations of 0.1, 0.3, 1 or 3 mg/m³ CeO_2 or 50 mg/m³ $BaSO_4$ for two years. A control group breathed filtrated air. Animals were examined after three months, one year and two years; a sub-group was kept exposure-free after two years of exposure and was examined after six months (a total of 30 months). The in-life part of the study was performed at the Experimental Toxicology of BASF. The histological evaluation is currently performed by Fraunhofer ITEM and the particle load of the organs is measured by the Federal Institute for Risk Assessment (BfR). The results of the in-life part and the examinations after 3 months and 1 year are presented here: The sizes of the aerosol particles were within the respirable range (1.4-2.3 µm) for the rats. The CeO_2 contents in the lungs were 12 µg (0.1 mg/m³) and 1,4 mg (3 mg/m³) after three months of exposure and 42 µg (0.1 mg/m³) and 2.6 mg (3 mg/m³) after one year of exposure. In contrast, animals exposed to $BaSO_4$ showed a lower lung content (1.7 mg per lung) after three months of exposure. This indicates an extraordinarily fast removal of the particles from the lung with an estimated half-time of one week. After one year of exposure to $BaSO_4$ this changed: The lung burden increased disproportionately to 10 mg per lung. During the exposure and the exposure-free follow-up; the animals did not show any clinical signs of toxicity. The lung lavage revealed an increase of inflammatory cells in the lung (PMN) in animals exposed to all concentrations of CeO_2 at all time points. Animals exposed to $BaSO_4$ did not show lung inflammation after 3 months; however, after one year an increase of inflammatory cells was observed. No systemic genotoxicity was observed [6]. The lungs of the animals aged 12, 24 and 30 months were processed histologically by Fraunhofer ITEM, Hannover (UBA: FKZ 37 1261206 and BAuA: F2325). Preliminary results after 12 and 24 months of exposure did not show increased incidences for lung tumor.

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GLOBAL AND GENE SPECIFIC DNA METHYLATION IN WORKERS EXPOSED TO MULTI WALLED CARBON NANOTUBES

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Background: Recently, a working group of the IARC classified a particular rigid MWCNT (MWCNT-7) as "*Possibly carcinogenic to humans*" (Group 2B). Other CNTs, have been classified as "*Not classifiable as to its carcinogenicity to humans*" (Group 3) due to a general lack of data especially among humans. In addition, recent studies (*in vitro* and *in vivo*), including that from our group have reported CNT induced epigenetic changes as a key player in patho-physiological response.

Objective: We therefore designed a cross sectional to study the association between occupational exposure to MWCNT and epigenetic alterations.

Material and Methods: We were able to recruit 24 MWCNT exposed workers and 43 control subjects for the study. We measured global DNA methylation/ hydroxymethylation levels using a validated LC-MS/MS method. Sequence specific methylation of LINE-1 elements, and promoter regions of important genes associated with epigenetic regulation (*DNMT1* and *HDAC4*), DNA damage/repair pathways (*NPAT/ ATM, SKI*) were studied using bisulfite pyrosequencing.

Results: We observed no differences in global methylation of the genomic DNA or repetitive element (*LINE*-1). Significant changes in methylation of CpG sites in promoter region of *DNMT1, HDAC4, NPAT/ ATM* and *SKI* were observed for the MWCNT exposed group.

Conclusions: We observed epigenetic alteration in the exposed group for the selected genes. We believe that the study provides important insight into the epigenetic alterations as a result of MWCNT exposure and could be used as a stepping-stone towards biomarker development.

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HUMAN BIOMONITORING DATA COLLECTION FROM OCCUPATIONAL EXPOSURE TO PESTICIDES

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Background: Human biomonitoring (HBM) as a tool for occupational exposure assessment has been reviewed, with a specific focus on pesticides.

Methods: The databases Scopus, Web of Science and PubMed were searched for the period 1990 to 2015; grey literature was also examined. A two tiered screening process that included quality scoring for HBM, epidemiological and toxicological aspects, was used to identify those studies of most relevance; resulting in 178 studies being identified for critical review. A Master Spreadsheet was designed to collate data from these papers, which contained information relating to: study type; study participants; chemicals under investigation; biomarker quality check; analytical methodology; exposure assessment; health outcome/toxicological endpoint; period of follow-up; narrative of results; risk of bias etc.

Results: Although HBM has been extensively used, epidemiological studies of occupational pesticide use were seen to be limited by inadequate or retrospective exposure information. Very limited data was identified examining seasonal exposures and the impact of PPE, and many of the studies used HBM to assess only one or two specific compounds.

From the 178 publications identified to be of relevance, 41 individual studies included herbicides, 79 included insecticides, and 20 included fungicides. Remaining studies related to mixtures or non-specific biomarkers for groups of pesticides. Despite current limitations, there was evidence for a role of HBM in occupational health and safety strategies, as both a tool for refined exposure assessment in epidemiology studies and to contribute to the evaluation of potential health risks from occupational exposure to pesticides.

Short discussion/conclusions: Some key issues were identified to enable implementation of HBM as part of the occupational health surveillance for pesticides in Europe. These included issues around priorities for the development of new specific and sensitive biomarkers, the derivation and adoption of health-based guidance values, development of QA schemes to validate inter-laboratory measurements, good practice in field work and questionnaire design, and the use of HBM for post-approval monitoring of pesticide safety.

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PESTICIDE URINARY BIOMARKER DISCOVERY IN SMALL-SCALE HUMAN VOLUNTEER STUDIES USING LC-FULL SCAN HRMS

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Background: Humans are exposed to pesticides through food, use of home and garden pesticides/biocides, and the environment (air, dust, especially in rural areas due to nearby agricultural activities). Human biomonitoring allows to assess the exposure integrating all sources and exposure routes. Urine is the most frequently used matrix because of the ease of collection. Most pesticides are rapidly metabolised and excreted. Therefore, analysis typically comes down to measurement of metabolites as biomarkers of exposure. While for classical pesticides, such as organophosphorus pesticides and pyrethroids, the biomarkers for human biomonitoring have been established, a lack of human data exists for many modern pesticides. A major bottleneck in biomonitoring of pesticides is that the optimum biomarkers of exposure in humans are not known. The improvements in analytical instrumentation enables biomarker discovery/verification in urine from volunteers exposed to pesticides at low (<ADI) levels.

Aim of the study: To find the most suitable urinary biomarker of exposure for pesticides in humans.

Methods: Controlled exposure was done through small-scale human volunteer studies (3 males and 3 females; oral or dermal exposure in separate events), approved by the Medical Ethical Review Committee. Urine was collected before and after exposure. Analysis of urine samples was done with and without deconjugation. After ultrafiltration, samples were analysed by LC-full scan HRMS. Multiple workflows using dedicated software tools were employed for biomarker detection.

Results: Are presented for two example pesticides. In both cases, multiple metabolites were (tentatively) identified. Most metabolites found in human urine had also been reported in animal studies. As was to be expected, higher responses were observed after oral intake compared to dermal application. For the pesticides studied, no clear differences were found between oral and dermal exposure in terms of relative responses of metabolites.

Short discussion/conclusions: Current LC-HRMS instrumentation and software tools are suited for human biomarker discovery/verification in urine from subjects exposed to low levels of pesticides. This facilitates selection of the most suited biomarker, i.e. the biomarker that is specific to the parent and most sensitively detected. For final selection of the biomarker, an additional requirement is that an analytical standard can be obtained.

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BIOMONITORING LONG AND SHORT TERM EXPOSURE TO PENCONAZOLE USING HAIR AND URINE SPECIMENTS

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Background: Penconazole (PEN) is a fungicide widely used in vineyards.

Aim of the study: To identify urinary metabolites for biological monitoring of occupational exposure to PEN; to assess the suitability of hair as a matrix of long-term exposure to PEN.

Methods: Winegrowers (16 subjects) exposed to PEN collected cumulative urine sample respectively for: 24 h before the first application (Pre-WS), during the application (WS1, WS2, and WS3), after the application since the next work shift (Post 24h 1 and Post 24h 2), the last work day for 24h after the application (Last post 24h), and a last sample from 25th to 48th hour after the application (Post 25-48h). Urine samples were analyzed by LC-MS/MS to obtain a profile of candidate metabolites. Based on the presence of the triazole moiety in the full scan mass spectra major candidates were found. From their mass spectra hydroxy and carboxy-penconazole (PEN-OH and PEN-COOH), both as free molecules and as glucuronide conjugates, were identified. The free molecules were quantified in urine samples after hydrolysis. Hair samples of 13 winegrowers, 3 family members of winegrowers, and 5 technicians involved in samples collection were collected before and after the treatment season (samples PRE- and POST-EXP). PEN in hair was desorbed with acetonitrile and extracts were analyzed by LC-MS/MS.

Results: PEN-OH was the most abundant metabolite with a wide inter-subject variability. Mean PEN-OH and PEN-COOH levels were in the 1.3-237.0 µg/L and 0.5-54.1 µg/L ranges, respectively. Excretion of PEN metabolites increased with consecutive work shifts. Urinary metabolites were correlated with the potential and actual dermal exposure assessed measuring PEN on the work clothes and on the skin, with Pearson r up to 0.428 in Post 25-48h samples. In hair samples, PEN was quantifiable in most PRE-EXP samples (0.010 ng/mg hair) and in all POST-EXP samples (0.060 ng/mg hair) with a significant increase ($p=0.005$). PEN was quantifiable in all POST-EXP families (0.011 ng/mg hair) and technicians (0.005 ng/mg hair); in winegrowers it was higher than the other two groups ($p=0.022$).

Conclusions: The results obtained suggest that PEN-OH in post-exposure urine sample and hair PEN are promising candidate for biomonitoring short- and long-term exposure to PEN in agriculture workers.

URINE COLLECTION METHODS FOR NON-TOILET TRAINED CHILDREN IN ENVIRONMENTAL EXPOSURE ASSESSMENT OF PESTICIDES

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Background: Young children differ from adults in their exposure and susceptibility to environmental chemicals (e.g. pesticides) because of various factors such as biometry, physiology, behavior and diet. Their heightened vulnerability to environmental stressors makes it important to obtain appropriate urine samples for biomonitoring. However, collecting urine from non-toilet trained children has been shown to be methodologically and practically challenging. Collection methods should not introduce contamination or affect the sample integrity and must be acceptable to the participants.

Aim of the study: Evaluation of various urine collection methods for non-toilet trained children which could be applied in a non-clinical setting to obtain pesticide biomonitoring data.

Methods: Selected urine collection methods include a disposable polyacrylate diaper, a urine bag, a collection pad containing a hygroscopic polymer and the clean catch method. Advantages and limitations of these methods were evaluated with respect to minimum required sample volume, potential for contamination, timing of collection, and burden on participants. The success rate was defined as the percentage of suitable samples from the total number of sample collection attempts. An attempt was considered successful if it yielded a urine sample of at least 5 mL and free of faeces contamination. In addition, the user rating of each method was evaluated.

Results: In total 24 samples were obtained for each of the urine collection methods. The success rates were 67%, 21%, 17% and 4% for the disposable diaper, urine bag, collection pad and clean catch, respectively. The average user ratings on a scale of 0-10 were 9.0, 4.7, 7.3 and 2.5, respectively. This indicates that a disposable polyacrylate diaper is a proper urine collection method among non-toilet trained children and therefore this method will be further evaluated for the storage stability of the analytes of interest, including clinical parameters such as creatinine and urea.
Preliminary results showed that urine stored in the polyacrylate granules could be extracted using an aqueous solution of 15% calcium chloride. The recovery of creatinine was 92% to 95%.

Conclusion: The disposable polyacrylate diaper is the most suitable method for collection of urine from non-toilet trained children for pesticide biomonitoring. This study will be continued by analysis of the recoveries from the diaper of a range of xenobiotic metabolites (e.g. pesticides, metals, and polyaromatic hydrocarbons).

SIMULTANEOUS ASSESSMENT OF PHENOLIC METABOLITES IN HUMAN URINE FOR A SPECIFIC BIOMONITORING OF EXPOSURE TO ORGANOPHOSPHATE AND CARBAMATE PESTICIDES

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Background: Organophosphate pesticides (OPP) and carbamates are still counted among the most prominent agents used for crops protection. Up to date the determination of dialkylphosphates and their thio derivatives in urine is established for the OPP biomonitoring. However, this approach does not provide information on the exposure to specific OPP agents. A lot of OPP as well as some carbamates also provide specific urinary biomarkers indeed.

Aim of the study: For a specific biomonitoring approach we developed an analytical procedure, which enables the assessment of a wide spectrum of phenolic metabolites of OPP and carbamates in human urine using gas-chromatography with tandem mass spectrometry (GC-MS/MS).

Methods: After enzymatic cleavage of possible glucuronide and sulfate conjugates, the analytes were extracted by solid phase cartridges and derivatized with N-Methyl-N-(trimethylsilyl)trifluoroacetamide. The prepared samples were determined by GC-MS/MS using electron impact ionization and multiple reaction monitoring mode. The validated method was applied to samples used for several pilot studies about the efficiency control of dietary pesticide intake reduction [1, 2].

Results: The method showed detection limits between 0.1 and 0.3 µg/l. Variation coefficients ranged from 2 % to 10 % for precision in series and 2 % to 14 % for inter-day precision. Furthermore, recovery rates between 87 % and 120 % were determined. The urinary levels of phenolic metabolites were very similar in the application studies. Frequent detection and high levels were found for 3,5,6-trichloro-2-pyridinol (parameter of chlorpyrifos and chlorpyrifos-methyl), 4-nitrophenol (parameter of parathion and paraoxon), 2-isopropoxyphenol (parameter of propoxur), 2-(diethylamino)-6-methylpyrimidin-4-ol (parameter of pirimiphos-ethyl, pirimiphos-methyl), 1-naphthol (parameter of carbaryl and naphthalene) and 2-naphthol (parameter of naphthalene).

Short discussion/conclusions: The method showed high reliability and robustness. It enables the simultaneous and specific monitoring of a wide spectrum of pesticides and biocides whose structures contain aryl moieties. The results of the application emphasize the need of such a multicomponent approach.

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COMPARISON OF PCB INDUCED INHIBITION OF TELOMERASE GENE EXPRESSION BIOASSAYS AND PCB CONCENTRATIONS IN HUMAN PLASMA

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Background: Polychlorinated biphenyls (PCBs) are toxic, industrial chemicals. Germany has established a biological tolerance value at the work place (BAT value) for the combined plasma levels of the PCB congeners 28, 52, 101, 138, 153, and 180 at 15 µg/L blood plasma. However, the evaluation of the BAT value was based on studies in rats only.

Aim of the study: To establish a BAT value for PCB contamination based on the deviation from telomerase gene (*hTERT*) expression induced by PCBs in exposed humans and to establish a bioassay as inexpensive prescreening tool.

Methods: We assessed PCB contamination in blood plasma collected 4 years apart from the same individuals with a high PCB body burden. For chemical analysis major PCB congeners were quantified using gas chromatography. For evaluation of *hTERT* expression we used two different cellular models: 1. Peripheral blood mononuclear cells (PBMCs) stimulated with tetanus toxoid (TT) for 5 days and 2. the B6B5.1 cell line stably expressing a luciferase reporter gene construct under the control of the *hTERT* promoter. After incubation with PCB contaminated plasma for 48 hrs, PBMCs were analyzed for *hTERT* expression using qRT-PCR and the B6B5.1 cell line was subjected to luciferase measurement. Results are represented as ratios in relation to the expression of a control gene.

Results: Blood plasma from PCB exposed individuals inhibited *hTERT* expression depending solely on the concentration of lower chlorinated PCBs (PCB congeners 28, 52, 101). In TT-stimulated PBMCs (N=122) a high concentration of lower chlorinated PCBs (mean concentration = 0.74 µg/L) caused a tenfold inhibition of *hTERT* expression levels (mean ratio = 0.02) as compared to a lower concentration of lower chlorinated PCBs (mean concentration = 0.24 µg/L; mean ratio = 0.21). Similar results were obtained using luciferase measurements. The capability of plasma to inhibit *hTERT* expression within identical individuals was relieved over time paralleling the elimination of lower chlorinated PCBs due to biotransformation.

Short discussion/conclusions: A high concentration of lower chlorinated PCBs reduces *hTERT* expression by a factor of 10. We therefore strive for a sole BAT value for the combined plasma level of lower chlorinated biphenyls excluding the PCB congeners 138, 153, and 180. Based on our results, we propose a BAT value for lower chlorinated PCBs at 1 µg/L plasma.

GENOTOXICITY ASSESSMENT OF ORGANIC DUST EXPOSURE – A HUMAN BIOMONITORING STUDY DEVELOPED IN BAKERIES

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Exposure to flour particles occurs in a range of food industries, including in particular industrial and artisan bakeries, cakes and biscuits, and pastry production. Epidemiological studies have shown an increase in mortality associated with exposure to particles with a diameter of less than 10 µm. This association is most evident in fine particulate matter, which normally exhibits physical and chemical characteristics that potentiate adverse effects on human health. Whereas coarse particles (larger caliber) are more likely to be deposited in the bronchial region, and do not reach the alveolar structures, the fine particles tend to deposit in the periphery of the lungs, especially in the bronchioles and pulmonary alveoli, from where its removal after deposition is very slow. Ultrafine particles can cause alveolar inflammation that potentiates lung diseases and increases blood clotting. This process may explain the increase in deaths associated with cardiovascular problems in episodes of urban pollution.
The aim of this study was to characterized possible genotoxic effects of particulate matter by the measurement of DNA damage and DNA oxidative damage by comet assay in workers in bakeries.
Peripheral blood lymphocytes were obtained from venipuncture from 10 workers, who also completed a characterization survey, and compared with a control group without exposure (n=43). Alkaline comet assay was performed to access DNA damage and a modification with FPG to measure DNA oxidative damage.
The results obtained verified that the workers had lower DNA damage mean in comparison with controls (7.70±2.09 vs 12.00±8.54), being the same situation for DNA oxidative damage (3.03±2.38 vs 6.86±6.01). Therefore, until now, no relationship was observed between particulate matter exposure of bakers and DNA damage and also, specifically, oxidative DNA damage. However, this is a preliminary study and more bakeries will be studied and exposure assessment data will be added.
In conclusion, our results suggest that particulate matter in this occupational context did not induce DNA damage and oxidative damage in peripheral blood lymphocytes.

Keywords: Human biomonitoring, Particulate matter, genotoxicity, comet assay, DNA damage, occupational exposure.

EVALUATION OF A CHALLENGE ASSAY AS AN EFFECT BIOMARKER IN ENVIRONMENTAL OR OCCUPATIONAL BIOMONITORING STUDIES

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Background: Human long-term exposure to environmental or occupational stressors may produce adverse effects, e.g., genotoxic effects, that underlie the onset of diseases, among which cancer is the most severe. Besides the instability that a wide variety of genotoxic and carcinogenic agents directly exert on the cell genome, also indirect effects, e.g., interference with the DNA repair capacity deserve to be studied. Such effects have been described for heavy metals, low doses of ionising radiation or complex mixtures, among others.

Aim: This work aimed to evaluate the usefulness of the *ex vivo* challenge assay as a functional biomarker of early biological effects, based on data previously obtained in molecular epidemiology studies.

Methods: The first study (S1) comprised a group of individuals with environmental exposure to ionising radiation and heavy metals (IR Group) and a non-exposed group (NIR Group), whereas the second study (S2) included individuals occupationally exposed to environmental tobacco smoke (ETS Group) and the respective control group (NETS Group). In S1, whole blood samples were subjected to 2Gy of ionising radiation before culture set up for translocation analysis through FISH. Blood samples from S2 participants were exposed to an alkylating agent (EMS) and then processed for the comet assay; the level of DNA damage was quantified by the percentage of DNA in tail. The level of chromosome or DNA damage measured after the ex vivo challenge with the genotoxic agents was compared to the basal level obtained for the same individuals in S1 and S2, respectively.

Results: The results from both studies showed that peripheral blood lymphocytes (PBLs) exposure to a medium/high dose of a genotoxicant (ionising radiation or ETS), followed by an assessment of chromosome or DNA damage, respectively, allowed to distinguish exposed and control groups. The distinct response consisted of a lower frequency of ionising radiation-induced translocations in the IR comparatively to the NIR group (S1) or a lower level of EMS-induced DNA breaks in the ETS comparatively to the NETS Group (S2).

Discussion and Conclusions: The results from both studies agreed in that the use of an ex vivo challenge of PBLs with a genotoxicant allowed the detection of a differential response between the exposed and non-exposed groups. Interestingly, PBLs from exposed individuals were less affected by the ex vivo exposure to a genotoxicant suggesting an adaptive response instead of a reduced DNA repair capacity. These findings are expected to contribute to the development of new biomarkers of early biological effects for human biomonitoring studies.

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SCHOOL-AGED GIRLS' INTELLECTUAL FUNCTION IS MORE AFFECTED BY LOW LEAD EXPOSURE THAN BOYS'

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Background and aim: School-aged children, 93 boys and 73 girls, living in the town of Simões Filho, Bahia, Brazil, a moderately industrialized area, near a manganese transformation plant and crossed by heavily traffic highways, were evaluated for lead (Pb) exposure and effects on intellectual performance.

Methods: Environmental Pb exposure was measured by evaluating Pb loading rates in settled dust in three elementary schools, using Petri dishes as passive samplers. Biological monitoring was performed by measuring blood lead levels (PbB) by graphite furnace atomic absorption spectrometry (GPAAS) and other hematological parameters. As manganese (Mn) is also a potential neurotoxin Mn levels were measure in blood, occipital hair and toenails. Children's IQ was estimated by Wechsler Abbreviated Scale for Intelligence (WASI) using the subtests of vocabulary and reasoning matrix. Likewise, maternal or main caregiver's IQ was also evaluated using the adult version of WASI. Data were analyzed by stepwise multiple regression analysis using SPSS version 22, with significance level of $p<0.05$.

Results: Pb loading rates in interior environments median and range were 48.8 (7.3-307.8) $\mu g/m^2/30$ days. These levels are lower than those observed in daycares in Sydney. Children´s PbB median (P25-P75) was 1.2 (0.6 – 2.3) µg/dL. From the 177 children, 98.3% had PbB levels below or equal to 5 µg/dL, the attention level set by the Center of Disease Control. Despite this low exposure level, a significant inverse association was observed between the log transformed PbB and children´s IQ (β-coefficient -10.5; 95%CI= -16.5 to -3.9; p=0.002) after adjusting for maternal IQ and age. However, after stratifying for sex, girls' IQ was much more affected by variation in the logPbB (β-coefficient= -14.3; 95%CI= -24.3 to -6.4; p=0.006) and association with boys IQ was no longer significant (β-coefficient= -7.4; 95%CI= -15.9 to 1.2; p=0.089). Mn biomarkers were not significantly associated with children´s IQ.

Conclusions: Our data suggest a possible interaction of sex and low lead exposure affecting much more girls than boys. Our hypothesis is that divalent metal transporters are more expressed in girls' cell walls, due to the physiological need for iron.

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URINARY BIOMARKERS OF EXPOSURE TO PAHS AND ASSOCIATION WITH OXIDATIVE DAMAGE TO NUCLEIC ACIDS

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Background: Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic pollutants produced by gasoline vehicles, cooking, heating, environmental tobacco smoke. Oxidative damage to DNA and RNA are biomarker of oxidative stress caused by lifestyle, diseases and environmental contaminant exposure: higher levels of urinary 8-OHdG have been found in populations exposed to high levels of PAHs.

Aim of the study: The determination of oxidative damage/repair markers of DNA and RNA in the urine of volunteers in order to study their association with the urinary exposure biomarkers of environmental pollutants like the metabolites of PAHs.

Methods: Simultaneous determination and quantification of 1-Hydroxypyrene (1-OHPy), 1and 2 –Hydroxynaphathalene (1 and 2-OHNAP), 3-Hydroxybenzo[a]pyrene (3-OHBaPy) and 6-Hydroxynitropyrene (6-OHNPy) in the urine of 110 volunteers, 25 smokers, 49 males, was carried out using solid phase extraction and HPLC-MS/MS. 8-hydroxy-guanine (8oxoGua), 8-hydroxy-2'deoxyguanosine (8oxoGuo) and 8-oxo-7,8-dihydroguanosine (8oxodGuo) were determined on the same urine samples by isotopic dilution HPLC-MS/MS**.**

Results: Urinary concentrations of 1-OHPy, 1and 2 -OHNAP in smokers (median 64.3, 3470, 6090 ng/g creatinine) are higher (t test, $p<0.001$) than in non-smokers (24.2, 709, 2444.3 ng/g creatinine). 3-OHBaPy and 6-OHNPy levels are very much lower and seem to be independent from smoking (non-smokers 0.38 and 0.08; smokers 0.31and 0.07 ng/g creatinine) but Pearson's correlation with urinary cotinine is $r=0.6$. The association the oxidative damage was studied both by Pearson' s correlation and by means of Principal component analysis (PCA) highlighting that 3-OHBaPy and 6-OHNPy are strongly related to 8oxoGuo, and 3-OHBaPy is also linked with 8oxodGuo (Pearson's only) in smokers and in women.

Short discussion/conclusions: The influence of smoking on 3-OHBaPy and 6-OHNPy is not clear and should be confirmed on a larger number of samples. The correlation of 3-OHBaPy, classified IARC class 1 carcinogen, with the oxidative damage biomarkers confirms the effect also of low levels exposure to PAHs (general population). The higher correlation in women than in men is worth of further investigation.

BIOMONITORING OF OCCUPATIONAL EXPOSURE TO STYRENE: DETERMINANTS OF EXPOSURE AND RISK MANAGEMENT MEASURES

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Background: Styrene, widely used in several industrial sectors including fiber reinforced plastic industry, is a neurotoxic and a suspected carcinogen.

Aim of the study: to assess occupational exposure to styrene in different activity sectors and identifying the factors which could influence exposure levels in order to build out prevention recommendations.

Methods: Biological monitoring was conducted in workers from sectors of fiber reinforced plastic manufacturing (polyesters), copolymers and garages. Two urine samples per worker were collected at beginning of the shift - beginning of the week (BS-BW) and at end of shift - end of week (ES-EW), along with the collection of information concerning the work post. Mandelic (MA) and phenyglyoxylic acids (PGA), styrene urinary main metabolites, were quantified using High Performance Liquid Chromatography (HPLC) coupled with an ultraviolet (UV) detector. Linear regression models were used to identify determinants of exposure.

Results: 273 urinary samples were collected from 137 workers in 16 companies, which 87 % worked in polyesters. Highest concentrations were observed in the sector of polyesters, either at BS-BW (geometric mean (GM) = 18 and maximum (max) =199 $mg.g^{-1}$ of creatinine) or at ES-EW (GM = 83 and max=1106 $mg.g^{-1}$ of creatinine), with average levels 5 fold higher at BS-BW and 20 to 40 fold higher at ES-EW than in copolymers or garages. ES-EW levels were significantly higher than BS-BW in polyesters but not in other sectors, and 3.5 % of those working in polyesters had levels above acceptable values in occupational settings (sum MA + PGA < 600 $mg.g^{-1}$ of creatinine). In polyesters, open molding processes were associated with higher exposures than closed molding, and the spray-up molding was responsible for the highest exposure levels. The factors identified as influencing concentrations at the end of the week were base levels at beginning of week, nature of process, proximity to the emission source, use of respiratory protection, styrene proportion in resin, and type of mold.

Short discussion/conclusions: While exposures to styrene in the implementation copolymers or in garages are low, they are still high in the polyester sector. Intervention on process (styrene proportion, closed molding), protective equipment (LEVs, respiratory protection) and individual practices (removal of the source, compliance with safety rules) are expected to decrease exposures and help managing health risks of workers.

ENVIRONMENTAL AND BIOLOGICAL MONITORING OF OCCUPATIONAL EXPOSURE TO POLYNUCLEAR AROMATIC HYDROCARBONS DURING HIGHWAY PAVING IN ITALY

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Background: Hot bitumen applications during road paving lead to the emission of a variety of volatile organic compounds, released as vapours, which condensate to aerosol in the air. Potential health hazards associated with exposure to vapours and aerosols of bitumen have been attributed to the presence of polynuclear aromatic hydrocarbons (PAHs), some of which are classified in EU as carcinogenic 1B, whereas occupational exposure during road paving has been classified by IARC as possibly carcinogen to humans (2B).

Aim of the study: We performed a cross-sectional study with the main aim of evaluating occupational exposure to PAHs by a combined approach of air and biological monitoring in workers involved in the paving of a new highway in Northern Italy, where mastic asphalt was used.

Methods: Four air monitoring campaigns were performed for a total of 16 air samples. Both aerosols and vapours of bitumen were collected applying the NIOSH 5506 method. The 16 PAHs listed as high priority by EPA were determined by HPLC-UV. End of shift urine samples were collected from 144 workers to determine 1-hydroxypyrene (1-OHP) and 2-naphthol (2-NAP) concentrations after enzyme digestion and HPLC-UV analysis. Creatinine concentration was also assessed by the Jaffè method. Socio-demographic and lifestyle information was collected by a questionnaire.

Results: Air monitoring demonstrated that paving workers were heavily exposed to PAHs, including carcinogenic compounds, that were measurable only in the aerosol phase. Higher exposure as well as dose levels were measured for the paver-drivers group. Logistic regression analysis demonstrated a significant association between 1-OHP values higher than the upper limit of Italian reference values and the job task, whereas 2-NAP values were mainly confounded by smoking habits. Such results were further supported by a neural network statistical approach.

Discussion: The present study provides new evidence of heavy exposure levels to PAH during road paving, in particular when mastic-asphalts are used in highway building. In 7 out of 11 air samples, the air levels of benzo(a)pyrene exceeded the acceptable concentration established by the German Federal Institute for Occupational Safety and Health (BAuA), in particular among paver drivers. Such workers are thus exposed to a tolerable carcinogenic risk (more than $4x10^{-5}$ additional deaths). Biological monitoring confirmed that 1-OHP was less affected by smoking habits as compared to 2-NAP and showed a higher association with occupational factors.

Conclusion: In conclusion the study shows a significant exposure to PAH in the investigated worker group. Carcinogenic PAH compounds were detectable only in the aerosol phase and this must be taken into account in the adoption of preventive measures. Biological monitoring supported the superiority of 1-OHP as compared to 2-NAP in these workers.

URINARY TRIMETHYLTIN REFLECTS BLOOD TRIMETHYLTIN IN WORKERS

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Background: Recently reported cases of organotin intoxication showed severe encephalopathy with symptoms of memory disturbance. The study showed higher ratio of urinary trimethyltin to urinary dimethyltin in the workers than that in the previous cases exposed to only dimethyltin, suggesting co-exposure to dimethyltin and trimethyltin in the present cases.

Aim of the study: The present study subsequently investigated blood dimethyltin and trimethyltin to understand the relationship of urinary and blood dimethlytin/trimethyltin for evaluation of validity of them as exposure markers.

Methods: Urinary and blood dimethyltin and trimethyltin at different time points in three workers were measured with HPLC-ICP/MS. Regression analyses were conducted with independent values of blood dimethyltin and trimethyltin and dependent values of urinary dimethyltin and trimethyltin respectively. Multiple regression analysis with dummy variable of individual was also conducted for adjustment of individual factors.

Results: Regression analysis showed significantly positive relation of urinary trimethyltin to blood trimethyltin, but did not show significant relation of urinary dimethyltin to blood dimethyltin. Multiple regression analysis with individual factor also showed significantly positive relation of urinary trimethyltin to blood dimethyltin.

Short discussion/conclusions: The study shows that urinary trimethyltin reflects blood trimethyltin. In co-exposure to trimethytin and dimethyltin, urinary trimethyltin can be an internal exposure marker of trimethyltin, which is considered to be not only derived from external exposure to trimethyltin but also the ultimate toxicant converted from dimethyltin, in human body.

HUMAN BIOMONITORING OF RESORCINOL EXPOSURE IN FINLAND

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Resorcinol (1,3-dihydroxybenzene, CAS 108-46-3) is used in, for example, the manufacture of rubber products, wood adhesives, flame retardants, UV stabilizers, and dyes. It is also used in personal care products such as hair colorants, anti-acne preparations, and peels. Resorcinol is a suspected endocrine disruptor that works via thyroid effects. In addition, resorcinol is irritating to the eyes and skin, and may cause sensitization through skin contact.

The aim of this study was to assess both environmental background exposure and occupational exposure to resorcinol. Occupational exposure was investigated in hairdresser work, in the manufacture of tyres, adhesive resins, and glue-laminated timber, by biomonitoring total resorcinol concentration in urine samples. The biomonitoring results were compared to the background urinary levels of occupationally non-exposed volunteers, and to "biomonitoring equivalents", which were estimated on the basis of available health-based limit values.

Almost all the urine samples (99%) of the non-occupationally exposed volunteers contained measurable amounts of resorcinol. The urinary resorcinol data were rather scattered, and resorcinol concentrations of women were clearly higher than the respective concentrations of men. The reason for this difference remains unclear. Some of the highest results exceeded the lowest health-based biomonitoring equivalent.

According to the results, hairdressers' exposure to resorcinol was at the same level as that of the reference population of occupationally non-exposed volunteers. All hairdresser's values remained below the biomonitoring equivalent of resorcinol, which means that health risks related to exposure are low.

The urinary resorcinol levels of the industrial workers were also at the same level as those of the reference population. When the 95th percentile of the urinary concentration data of the non-occupationally exposed population is used as a biological guidance value for occupational exposure, the results of some workers in the tyre manufacturing company suggest low occupational exposure to resorcinol. In this case also, exposure was below the biomonitoring equivalents estimated on the basis of health-based limit values. The workers in phenolic resin manufacturing and glue-laminated timber manufacturing were not occupationally exposed – the urinary resorcinol concentrations remained at the level of the occupationally non-exposed population.

URINARY ELIMINATION OF S-PHENYLMERCAPTURIC ACID AND URINARY BENZENE 16 HOURS AFTER THE END OF THE EXPOSURE TO LOW CONCENTRATIONS OF BENZENE

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Background: S-phenylmercapturic acid (SPMA) and urinary benzene (U-Ben) are biomarkers of exposure to benzene usually measured in the urine at the end of the work shift (ES). However, studies in workers exposed to high benzene concentrations suggest that SPMA might not be completely eliminated within 16 hours after the end of exposure, while there is no evidence on this point as regards U-Ben.

Aim of the study: To assess SPMA and U-Ben concentrations in urine samples collected in 93 male workers at a metallurgical coking plant, 16 hours after the end of the occupational exposure to low benzene concentrations.

Methods: Exposure to airborne benzene (A-Ben) during the work shift was measured by personal passive samplings, while biological monitoring was performed on urine samples collected from all the workers at ES and then again the next day, 16 hours later, before the beginning of the next work shift (next BS). In all the samples, SPMA, U-Ben, free cotinine (U-Cot) and creatinine were determined. All enrolled workers were administered a questionnaire collecting specific information about job and any other source of non occupational exposure to benzene.

Results: A-Ben concentrations ranged between 3.9 and 105.5 $\mu g/m^3$ (median 17.2 $\mu g/m^3$). SPMA was always detectable, whereas U-Ben was below the limit of quantification (LOQ) in 26.7% of the ES and 35.6% of the next BS samples. Smokers showed significantly higher concentrations of SPMA and U-Ben than non smokers at both the ES and the next BS. At both the sampling times, SPMA and U-Ben concentrations showed a positive dependence on personal A-Ben levels, as well as on the creatinine and U-Cot values.

Conclusions: In workers exposed to low benzene concentrations, the elimination of SPMA and U-Ben, in urine samples collected 16 hours after the end of the work shift, was yet dependent on the occupational exposure suffered in the previous work shift.

WORKPLACE DRUG TESTING IN AUSTRALIA. A SNAPSHOT AND EMERGING ISSUES

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Background: With the increasing prevalence of illicit drug consumption and the potential for misuse of prescription and non-prescription medicines, many workplaces have adopted policies requiring workers to submit to random drug testing as a way of ensuring a safe workplace. Urine and oral fluid specimens are most amenable media for testing, and sensitive, antibody-based screening tests, supported by gas- and liquid-chromatographic/mass spectrometry confirmation analysis results in testing regimes that are reliable, accurate, and defensible. Workplaces employing these strategies depend upon three main elements to ensure best-practice outcomes. These are a written policy, stating the intended aim of the testing strategy; a testing procedure that meets the requirements of the policy; and an education program, to alert workers to the risks associated with drug use. These may be underpinned by a support program to assist workers experiencing problems with drug misuse and abuse.

Aims and method: This paper describes a survey of drug tests performed during a single year (2016-2017) by one drug and alcohol testing business in Australia providing a service to a broad range of national clients, from sectors ranging from transport and logistics to construction, and from law enforcement to mining. Data include results of on-site screening immunoassays and LC-MS confirmation results of non-negative samples.

Results and conclusions: The survey identifies differences in the rates of detection and the nature of the drugs detected between industry sectors, between Australian States and Territories, and between rural and metropolitan areas. These data provide a valuable baseline against which future trends in drug use may be compared, especially with respect to changes associated with;

(a) changes in legal status of drugs such as marijuana for medicinal or recreational use,
(b) changes in prescription availability of drugs (such as codeine),
(c) changes in standards or guidelines for drug testing and the concentration thresholds for reporting, and
(d) changes in the regional availability of illicit drugs associated with their supply, manufacture, and importation, and the efforts of enforcement agencies to disrupt these activities.

AIR POLLUTION STRESS AND THE AGEING PHENOTYPE

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Ageing is a complex physiological phenomenon. The question why some subjects grow old why remaining free form disease whereas others prematurely die remains largely unanswered. We focus here on the role of air pollution in biological ageing. The trajectory form healthy to unhealthy aging often comprise telomere length and a few studies addressed a role of air pollution on the telomere length. Recent studies in adults show that the estimated effects of particulate air pollution exposure on the telomere mitochondrial axis of ageing may play an important role in chronic health effects of air pollution. In the ENVIR*ON*AGE (ENVIRonmental influence *ON* AGEING in early life) mothers with higher residential exposure to particulate air pollution ($PM_{2.5}$) gave birth to newborns with lower telomere length, which could not be explained by other factors including social economic class. For a 5 $\mu g/m^3$ increase in residential $PM_{2.5}$ exposure during pregnancy, cord blood telomeres were 7% shorter and placental telomeres 13% shorter. Improved air quality may promote molecular longevity from birth onwards.

PROMOTING HEALTH IN SMALL AND ARTISANAL MINING OF GOLD (PROSAMIGO) – A FEASIBILITY STUDY FOR HUMAN BIOLOGICAL MONITORING OF MERCURY EXPOSURE

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Background: The use of mercury in artisanal and small-scale gold mining (ASGM) has consequences for human health and the environment. We conducted the study PROmoting health in Small and Artisanal MIning of GOld (PROSAMIGO) to explore the situation for mercury and health in the gold mining areas in the inland of Suriname, South America.

Aim of the study: To study the feasibility of introducing a human biological monitoring (HBM) program to assess mercury exposure for gold miners and local residents, including children.

Methods: A literature study was performed to determine the most suitable biological media for HBM. A health education program was developed and introduced in gold mining areas and villages in the inland of Suriname. We interviewed 30 gold miners and 76 local residents to find out about their interest to participate in a HBM program and included questions related to what they could do themselves to reduce their uptake of mercury.

Results: Recent exposure to metallic mercury can be assessed by blood sampling within 24 h after exposure. Urinary mercury levels mainly reflect long-term inhalation exposure to elemental mercury vapors and divalent mercury. Mercury in blood and hair reflects mid- and long-term exposure to methyl mercury, whereas analysis of a hair segment close to the scalp indicates recent exposure. A diagram was prepared that can support decision-making, regarding the most suitable biological medium for HBM (Boerleider et al., 2017). The respondents expressed an interest to know their mercury body burdens. Respondents gave consent for collection of urine, hair and breath samples, also from their children. Some respondents did not want to provide blood samples because of cultural beliefs.

Short discussion/conclusions: We consider it feasible to prepare a HBM program in the inland of Suriname. For the successful introduction of HBM it is important to carefully report back the lab results to each participant, together with information on possible solutions and adequate care, tailored to the person's situation.

Acknowledgement: Funding was received from the Uitvoeringsorganisatie Twinning Suriname Nederland (UTSN) of the Dutch Ministry of Foreign Affairs.

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URINARY MERCURY EXCRETION IN BOLIVIAN WORKERS AND FAMILIES ENGAGED IN SMALL SCALE GOLD MINING

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This presentation describes a cross sectional study which examined the association of geospatial, environmental, and workplace factors with the urinary excretion of mercury. Artisanal gold mining is attractive to economically disadvantaged communities because it requires little capital investment and uses relatively simple equipment. The prevailing method involves the addition of elemental mercury to pulverized rock to form a gold-mercury amalgam which is then further processed to remove the mercury. Individuals involved in gold extraction are at risk of adverse health effects, including damage to their nervous systems and kidneys, when they inhale mercury vapor. Biological monitoring based on the urine concentrations of mercury was performed to assess the individual exposures sustained by members of a community engaged in small scale gold mining. The analytical method employed ICP-DRC-MS using CDC method # ITU007B. Spot urine samples and questionnaire responses were obtained from 89 men, 24 women, and 21 children. Results were categorized using a scheme developed by the German HBM Commission (Angerer et al., 2011): ACCEPTABLE (less than 7 µg/L urine), CAUTION (7-25 µg/L urine), and HEALTH ALERT (> 25 µg/L urine). While over half of the participants had acceptable levels of mercury exposure, 17% of the men (n=11/89), 25% of the women (n=6/24), and 25% of the children (n=5/21) were found to have levels placing them in the HEALTH ALERT range. 22% (14/63) of adults reporting occupational contact with mercury were in the HEALTH ALERT range compared with 13% (3/23) among those without such exposure. Initial results indicate that urinary mercury levels are highly correlated within family groupings. These findings will be used to develop interventions in collaboration with the community, the mining cooperatives, and PLAGBOL, a Bolivian organization seeking to minimize exposures to environmental toxicants.

This project has approval from the Comite Nacional de Bioetica (Bolivia) and the CUNY Institutional Review Board for the Protection of Human Subjects (USA).

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BIOLOGICAL MONITORING OF EXPOSURE TO SELENIUM COMPOUNDS IN WORKERS OF THE SELENIUM PROCESSING INDUSTRY

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Selenium (Se) is an essential trace element for humans, but exhibits a very small safety margin between recommended daily intake and toxic dosages. Volunteer exposure studies have indicated different metabolisms for organic and inorganic Se compounds. Thus, a comprehensive biomonitoring approach for Se exposure at workplaces and particularly the correlation between Se biomarkers and occupational Se exposure has to be established.

In a case-control study, we investigated 20 male employees of a Se processing plant and 20 age matched men without occupational Se exposure. The average shift level of total Se and of the water-soluble Se-fraction in air was measured for each worker. Blood samples were taken at the end of the shift and urine samples at the beginning and at the end of the shift. Total Se was determined in blood plasma as well as in the urine samples by ICP-MS. Moreover, Se speciation analysis was performed in the urine samples by LC-ICP-MS.

The air exposure to total Se ranged between <LOD and 2394 µg/m^3, whereas soluble fraction ranged between <LOD and 72 µg/m^3. Se plasma levels of the exposed individuals ranged between 62 and 123 µg/l, and were significant higher than the levels in the controls. Se levels in post-shift urine of the exposed ranged between 22-222 µg/g creatinine and were significantly higher than Se levels in pre-shift urine (21-126 µg/g). Moreover, several Se species levels in urine showed an increase during the shift. None of the biomarker levels was significantly correlated with the total Se air exposure, whereas the daily increment of urinary Se levels showed a strong and significant correlation with the soluble fraction in the air samples.

At workplaces in the selenium processing industry workers were exposed to considerable high air concentrations of Se which exceeded the German exposure limit (MAK 20 µg/m^3). In contrast, the biological tolerance limit (BAT 150 µg Se/L plasma) was not exceeded in any of the workers. The selenium species analysis confirms that there are differences in metabolism between inorganic Se compounds and organic Se compounds, e.g. from dietary sources. Finally, the correlation of biomarker levels with the soluble fraction of the air exposure indicates a more specific toxicological assessment of occupational exposure to Se and inorganic Se species.

METAL EXPOSURE AND OXIDATIVE STRESS IN TUNISIAN ELECTRIC STEEL FOUNDRY WORKERS

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Background: Electric steel foundry workers are potentially exposed to several toxic chemicals including metals.

Aim of the study; To assess metal exposure in electric steel foundry workers and its association with the oxidative DNA damage evaluated as urinary 8-oxo-7,8-dihydro-2'deoxyguanosine (8-oxodG).

Methods: Urinary metals [Arsenic (As), Barium (Ba), Cadmium (Cd), Chromium (Cr), Cobalt (Co), Copper (Cu), Manganese (Mn), Nickel (Ni), Lead (Pb), Tallium (Tl), Vanadium (V), and Zinc (Zn)] were investigated by ICP-MS in spot samples collected from 89 male workers from an electric steel foundry in Tunisia, while 8-oxodG was assessed by LC-MS-MS.

Results: Median levels were 29, 3.5, 1.43, 0.75, 0.43, 25, 0.6, 2.4, 5.0, 0.4, 0.85, and 895 µg/L for As, Ba, Cd, Cr, Co, Cu, Mn, Ni, Pb, Tl, V, and Zn respectively. The levels of Cd, Cr, Cu, Pb, V, and Zn were higher than the reference values for the Italian general population in 47-82% of samples. Median levels of all analytes were also higher than those found in other studies for the same industrial setting in Italy. No difference was found between smokers and non-smokers. Urinary metals were generally correlated each other's ($0.283<r<0.850$), with the highest values for Cd vs. Pb ($r=0.850$), Cd vs. Tl ($r=0.838$), and Tl vs. Pb ($r=0.836$). Differences were found among job titles for almost all metals, with maintenance workers and workers from the galvanization and rolling workshop more exposed than steel smelter workers.
The median 8-oxodG level for all subjects was 3.20 µg/L (1.85 µg/g creatinine), and in the range of values reported in other occupational fields or in the general population. Significant linear correlations between 8-oxodG and As, Ba, Cr, Co, Mn, Pb, Tl, and Zn ($0.213<r<345$) were found. Multiple correlation models, corrected for smoking habit, age, body mass index, and urinary creatinine confirmed the influence of As, Mn, Pb, Tl, and Zn to 8-oxodG.

Conclusions: The results of this study show that the workers from this foundry were occupationally exposed to metals, while the oxidative DNA damage assessed by urinary 8-oxodG was in line with that observed in non-occupationally exposed subjects. The exposure to metals contributed to the oxidative DNA damage.

ANNUAL TRENDS IN GENERAL POPULATION EXPOSURE TO CADMIUM: STUDIES IN JAPAN, KOREA AND CHINA

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Background: Cadmium (Cd) has toxicities on kidney and bones after chronic exposures. Daily foods are exclusive exposure sources for general public.

Study aim: Annual trends in dietary Cd exposure (Cd-D) were studied in Japan, Korea and China.

Materials & Methods: Literatures since 1960 were collected. Data were limited to adults in areas with no known Cd pollution; 19, 16 and 19 reports were available for Japan, Korea and China, respectively. Food sampling was by food duplicate collection or market basket methods. Flame/flameless AAS were used in the past and ICP-MS became more prevalent. GMs in µg/day were taken as representative, and regression analyses were applied to examine annual trends.

Results and discussion: In Japan, the earliest reports on Cd-D in late 1960s gave high Cd-D values of 81~116 µg/day. A large-scale survey in 1977-81 resulted in a lower Cd-D of 37 µg/day. Later studies in 2003-7 gave Cd-D of 11.5~16.5 µg/day. The reduction in 30 years was after regulatory and technical efforts to remove high-Cd rice from markets. Rice consumption was also reduced. In Korea, a high Cd-D of 70.5 µg/day was first reported in 1980. High values of >20 µg/day followed in 1990s, but low values of 10~20 µg/day were reported in 2000s. The latest value was 5~7µg/day in 2011. Thus, similar reductions occurred in Korea. Application to the modified exponential curve suggests that Cd-D will be reduced to 11.2 and 7.5 µg/day in Japan and Korea, respectively, by 2040. In China, the first report was available in 1990; Cd-D was 13.8 µg/day. The latest publication (2012) reported a Cd-D of 15.5 µg/day. However, there was a wide variation up to 41.8 µg/day. Analyses are difficult for China, because the time span was too brief for precise trend analysis, scattering was wide in data, and the number of studies may be too limited to cover the vast country.

Conclusion: Analyses for Japan and Korea revealed that Cd-D has been gradually decreasing to the level well <20 µg/day. Further follow-up studies are necessary for the analysis in case of China.

MANGANESE AND LEAD LEVELS IN SETTLED DUST IN ELEMENTARY SCHOOLS ARE CORRELATED WITH BIOMARKERS OF EXPOSURE IN SCHOOL-AGED CHILDREN

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Background: Manganese levels in settled dust in elementary schools near a manganese transformation plant in Brazil increased at a rate of 34.1% per km closer to the plant during the rainy season.

Aims: In this study, we investigated how this environmental pollution indicator varies in the dry season and if there is a correlation with Mn biomarker levels in school-aged children.

Methods: Dust samples were collected with passive samplers (disposable Petri dishes) placed in interior and exterior environments of 15 elementary schools. Occipital hair, toenail and blood samples were collected from 173 students aged 7 to 12 years at three of these schools with varying distance from the plant. Mn and Pb levels were measured by graphite furnace atomic absorption spectrometry.

Results: The Mn concentration geometric means (GM) in dust fall accumulation in interior environments of schools located in 2, 4, 6 and >6 km-radii from the plant were 2212, 584, 625 and 224 µg Mn/m^2/30 days. The modelled rate of change of Mn levels in dust with distance was 60.7% for each km towards the plant. Lead levels in settled dust varied between 17.8 and 81.1 µg/m^2/30 days with no association with distance from the industrial plant. Median (range) of Mn in hair and toenail were 0.59 µg/g (0.16 – 8.70) and 0.69 µg/g (0.15 – 13.30), respectively. They were significantly correlated ($p<0.001$) with Mn loading rates in the interior environment (Spearman rho=0.469 and rho=0.532, respectively) and different between schools, with the lowest concentrations in a school located 3.95 km upwind and the highest in the school located downwind 2.79 km from the plant.

Conclusions: Children´s biomarkers of exposure to manganese were significantly associated with Mn levels in dust fall accumulation at schools, which are a function of the wind direction and distance from the Mn transformation plant.

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USING BIOMARKER DATA TO ESTABLISH A BENCHMARK DOSE LIMIT FOR PERFLUORO-OCTANOIC ACID (PFOA)

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Background: Exposure limits set for exposure to PFOA and other perfluorinated alkyl substances (PFAS), whether as daily intake, drinking water concentrations or target serum concentrations, have mostly relied on extrapolation from animal experimental results. Because of uncertainties in interspecies extrapolation, establishing guideline values based on epidemiological data has obvious advantages. Under certain conditions data on biomarkers of exposure and effect may provide evidence of a causal association when the exposure biomarker is associated with environmental exposure, and the effect marker being predictive of disease. The association between serum cholesterol and PFOA meets these conditions, and its application to derive a bench mark dose as a basis for limit setting is shown using the largest available database with a wide range of serum PFOA concentrations.

Aim: To set a benchmark dose for serum PFOA based on its association with cholesterol.

Methods: The C8 study of a population exposed to drinking water contaminated with PFOA included 45606 adults with a significant association between serum PFOA and cholesterol, adjusted for potential confounders. This association has been found in numerous studies. By logistic regression modelling the exposure-response for the relation between serum PFOA and hypocholesteremia (total cholesterol>240 mg/dl), the benchmark dose (BMD) and its lower 95% bound (BMDL) associated with a benchmark response rate (BMR) of a 1% change in response is obtained.

Results: At the lower end of the exposure range, the exposure response is close to simple linear. The BMD for PFOA at a the 1% BMR, (in models not including other PFAS), in terms of serum concentration of PFOA was 4.1, with corresponding BMDL at the lower confidence level of 2.8 ng/ml.

Discussion The association is attenuated by including other PFAS in the model, yielding larger values of BMDL. The BMD of 1% is arbitrary: while higher than that used in animal studies it has been recommended for epidemiological outcomes, but 0.1%, 5% and even 10% are used. It can however serve as a Point of Departure from which a guidance value can be established, after any adjustment factors are applied. If it is directly converted to a target exposure limit, this would be close to current general population average serum levels.

Conclusion: A BMD can be set for serum PFOA based on its effect on cholesterol, is about 3 ng/ml and can form the basis for a Tolerable Daily Intake.

LEVELS OF SPECIATED ARSENIC IN URINE FROM THE CANADIAN HEALTH MEASURE SURVEY AND USING BIOMONITOIRNG EQUIVALENTS FOR ESTIMATING RISK

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Background: In Canada, biomonitoring data is collected on an ongoing basis through the Canadian Health Measures Survey (CHMS). Health Canada released nationally- representative levels of speciated arsenic in urine in 2013, 2015 and 2017. The Canadian general population is exposed to inorganic arsenic mainly through food. After absorption, inorganic arsenic is metabolized through initial reduction of pentavalent arsenate (As^{5+}) to trivalent arsenite (As^{3+}) followed by oxidative methylation to monomethylarsinic acid (MMA) and dimethylarsinic acid (DMA) metabolites.

Aims: Nationally-representative levels of speciated arsenic in urine were examined to assess the influence of demographic variables (e.g., age, sex, smoking status) and other factors (drinking water source, fish and rice consumption). Cancer risk estimates were derived, using the biomonitoring equivalents (BE) for inorganic arsenic.

Methods: Geometric means and selected percentiles for urinary levels of arsenate, arsenite, DMA and MMA (µg As/L) were calculated for 2563 participants aged 3–79 years collected in 18 sites across Canada from August 2009 to November 2011. Multiple regression models were conducted to assess the influence of various factors. The biomonitoring equivalent (BE) was used to derive cancer risk levels associated with the sum of MMA and DMA.

Results: Inorganic arsenic species and metabolites were detected in 0.5% of samples as arsenate, in 24.4% as arsenite, 27% as MMA and 96.2% as DMA. The geometric mean of DMA in the Canadian population was 3.5 µg As/L (95%CI: 3.0-4.0). Associations were found between the sum of urinary DMA and MMA and factors such as age (p=0.01), rice consumption (p=0.04) and detection of arsenobetaine and arsenocholine (p=0.0001) in a multiple regression model (R2 =0.36). Based on the BE, the sum of MMA and DMA for all age groups exceeded the negligible cancer risk threshold of $1x\ 10^{-5}$.

ITALIAN REFERENCE VALUES OF ELEMENTS IN URINE: THE EXAMPLE OF CHROMIUM AND NICKEL

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Background: Chromium (Cr) and Nickel (Ni) are elements with significant toxicological characteristics and in some of their chemical forms they have been classified by the IARC as human carcinogens. These elements are widespread in nature and can be introduced into the atmosphere either from natural sources or because of anthropic activity (combustion, melting and refining of metals). Urine concentration is considered a good indicator of exposure to the two elements. International Reference Values (RV) in urine for the general population have been defined, but data for the Italian population are scarce and fragmentary.

Objectives: To present the results of a collaborative study involving 13 collection centers, located in the Northern, Central and Southern Italy, and 6 analysis laboratories, previously selected in the basis of the results of 5 inter-laboratory analytical exercises.

Methods: Each sampling center has selected from a minimum of 8 to a maximum of 25 subjects: a total of about 250 adults (18-60 years, males and females) non-smokers and not professionally exposed to metals. The urine was collected on all subjects in two periods of the year (June and November) for a total of about 500 samples. At the two collection times an ad hoc questionnaire was administered to assess individual situations that could be related to abnormal metal absorption, such as personal characteristics, frequency and mode of exposure to emissions from traffic, and so on. In all urine samples the concentration of Cr and Ni was determined using an ICP-MS method, previously submitted to a protocol to assure comparability and comparability of the measurements. The results were evaluated by parametric statistical methods after logarithmic transformation of the data. Values below the detection limit (LoD) have been transformed into half of the LoD.

Results: The distribution of Ni and Cr in the urine appears asymmetrical and adaptable to a log-normal pattern. The 95th percentile of data mediated in the two periods of the year is 4.44 µg/L (geometric mean 1.47 µg/L) and 0.600 µg/L (geometric mean 0.221 µg/L) respectively for Ni and Cr. The analysis of the variance by site of sampling revealed data groups significantly different from the others for both elements. The statistically significant ($p < 0.001$) linear regression analysis between the two elements highlights a probable common source of exposure.

Discussion and Conclusions: Comparison of the data of this study with those previously published by the Italian Society of Reference Values shows a slight increase for Cr and a non-variation for Ni. The obtained values are useful to interpret exposure levels to xenobiotics in the environmental and occupational field.

IMPROVING RISK ASSESSMENT OF VINEYARD MANCOZEB APPLICATORS BY INTEGRATING ENVIRONMENTAL AND BIOLOGICAL MONITORING RESULTS

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Background: Ethylene-bis-dithiocarbamate (EDBC) fungicides have been used for decades, and although they have a low acute toxicity, there have been reports of possible negative health effects. Due to the specificities of agriculture, it is necessary to perform real-life risk assessment to validate the pre-marketing results. Additionally, many authors have raised concerns about using fixed absorption coefficients, together with other generic estimates, in absorption assessment. Implications are especially severe in modeling efforts, as the estimated absorbed dose is the key variable.

Aim of the study: The aim of this study was to estimate the absorbed dose and risk using a fixed absorption coefficient and a first-order kinetics model and evaluate environmental and biological monitoring endpoints' potential for modeling purposes.

Methods: This study was carried out in 2011 in the Region of Lombardy (Italy). Environmental monitoring was done using the "patch" method and by collecting hand wash liquid, and biological monitoring was done by collecting 24-hour pre- and post-exposure urine samples. The determination of mancozeb and ETU in different samples (pads, hand wash, and urine) was done by liquid chromatography-mass spectrometry. Risk assessment was done by comparing the estimated absorbed dose to the Acceptable Operator Exposure Level (AOEL) and repeated after accounting for the duration of exposure. Suitability of different exposure variables for modeling purposes was assessed using Spearman correlation coefficients.

Results: 29 healthy male farmers applied mancozeb on 38 work-days. Median total absorbed dose was 3 ng/kg body weight. Expressed as risk, the median absorbed dose was more than 10,000 times lower than the AOEL. After accounting for the duration of exposure, hand dose was reduced by more than 80% and body dose by around 50%. In general, best correlations were seen between the total dose and body dose, and the 24-hour post-exposure ETU urine levels (with and without correction for creatinine). The total absorbed dose and body dose had correlation coefficients with 24-hour post exposure ETU levels of 0.67 and 0.66, respectively ($p<0.05$).

Short discussion/conclusions: Workers' exposure to mancozeb is significantly below the AOEL. Using a first-order kinetics model for dermal absorption led to a major decrease in the estimated absorbed dose. This reduction could play a crucial role when environmental and biological monitoring results are used for modeling purposes.

BIOMONITORING OF LEUKOTRIENE-MEDIATED NEUROINFLAMMATION FOR RISK ASSESSMENT OF THE TOXIC BRAIN DAMAGE IN METHYL ALCOHOL EXPOSURE

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Background: Methanol is one of the most widely industrially used toxic alcohols throughout the world. The role of leukotriene-mediated neuroinflammation in methanol-induced toxic brain damage has not been studied.

Aim of the study: We studied acute and chronic concentrations and the dynamics of leukotrienes (LT): cysteinyl-LTs (LTC4, LTD4, LTE4) and LTB4 in peripheral blood serum in the patients with methanol exposure.

Methods: Series of acute cysteinyl-LT and LTB4 concentration measurements were performed in 28 hospitalized patients with acute methanol exposure (mean observation time: 88±20 h). In 36 survivors of acute methanol exposure, control LT measurements and the follow-up clinical examinations were performed two years after discharge. LT were measured by LC-ESI-MS/MS. The clinical tests included optical coherence tomography with RNFL, visual evoked potentials, magnetic resonance imaging of the brain, complete ocular and neurological examination, and biochemical tests.

Results: The acute maximum (Cmax) LT concentrations were higher than concentrations in survivors: Cmax for LTC4 was 80.7±5.6 versus 47.9±4.5pg/mL; for LTD4, 51.0±6.6 versus 23.1±2.1pg/mL; for LTE4, 64.2±6.0 versus 26.2±3.9pg/mL; for LTB4, 59.8±6.2 versus 27.2±1.4pg/mL (all $p<0.001$). The patients who survived had higher LT concentrations than did those who died (all $p<0.01$). Among survivors, patients with CNS sequelae had lower LTE4 and LTB4 than did those without sequelae (both $p<0.05$). The LT concentrations increased at a rate of 0.4–0.5pg/mL/h and peaked four-five days after admission. The patients with better clinical outcomes had higher cys-LTs (all $p<0.01$) and LTB4 ($p<0.05$). More severely poisoned patients had lower acute LT concentrations than those with minor acidemia.
The follow-up LT concentrations in survivors with and without CNS sequelae did not differ (all $p>0.05$). The mean decrease in LT concentration was: 30.9±9.0pg/mL for LTC4, 26.3±8.6pg/mL for LTD4, 37.3±6.4pg/mL for LTE4, and 32.0±8.8pg/mL for LTB4.

Short discussion/conclusions: Our data suggest that leukotriene-mediated neuroinflammation plays an important neuroprotective role in the mechanisms of brain damage in acute methanol exposure in humans. Acute elevation of LTs concentration during hospitalization was moderate, adaptive, transitory, and was not followed by chronic neuroinflammation in the survivors two years after discharge from hospital.

IN-FIELD PERSONAL CHOLINESTERASE ASSESSMENT PROJECT (PCAP). A METHOD OF MEASURING CHOLINESTERASE INSECTICIDE EXPOSURES AMONGST FARMERS IN WESTERN VICTORIA, AUSTRALIA

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Background: Farm workers face significant risks from the use of cholinesterase-inhibiting insecticides, such as organophosphorus (OP) and carbamates (Carb). Plasma and erythrocyte cholinesterase measurement (PChE and EAChE) are useful tools to estimate exposure to OP and Carb in farm workers. However, their use is limited by the absence of a relevant standard against which field measurements may be compared. Data that are gathered must be compared with population reference data, and this may lead to misclassification of the extent of cholinesterase inhibition. Using an adaptation of a commercially-available in-field cholinesterase test kit (EQM Test-Mate), we have developed a sensitive personalized measurement of plasma cholinesterase.

Aims and method: We have used measurements of PChE performed in duplicate in the presence and absence of oxime that regenerates PChE *in vitro*. The difference between the two measurements represents the individual inhibition of PChE for each participant. We compared these individual measures in farm workers in different areas of western Victoria, where each area included different agricultural practices (e.g. cropping, livestock, mixed agriculture).

Results and conclusions: Different study areas demonstrated different levels of inhibition of PChE consistent with factors including;
(a) seasonal agricultural practices associated with rainfall, sowing, harvest,
(b) the identity and rate of use of OP and Carb insecticides
(c) use of personal protective equipment.
In addition, there were suggestions from the data that there were effects associated with non-OP or Carb products that stimulate further research into the effects of insecticide exposure. The individual repeated measurements were reported to participants and this resulted in improvements in understanding of agricultural chemical risks and improvements in workplace practices.

BIOMONITORING OF ANTINEOPLASTIC DRUGS IN URINE: PROS AND CONS

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Occupational exposure to cytostatic drugs has been recognized as a potential health hazard since the seventies. Hospital and pharmacy personnel may be exposed to cytostatic agents by inhalation, direct skin contact or accidental events. Since 40 years, several biological monitoring studies have confirmed the uptake of cytostatic drugs in blood and urine samples. Evaluation of these studies (T Kibby, J Occup Environ Hyg, 14 (3): 159 (2017)) shows that during the last decades there has been a very good progress in analytical methods for cyclophosphamide (CP), ifosfamide (IF), fluorouracil (FBAL), gemcitabine (Gc), epirubicin (epi) and platinum-as a marker of cis-, carbo-, oxaliplatin. Now, it is a good opportunity to describe strengths and weaknesses of biological monitoring.

Strengths

- direct proof for uptake of cytostatic drugs (e.g. after accidents)
- sensitive techniques for CP/IF, FU, Gc, Epi, Pt-drugs (cis-, carbo-, oxaliplatin)
- “education effect” for staff
- database for derivation of limit values

Weaknesses

- timing of sampling difficult (most drugs are eliminated within hours)
- sensitive analytical methods limited to specific substances only
- no information about source and/or pathway of drug-uptake
- challenging communication of positive results to personnel
- interpretation in terms of personal risk is not possible at present

In conclusion, biological monitoring is an extreme powerful tool in case of quantifying potential uptakes of cytostatic drugs under distinct circumstances (spillages, accidents, inspection of new techniques). As a routine method for risk assessment at workplaces it cannot be recommended at present. But this would change notably if threshold limit values for safe handling of cytostatic dugs could be established in future.

SPECIATION ANALYSIS OF ANTINEOPLASTIC PLATINUM DRUGS IN URINE

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Biomonitoring of cancerostatic platinum compounds in urine demands for rapid and accurate analytical strategies. During the recent years, inductively coupled plasma mass spectrometry (ICP-MS) has emerged as the state-of-the-art technology for analysis of platinum at ultra-trace concentrations in biological and environmental samples. As the method detects total platinum only, and cannot distinguish between different platinum compounds or oxidation states, further developments aimed at the enhancement of selectivity. Nowadays, high selectivity is achieved via combining the sensitive ICP-MS detector with separation techniques as liquid chromatography (LC) or capillary electrophoresis (CE).

The first part of the presentation gives an introduction to the general concepts and instrumental strategies for LC-ICP-MS and CE-ICP-MS based speciation of platinum compounds in biological and environmental samples.

In the second part three advanced methods for platinum monitoring in human urine will be presented and discussed, i.e. (i) a quantitative high-throughput method for ICP-MS analysis of total platinum in occupational samples based on flow injection without the need of sample preparation, (ii) the accurate quantification of carboplatin in human urine employing LC-ICP-MS and LC-MS with quantification via species specific isotope dilution analysis, (iii) the implementation of sub-2 µm particles as stationary phase in LC for high-throughput speciation of oxaliplatin in patient urine.

BIOMONITORING OF PLATINUM IN URINE AFTER DRUG APPLICATION BY HYPERTHERMIC INTRAPERITONEAL CHEMOTHERAPY AND PRESSURIZED INTRAPERITONEAL AEROSOL CHEMOTHERAPY

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Background: Hyperthermic intraperitoneal chemotherapy (HIPEC) has been introduced over the last decade for the treatment of peritoneal carcinomatosis. In this procedure, heated cytotoxic agents are administered directly in the abdominal cavity, ensuring drug exposure of cancer cells while reducing systemic toxicity. More recently, Pressurized intraperitoneal aerosol chemotherapy (CIPPA) has been introduced to improve the drug penetration into the tissue by nebulizing the drug with carbon dioxide.
Both HIPEC and CIPPA might cause a risk of exposure of surgical staff to cytotoxic drugs which are considered as toxic substances. In this study, we investigated external exposure and internal contamination of surgeons, anesthetists, nurses and auxiliary nurses to platinum during HIPEC and CIPPA.

Method: Surface samples were collected from various locations in the operating rooms including gloves, hands and injectors. Urines samples were collected from 10 volunteers of the surgical staff and from a control group of 5 persons. The platinum analysis was performed by inductively coupled plasma mass spectrometry (ICP-MS).

Results: Platinum was not detected in more than 50 % of urine samples of the surgical staff after HIPEC or after CIPPA. No significant difference was observed between HIPEC, CIPPA and control groups for platinum exposure. For surfaces samples, significant contaminations were observed on the floor, gloves, shoes and injectors.

Conclusion: In the light of these results, the risk of platinum exposure of surgical staff during HIPEC and CIPPA appears to be rather low when safety measures are implemented.

A NEW, SENSITIVE AND VERSATILE ASSAY FOR QUANTITATIVE DETERMINATION OF α-FLUORO-β-ALANINE (FBAL) IN HUMAN URINE BY USING THE REVERSED-PHASE ULTRAHIGH PERFORMANCE-TANDEM MASS SPECTROMETRY (RP-UPLC-MS/MS) SYSTEM

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Background: A method for the quantitation of FBAL, the main metabolite of capecitabine (Cape) and 5-fluoruracil (5-FU), is described. Among antineoplastic drugs (ADs), 5-FU and Cape (the new oral prodrug) are the most applied drugs in cancer therapy.

Aim of the study: The main objective was to develop a reliable method that was easy to run on a reversed-phase UPLC system coupled to tandem mass spectrometry. FBAL was derivatized with Sanger's reagent (dinitrofluorobenzene, DNFB) to ensure a complete yield of a stable 2,4 dinitrophenil-α-fluoro-β-alanine derivative (DNB-FBAL). This new procedure was based on the use of a mixed-mode anion exchange solid phase extraction (MAX-SPE) enabling urinary extracts to be clear from endogenous interferences affecting quantitative results.

Methods: The assay was validated in human urine according to FDA criteria with the use of an internal standard (β-alanine-d4) to minimize experimental error.

Results: Good accuracy and precision were demonstrated by determination of spiked urine QC samples in four consecutive days. The recovery of FBAL was between 73.8 and 84.1%, with a minimum matrix effect. The intra-day and inter-day precisions were less than 12.1%. The lower limit of quantitation (LOQ) was 0.5 ng/mL.

Short discussion: As the LOQ value of FBAL-derivative was lower than that reported in the literature[1], this method was successfully applied to determine levels of this metabolite in a large number of urine samples taken from personnel who were occupationally exposed to ADs. Samples were collected at a specific time in relation to the end of shift and the half-life ($T_{½}$) of the studied drugs. The specific time was determined by analyzing samples taken from patients who were given 5-FU or Cape. Surprisingly the results showed that the urine of one pharmacy technician who had been compounding 5-FU incorrectly was contaminated with FBAL. This value was set at a concentration of 0.8 μg/L.

Conclusions: Results from this study demonstrate that biological monitoring continues to be the method of choice for determining whether the exposure risks in hospital wards are being controlled appropriately. Also, surface load results detected in work environments by monitoring surveys may be best interpreted by using reliable and sensitive methods.

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BIOMONITORING OF ANTINEOPLASTIC DRUGS IN URINE OF CLINICAL STAFF

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Introduction: Antineoplastic drugs are carcinogenic, mutagenic and have reproductive toxicity. After uptake they can have harmful effects on the health of healthcare workers. Because of increasing numbers of chemotherapies healthcare workers in hospital are at a potential health risk. A recent review (Kibby, J Occup Environ Hyg 2017) confirmed the presence of antineoplastic drugs in the urine of healthcare workers at several hospitals. The goal of this study was to assess the potential exposure of healthcare workers by antineoplastic drugs in oncology wards in Germany.

Methods: During five consecutive days 237 wipe samples were collected by validated methods. Furthermore, 27 hospital nurses (81% females) and 4 medical practitioners collected 153 pre and post shift urine samples. Residues of platinum drugs (PT) were determined by voltammetry. Contamination of 5-fluorouracil (FU; in urine metabolite FBAL), cyclophosphamide (CP) and ifosfamide (IF) were analyzed by validated GC/MS/MS methods.

Results: The wipe samples were tested positive to a high percentage (97% of 78 PT, 76% of 83 FU, 28% of 76 CP, 13% of 76 IF samples). Highest contamination was found on the surface of a disposal container (FU 12,600 pg/cm^2), the control panel of a flusher disinfector (Pt 181,000 pg/cm^2) and the skin of a patient after the application of a chemotherapy infusion (CP 259 pg/cm^2). In addition there were drug residues on the gloves used by health care workers after the administration of antineoplastic substances. Although high contaminations on surfaces were detected, no drug residues were found in any of the 153 urine samples (detection limits: 0.05 µg/l for CP/IF, 0.2 µg/l for FBAL). Platinum was detected in most samples (mean 2.3, SD 1.9 ng/l), but all results were below the reference value of 10 ng/l.

Conclusion: The workplace contamination studies in both German hospitals indicated that the precautions (e.g. gloves) which were taken by the personnel were sufficient to avoid measurable uptake of antineoplastic drugs. However, high contamination on some spots indicated a need for further improvement of working procedures during drug handling.

HYDROLYTIC CLEAVAGE PRODUCTS OF GLOBIN ADDUCTS IN URINE AS A NEW TYPE OF BIOMARKERS. STUDIES IN RATS AND HUMANS

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Background: Covalent adducts with blood protein globin proved useful as biomarkers of cumulative exposures to alkylating agents or other reactive compounds. However, the inconvenience due to sampling blood prevents this strategy from wider use in occupational or environmental biomonitoring.

Aim of the study: To verify a hypothesis that physiological removal of erythrocytes from the circulation includes hydrolytic cleavage of adducted globin to amino acid adducts and/or related products. These are excreted in urine and may be monitored as potential non-invasive biomarkers of exposure to parent compounds.

Methods: *In vivo* fate of amino acid adducts of selected adduct-forming chemicals (methylisocyanate, MIC; ethylene oxide, EO; styrene oxide, SO; acrylamide, AA) in globin was studied in rats. The animals were dosed intravenously with rat erythrocytes chemically modified by the tested compounds *in vitro*, or intraperitoneally with the parent chemicals (N,N-dimethylformamide, DMF, a metabolic precursor of MIC, was administered *in vivo* instead of MIC itself). Determination of the adducts in globin and their cleavage products in urine was carried out by HPLC/MS/MS.

Results: The adducts of tested compounds with N-terminal Val in globin were excreted in the form of adducted N-terminal Val-Leu dipeptides or adducted Val. Globin adducts with Cys, His and Lys were excreted as Nα-acetyl amino acid adducts. Toxicokinetics of the globin adducts in blood and their cleavage products in urine were closely associated. Some of the products identified in rats were also detected in exposed humans. N-(2-hydroxyethyl)valyl-leucine (EO-Val-Leu) was found in the urine of sterilization plant workers exposed to EO, correlating well with N-(2-hydroxyethyl)valine levels in their globin.

Conclusions: Globin adducts of various types of reactive chemicals do undergo hydrolytic cleavage to urinary products that represent a promising new category of exposure biomarkers.

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DIISOCYANATE-LYSINE CONJUGATES IN URINE. A SPECIFIC DIISOCYANATE METABOLITE?

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Background: Diisocyanates are a group of chemicals with two highly reactive N=C=O groups that react readily with polyols to form polyurethanes. The current widely used methodology for the biological monitoring of exposure to diisocyanates is based upon the analysis of the diamine derivatives in urine after acid hydrolysis. This method cannot distinguish between diisocyanate or diamine exposure. Diamines are used in the manufacture of diisocyanates and as industrial chemicals themselves. As the risks associated with exposures are different, diisocyanates are respiratory and dermal sensitisers and some diamines, such as methylene dianiline (MDA), are suspect carcinogens, a method to distinguish the exposures would be beneficial. A specific urinary metabolite is yet to be reported however isocyanate-lysine conjugates have been detected in plasma of diisocyanate-exposed workers.

Aim of the study: The work to be presented investigated the presence of methylenediphenyl diisocyanate (MDI) lysine (MDI-lys) conjugates and acetyl MDI lysine (acMDI-lys) conjugates in urine of workers exposed to MDI and workers exposed to MDA.

Methods: Urine samples were analysed for MDI-lys and acMDI-lys conjugates by hydrolysis with Pronase E, followed by solid phase extraction clean up and analysis by liquid chromatography tandem mass spectrometry. Aliquots of the samples had previously been analysed for MDA by gas chromatography mass spectrometry (GC-MS) after acid hydrolysis and derivatisation.

Results: MDI-lys and acMDI-lys conjugates were observed in samples (N=37) from persons with known exposure to MDI, peaks were also observed in samples (N=22) from persons with known exposure to MDA but to a much lesser extent. Total lysine conjugates on average were attributed to 63% of the GC-MS result for MDI exposed persons with a good correlation of $r^2>0.77$, compared to only 3.6% for MDA exposed persons ($r^2>0.39$). Samples that were not detected (<5 nM) for MDA by GC-MS were also analysed for lysine conjugates. Out of these 20 samples only 1 had a level of acMDI-lys over the limit of detection (LoD = 0.5 nM), no samples were positive for MDI-lys (LoD=0.5 nM).

Short discussion/conclusions: The work presents evidence that lysine conjugates of diisocyanates may be specific urinary diisocyanate metabolites. Urinary MDI-lys conjugates have not previously been reported and this work provides some insights to a long unknown part of MDI metabolism.

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SUITABILITY OF DIFFERENT NAPHTHALENE METABOLITES FOR THEIR APPLICATION IN BIOMONITORING STUDIES

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Background: Naphthalene occurs together with polycyclic aromatic hydrocarbons (PAH) on industrial workplaces as well as ubiquitary in the environment. For a biological monitoring of naphthalene exposures, up to now mainly the metabolites 1- and 2-naphthol are used. Recently, with 1,2-dihydroxynaphthalene (DHN) and 1- and 2-naphthyl mercapturic acids (NMA), new biomarkers were proposed.

Aim of the study: In a collective of nine occupationally exposed employees dealing with creosote, the naphthalene metabolites 1,2-DHN, 1- and 2-NMA as well as 1- and 2-naphthol were analysed in order to evaluate the suitability of the different parameters for their application in biomonitoring studies.

Methods: The urine samples were taken during one working week at several sampling times before and after the shift. Urine samples were analysed with a validated GC-MS/MS method for the parameters 1,2-DHN, 1- und 2-naphthol. Additionally, the mercapturic acids 1- and 2-NMA were analysed with a validated LC-MS/MS procedure. For all parameters, appropriate isotope-labelled internal standards were applied.

Results and discussion: In the analysed 51 urine samples, 1,2-DHN is the main metabolite with excretions ranging from 3.7-1497 µg/l (median 36 µg/l). For the sum of 1- and 2-naphthol, concentrations in the range of 2.6-294.1 µg/L (median 13 µg/l) were observed. 1-NMA concentrations were in the range of <limit of quantification (0.04 µg/l) – 4.1 µg/L and could be detected in 61 % of the samples. 2-NMA could not be detected in the analysed urine samples. The parameters 1,2-DHN, sum of 1- and 2-naphthol as well as 1-NMA show significant correlations ($p<0.001$), what indicates naphthalene as common exposure source.

Conclusions: 1,2-DHN is the most sensitive and specific parameter of a biological monitoring of naphthalene exposures at workplaces. Further studies with this parameter are needed for persons at different workplaces as well as for persons without occupational PAH exposure to generate data for the evaluation of assessment values in biological material.

KINETICS OF TRI-(2-ETHYLHEXYL) TRIMELLITATE (TOTM) AND ITS METABOLITES IN BLOOD AND URINE AFTER SINGLE ORAL DOSE EXPOSURE

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Background: Tri-(2-ethylhexyl) trimellitate (TOTM) is an alternative plasticizer to di-(2-ethylhexyl) phthalate (DEHP) and is predominantly used for medical devices consisting of PVC material. Since the plasticizer is not chemically bound to the PVC, it leaches out and migrates to the contact medium, e.g. blood for blood transfusion. Hence, the metabolization of TOTM and its effects to the human body are of great interest. However, few data about the human metabolism of TOTM are available.

Aim of the study: The study aimed at the investigation of the metabolization of the plasticizer TOTM in the human body.

Methods: A dose of 100 mg of TOTM was administered orally to a volunteer. Blood was collected before ingestion (T0) as well as 1, 3, 5, 7, 24 and 48 h after ingestion, respectively, and was stored at –20° C until analysis. The total volume of each urine void was collected until 70 h after ingestion. The volumes of all urine samples were determined and aliquots of 10 mL each were stored at –20° C until analysis. Both, blood and urine samples were processed and analyzed by liquid chromatography (HPLC) coupled with ESI-tandem mass spectrometry (LC-MS/MS).

Results: Blood TOTM, its monoester metabolites MEHTM (mono-(2-ethylhexyl) trimellitate) and its diester metabolites DEHTM (di-(2-ethylhexyl) trimellitate) were detected in the blood samples with maximum levels at 5 h, 5 h and 3 h, respectively, after TOTM intake. Out of the three possible regioisomers of MEHTM and DEHTM, the monoester isomers 1-MEHTM and 2-MEHTM and the diester isomers 1,2-DEHTM and 2,4-DEHTM, respectively, were detected. The monoester 2-MEHTM was still detectable in blood 48 h after the TOTM intake.

Urine: The monoesters 2-MEHTM and 1-MEHTM as well as two secondary oxidization products 1-M(5Cx-EP)TM (1-mono-(2-ethyl-5-carboxypentyl) trimellitate) and 2-M(5Cx-EP)TM were detected in urine with maximum levels at 6 h and 5 h, respectively, after the TOTM intake. The metabolite 2-MEHTM was detectable in urine 70 h after the TOTM intake.

Conclusions: The results demonstrate that TOTM was indeed absorbed and metabolized by the human body after oral ingestion. The elimination kinetic was found to be rather slow as 2-MEHTM was still detectable in blood and urine after 48 h and 70 h, respectively. To the knowledge of the authors this is the first study that enabled the investigation of the human metabolism of TOTM after oral exposure

URINARY EXCRETION OF HEPTANONES, HEPTANOLES AND 2,5-HEPTANE-DIONE AFTER CONTROLLED ACUTE EXPOSURE OF VOLUNTEERS TO N-HEPTANE

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Background: Acute inhalation exposure to the solvent n-heptane may cause mucosal irritation and affect the central nervous system. In Germany, an occupational exposure limit of 500 ppm applies to prevent acute effects of n-heptane exposure. Neither well-established parameters nor assessment values are currently available to evaluate internal exposure by biomonitoring.

Aim of the study: Using controlled inhalation exposures of volunteers, our study aimed at the collection of data on the association between external and internal n-heptane exposure. By this means, the validity of selected heptane metabolites to reflect internal exposure was investigated.

Methods: Twenty healthy, non-smoking males (aged 19 – 38 years, median 25.5) were exposed in a random order at three different days to 160, 330 and 500 ppm n-heptane each in an exposure chamber. Paired urine samples before and after 180 min of exposure were collected in 42 cases. The samples were analysed for the n-heptane metabolites heptane-2-one, 3-one, 4-one, 1-ol, 2-ol, 3-ol, 4-ol and 2,5-dione using headspace solid phase dynamic extraction gas chromatography/mass spectrometry (HS-SPDE-GC/MS) after enzymatic hydrolysis.

Results: Starting from median metabolite concentrations between < 0.5 (3-one) and 82.9 µg/l (4-one), an exposure related increase of metabolite excretion was observed for all parameters except 4-one (median post exposure 35.6 µg/l). After exposure to 500 ppm n-heptane, median metabolite excretions ranged between 11.7 (3-one) and 840.4 µg/l (2-ol). Correlation analysis (Pearson, n= 42, $p< 0.01$) revealed significant associations between external exposure and volume related metabolite excretion only for 2-one and 3-one ($R\geq 0.693$). Moreover, significant correlations with external exposure were also found for all heptanols ($R\geq 0.585$) when using creatinine corrected metabolite concentrations. Post exposure 2,5-dione was not associated with external exposure.

Short discussion/conclusions: A high background excretion and/or a lack of association with external exposure limit the validity of the parameters 4-one and 2,5-dione to reflect uptake of n-heptane immediately after a 3h inhalation exposure. In contrast, 2-one and 3-one as well as several heptanol metabolites seem to be more promising candidates for biomonitoring of n-heptane. When using heptanols to assess internal exposure, enzymatic hydrolysis of the samples and creatinine correction of the markers´ urinary concentrations is essential.

EXPOSURE TO MYCOTOXINS IN CORK INDUSTRY – THE IMPORTANCE OF A MULTIBIOMARKER APPROACH

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Occupational exposures to chemicals are associated to co-exposure to several chemicals and by different exposure routes. Occupational exposure limits are not intended to consider these complex occupational exposure scenarios and less consideration is given if the workers besides being exposed in the workplace have also exposure by food consumption. Although an important natural resource, working in cork transformation is associated with exposure to organic dust composed by particles, fungi and their metabolites such as mycotoxins. Mycotoxins are toxic compounds and several health effects are associated.

Nineteen urine samples of workers from a cork industry located in Portugal were analyzed. An improved "dilute and shoot" LC-MS/MS multibiomarker approach was used to monitor urinary excretion of 23 mycotoxins.[1] Alternaria toxin tenuazonic acid (TeA) and its isomer *allo*-tenuazonic acid (*allo*-TeA) were also analysed following a recently published protocol.[2]

Nine different mycotoxins were found in workers urine samples: dihydrocitrinone (DH-CIT), deoxynivalenol-3-glucuronide (DON-3-GlcA), several enniatins (EnA_1, EnB, EnB1), ochratoxin A (OTA) and ochratoxin degradation product (2´R-OTA), TeA and *allo*-TeA. Most of the samples presented more than one mycotoxin (63.1%) and there were samples presenting up to four different mycotoxins. The most reported mycotoxin was TeA (94%>LOQ) followed by allo-TeA (44.4% >LOQ). OTA was found in 5 samples (26.3%) and DH-CIT in 4 samples (21%).

This is the first study intended to assess the exposure to multiple mycotoxins of a group of workers. In fact it was possible to conclude that workers were exposed simultaneously to several mycotoxins. Probably exposure is occurring also by food intake since mycotoxins are also food contaminants. Considering a precautionary approach, it is possible to ponder that exposure below the reference limits of each substance can have more severe health effects due to possible interactions between mycotoxins. As a conclusion, this exploratory work claims attention to the need of consider the risk of human co-exposure to multiple mycotoxins and for the importance of using a multibiomarker approach since allows understanding the most common mixtures present in the workplaces and what substances interactions can be expected.

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BIOLOGICAL MONITORING OF LOW LEVEL EXPOSURE TO BENZENE IN A REFINERY: EFFECT OF MODULATING FACTORS

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Background: Biomarkers of occupational exposure to air benzene (AB), a known human carcinogen, are modulated by exposure to other volatile organic chemicals (VOCs) and other factors.

Aim of the study: To investigate the role of various factors on the metabolism of benzene, including the metabolic genotype GST, cigarette smoking and co-exposure to toluene, on the levels of different biomarkers in subjects exposed to low levels of AB.

Methods: The study involved 146 refinery workers (70 smokers and 76 non smokers). Exposure to AB and other VOCs was measured using Radiello. The analysis was performed by GC-FID. The biological indicators: urinary benzene (UB), S-phenylmercapturic acid (SPMA) and t,t-muconic acid (MA) were determined by GC-MS, HPLC-MS/MS and HPLC-UV, respectively. GSTT1 and GSTM1 polymorphisms were analyzed in oral mucosa cells by standard polymerase chain reaction (PCR)-based methods. Information on smoking was collected through the use of an individual questionnaire. An informed written consent to take part in the study was obtained from all subjects before enrollment.

Results: Exposure to benzene and other VOCs of oil refinery operators was low (mean values: benzene 32.6 $\mu g/m^3$, toluene 56.4 $\mu g/m^3$, xylenes 52.5 $\mu g/m^3$, UB 0.57 µg/L, MA 79.6 µg/creat, SPMA 4.13 µg/creat). Statistically significant correlations between environmental and biological monitoring data were found for all three biomarkers ($p = 0.011$, <0.0001 and <0.0001 for UB, MA and SPMA, respectively). The indicator which best correlated with AB was SPMA ($r=0.74$). The levels of all three biomarkers were significantly higher in smokers than in nonsmokers ($p<0.0001$). A statistically significant difference was found, only for SPMA, between individuals expressing the genotype GSTT1 "null" (lower SPMA) and subjects with genotype GSTT1 "non null" (higher SPMA). No significant difference was found in relation to GSTM1. A statistically significant decrease of the SPMA/AB ratio was observed with increasing environmental toluene concentration.

Short discussion/conclusions: The study confirmed the validity of SPMA as a good biomarker of AB exposure even at very low levels of exposure. It was also confirmed that cigarette smoking is the main confounding when assessing biological monitoring data of occupational exposure to AB. Our data indicate that the GSTT1 genotype significantly modulates the urinary levels of SPMA, suggesting that subjects with a "null" genotype may be more susceptible to the effects of AB, even at low levels of exposure. Finally, environmental toluene appears to inhibit the metabolism of benzene to SPMA even at very low concentrations, resulting in the underestimation by SPMA of the real exposure of workers to AB.

PESTICIDES AND HEALTH IN AGRICULTURE. GENETIC DAMAGE AND SUSCEPTIBILITY

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Background: Pesticides are natural or synthetic chemicals used to eliminate or reduce pests. Pesticide effects became a concern for their long term effects observable on environment, animals and ultimately on humans. These compounds play an important role in several sectors but are mainly used in agriculture.

Aim of the study: The objective of this work was to study occupational exposure to pesticides in a multistage approach in order to integrate information obtained with biomarkers of exposure, effect and susceptibility.

Methods: Biomarkers of exposure included determination of pesticides in urine, namely pyrethroids, organophosphates, carbamates, excretion of total thioethers in urine and enzymatic activity of plasma cholinesterase. Biomarkers of effect comprised the study of genetic damage with different assays: micronucleus (both in lymphocytes and reticulocytes), chromosomal aberrations test; DNA damage, evaluated by means of comet assay and also somatic mutation. In addition, alterations in the immune system were also studied using lymphocyte subsets analysis. The potential role of the genetic polymorphisms in genes related with the metabolic fate of pesticides (*EPHX1, GSTM1, GSTT1* and *GSTP1*) and DNA damage repair (*XRCC1* and *XRCC2*) in modulating individual levels of biomarkers related to pesticide exposure was also evaluated.

Results: Eighty-five farmers exposed to several pesticides and sixty-one unexposed controls took part in the study. Significant increases of micronuclei (both in lymphocytes and reticulocytes), chromosomal aberrations and DNA damage assessed by Comet assay were found in pesticide workers as compared with controls. Pesticide workers also presented significant alterations in the percentage of B lymphocytes in comparison with control group. Concerning the effect of the genetic polymorphisms on the different biomarkers studied, results suggest that positive genotype of *GSTT1* and *GSTM1*, *GSTP1* Ile/Ile and *XRCC1* codon 399 Gln/Gln genotypes are associated with increased genetic damage.

Short discussion/conclusions: Results confirmed the increased presence of DNA damage in farmers exposed to pesticides, and showed as exposure conditions influence observed effects. On the other hand, the present data should not be over-interpreted, since the analyzed biomarkers differ in their half-lives and biological complexity. All biomarkers of effect studied are rather non-specific and are able to reflect many types of various genotoxic exposures.

ASSOCIATIONS BETWEEN APOLIPOPROTEIN E GENOTYPES AND HG CONCENTRATIONS IN CORD BLOOD

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Apolipoprotein E (apo E, gene *APOE*) is a plasma glycoprotein with central roles in lipid and neuronal metabolism. Some studies also point on its metal-binding and antioxidative properties. It has three major isoforms apo E2, apo E3, and apo E4 encoded by alleles ε2, ε3 and ε4, respectively. Individuals with ε4 variant are supposed to be more susceptible to neurodegenerative diseases, but also to metal toxicity, including mercury (Hg). On the other hand, it is believed that apoE4 (ε4) associations with higher levels of cholesterol, vitamin D and calcium can be beneficial early in life.

The aim of present study was to estimate the potential relations between *APOE* polymorphisms and concentrations of total, methyl and inorganic Hg (tHg, MeHg, iHg) in cord blood (CB) of newborns whose mothers were exposed to Hg through fish consumption. We were focused on impact of *APOE* polymorphism of mothers and/or their newborns, since it is not clear whose genotypes could have greater impact on placental toxicokinetics.

Mothers (n=398) and newborns (n=573) from central Slovenia and coastal Croatia, participants of EU projects PHIME and CROME-LIFE+, were included in the study. We used existed data set of personal (and lifestyle) characteristics, tHg blood concentrations and archived DNA extracts from maternal leukocytes and cord tissues. CB MeHg was additionally measured by CVAFS (Tekran 2700), iHg estimated by subtraction of MeHg from tHg and DNA genotyped for *APOE* (rs429358 and rs7412) by TaqMan® SNP assay (Applied Biosystems). Statistics: Wilcoxon rank-sum test, multiple linear regression (STATA).

Mothers and their newborns were divided into *APOE* ε4 carriers (genotypes ε3/ε4 and ε4/ε4) and ε4 non-carriers (genotypes ε3/ε3, ε3/ε2 and ε2/ε2). We identified 15% and 19% ε4 carriers among mothers and newborns, respectively. Presence of ε4 in mothers was associated with higher tHg in CB than in ε4 non-carriers (4.1 ng/g versus 3.3 ng/g, p=0.006), as well as presence of ε4 in newborns (3.1 ng/g versus 2.6 ng/g, p=0.002). MeHg presented about 85% of CB tHg (Croatian population). After taking into account seafood intake, parity, age, body mass index and smoking the observed higher concentrations of tHg or MeHg in *APOE* ε4 carriers were no longer significant.

In conclusion, our data do not support the association of *APOE* genotypes with CB tHg neither with CB MeHg levels at low Hg exposure, while the possible association with iHg is yet to be established.

IDENTIFICATION OF SMOKING-INDUCED CHANGES IN DNA METHYLATION IN AN EPIGENOME-WIDE SCAN

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Background: Gene expression in eukaryotes is regulated in part by epigenetic modifications mediated through DNA methylation and stable histone modifications, an exquisite biological system that maintains cellular homeostasis. There is significant evidence that the dysregulation of epigenetic modifications is related to several complex diseases; thus, the study of the possible impacts of hazardous environmental stresses on epigenetic modifications has attracted interest because it allows a better understanding of disease pathogenesis.

Aim of the study: To identify the genes involved in smoking-induced epigenetic alteration, we assessed the DNA methylation levels of 473,864 autosomal CpG sites in a total of 384 subjects from Japanese general population-based cohorts.

Methods: Genomic DNA obtained from peripheral blood was bisulfite treated and genome-wide methylation profiles were obtained using the Illumina Human Methylation 450K BeadChip.

Results and discussion: After removing an outlier sample and low call rate sites, we detected 11 sites at 6 loci, including *NFE2L2*, *ALPPL2*, *GPR15*, *AHRR*, *IER3* and *F2RL3*, which had *P* values of less than 0.01 in comparisons between current and non-current smokers for the DNA methylation levels. To address whether the smoking-induced DNA demethylation is reversible, the difference in the DNA methylation levels among never, former, and current smokers was examined. In former smokers, the sites in *ALPPL2*, *AHRR*, *IER3* and *F2RL3* were demethylated to a degree between never and current smokers, whereas the sites in *GPR15* and *NFE2L2* showed the nearly the same DNA methylation levels observed in never smokers. The smoking-induced change in the DNA methylation levels is not dependent on smoking dose or years, but its restoration is dependent on the time since smoking cessation, suggesting that the epigenome adapts dynamically to smoking stress.

NMR-BASED METABOLOMICS OF EXHALED BREATH CONDENSATE FROM SUBJECTS AFTER LOW-LEVEL OCCUPATIONAL EXPOSURE TO CHEMICAL MIXTURES

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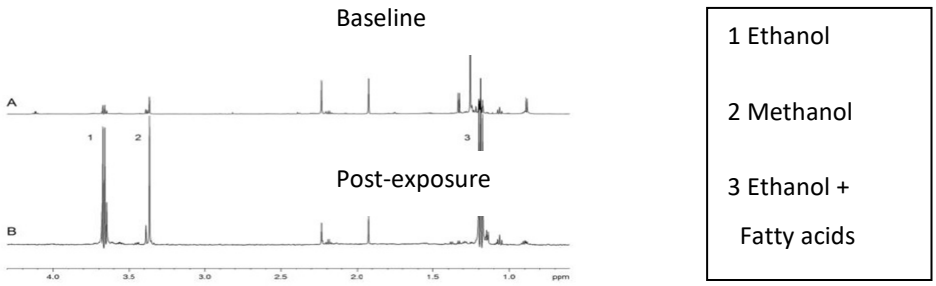
Background: Exhaled Breath Condensate (EBC) is a non-invasive matrix that allows access to the lung epithelial lining fluid. Therefore, it can provide useful information regarding the biochemical effects of exogenous toxicants following occupational exposure. Recently, nuclear magnetic resonance (NMR)-based metabolomics has been applied to EBC to identify new biomarkers and definite metabolic pathways in respiratory medicine.

Aim: We aimed at verifying if metabolomic analysis of EBC could possibly recognize specific biomarkers and/or metabolic patterns in individuals with low-level occupational exposure to complex chemical mixtures.

Methods: NMR-based metabolomics of EBC, combined with partial least squares discriminant analysis, was applied to a group of subjects in a high-tec metal mechanic industry before and after exposure to low or very low levels of carbon dust, phenols and other solvents present in glue (exposed group, n = 20) with and without wearing personal protective equipment. As a control group we have studied white collar individuals from the same industry with no exposure (control group, n = 12).
All NMR spectra were recorded on a 600 MHz Bruker Avance III spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) equipped with a CryoProbe™. EBC samples were collected using ECoScreen and TURBO-DECCS condensers.

Results: The NMR profiles of the two groups of subjects were undistinguishable before exposure. Similarly, those of the exposed group were undistinguishable before and after exposure with protective mask. They became clearly separated when the exposed group was not wearing the protective mask for 30 min. The group discrimination was due to variation in distribution of ethanol, methanol and fatty acids.



Conclusions: Our results showed that NMR-based metabolomics of EBC is highly sensible and can be successfully used in occupational medicine to distinguish between exposed and non exposed subjects even at very low levels of exposure.

DISCRIMINATION OF CARBON BLACK PARTICLES – LOADED WITH DIFFERENT CONCENTRATIONS OF DIESEL ENGINE EXHAUST – USING UNTARGETED METABOLOMICS COMBINED WITH CELLULAR ASSAYS

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Background: The lack of knowledge about the impact of many xenobiotics on human health requires new approaches to assess biological response. Recently, 3-nitrobenzanthrone (3-NBA), a product of incomplete combustion in diesel engines, has attracted a lot of attention due to its carcinogenic potential. Nevertheless, its mode of action is not well described particularly in the context of diesel soot particles. Therefore, carbon black (CB) particles were loaded with different environmental relevant 3-NBA concentrations. An untargeted metabolome analysis was combined with cellular assays to determine differentially expressed metabolites and to assess toxicological endpoints in human lung alveolar cells (A549).

Aim of the study: It should be tested if the biological response to the different loaded CB particles in a low dose range could be discriminated by combining metabolomics with cellular assays.

Methods: A549 cells were exposed for 24 h to CB particles and to low/high concentration of 3-NBA bound to CB (6 µg and 15 mg 3-NBA/g CB). 9 doses of all three particle types were selected (4 ng/ml – 500 ng/ml referring to CB). Reactive oxygen species, proliferation, mitochondrial membrane potential, cellular viability and integrity were assessed with assays. For untargeted metabolome analysis, cells were lysed after exposure, hydrophilic and lipophilic compounds were extracted and differentially expressed metabolites were identified by gas chromatograph-mass spectrometry. Enrichment analysis and principal component analysis (PCA) were performed.

Results: All types of particles induced moderate alterations of the observed toxicological endpoints consistent with the low dose range of the particles. PCA of the data obtained by cellular assays could not discriminate between the particles. Enrichment analysis of the differential expressed metabolites linked them to biological pathways and gave preliminary indications to the mode of action. For example, pure CB particles induced regulation of β-oxidation whereas loaded CB particles triggered oxidative defense at lower doses. In contrast to the assays, the metabolomics data enabled separation of the particles by PCA.

Discussion and Conclusions: A metabolomics profiling sensitively reflects the biological response to xenobiotics and provides insights into the underlying mechanisms. Nevertheless, this method has to be combined with cellular assays to anchor alterations on the metabolome level with toxicological effects.

DETERMINATION OF PHTHALATE METABOLITES IN AMNIOTIC AND CEREBROSPINAL FLUIDS

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Background: Phthalates are widely used industrial chemicals, which possess endocrine-disrupting properties and also possible neurotoxic effects. General population is exposed to phthalates through air, food, beverages, personal care products and home cleaners. After absorption they are metabolized to hydrolytic monoesters and oxidative metabolites and excreted as such or as more water soluble glucuronide conjugates. Monoesters present in the bloodstream could cross barriers like placenta or the blood-brain barrier and be found in amniotic fluid (AF) or cerebrospinal fluid CSF, even if at much lower levels than in urine.

Aim of the study: Urinary levels of metabolites are more frequently measured than the parent compounds because the risk of accidental contamination of samples during collection, storage and analysis is greatly reduced. In matrices like amniotic fluid or cerebrospinal fluid, where lower levels have been found, the contamination control is crucial and analytical methods must give emphasis to this issue.

Methods: 70 amniotic fluid samples were donated by pregnant women undergoing routine amniocentesis, and 14 CSF samples were obtained from hospitalized patients who underwent diagnostic procedures. Urine samples are subjected to enzymatic hydrolysis and purification on SPE before HPLC-MS/MS analysis for the determination of mono-benzylphthalate (MBzP), mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono butyl phthalate (MnBP) and mono-ethylphthalate (MEP). As even Milli-Q purified water contains traces of phthalate monoesters, in order to limit external contamination samples were filtered and injected into the HPLC-MS/MS, avoiding the SPE step. Blank results were obtained analyzing filtered methanol and subtracted from samples results.

Results: The concentrations of phthalate monoesters in amniotic fluid are about ten times lower than those found in maternal urine and the metabolites having a higher concentration in the amniotic fluid are MnBP (mean 3.53 µg/l) and MEHP (1.47 µg/l). The levels of monoesters in the amniotic fluid do not increase significantly with the enzymatic hydrolysis, indicating that they are present mainly in the free form. In analogy with this results, CSF samples were tested avoiding also enzymatic hydrolysis. Also in this case MnBP was the most abundant (mean 7.21 µg/l) followed by MEHP and MBzP.

Short discussion/conclusions: Results suggest that small molecules circulating in the bloodstream like phthalate monoesters can cross body membranes like placenta or the blood-brain barrier. Conjugation to glucuronic acid increases excretion velocity but also the molecular size, probably preventing the contamination of other body compartments. However, when trace analysis is carried out, great care must be paid during sampling, storage and sample preparation, in order to reduce and control sample contamination. To this purpose glass equipment must be used for sampling and storage and blank samples must be tested in the system.

CHROMIUM SPECIATION IN EXHALED BREATH CONDENSATE: IMPROVED RISK CHARACTERIZATION?

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Introduction: The biomonitoring of hexavalent chromium relies on the measurement of 'total' chromium in a urine sample. This is not ideal because there can be several sources of chromium in a urine sample and thus this exposure measurement is not as definitive as it could be.
This research investigates the feasibility of exhaled breath condensate (EBC) as a matrix for determining exposure to specifically hexavalent chromium. Using a newly developed analytical method (µLC-ICP-MS) for chromium speciation it was possible to simultaneously determine both trivalent and hexavalent chromium in EBC.

Method: A pair of samples (one EBC and one urine sample) was collected both pre (Monday morning) and post (Thursday afternoon) working week from workers (n=49) who were potentially occupationally exposed to hexavalent chromium through inhalation. Control samples (n=22) were also collected in the same way. Samples collected from occupationally exposed volunteers were predominantly from electroplating shops; which included electroplaters, anodisers, polishers, grinders, inspectors and other office administration staff. Samples were also collected from plasma cutters and blenders.

Results: The results show that it was possible to detect and quantify both trivalent and hexavalent chromium in EBC samples. Results showed, that both trivalent and hexavalent chromium were significantly higher in the pre and post EBC samples from workers than in controls. The urine samples also showed significantly higher levels of chromium in workers compared to controls.

Conclusion: For the first time it is possible to simultaneously measure both trivalent and hexavalent chromium in EBC using µLC-ICP-MS. This novel approach shows potential as a biological matrix to better assess and understand occupational exposure to hexavalent chromium by inhalation.

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DETERMINATION OF MERCURY IN HAIR OF CHILDREN

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Background: Although high or repeated exposure to different forms of Hg can have serious health consequences, the most important toxicity risk that Hg poses to humans is as methylmercury (MeHg) which exposure is mainly through consumption of fish. Once in the bloodstream, MeHg is able to across the blood-brain barrier and to accumulate in the brain causing neurotoxic effects.

Aim of the study: Generally, more than the 80% of Hg in hair is as MeHg, which is taken up by hair follicles as MeHg-cysteine complexes. Thus, hair can be used for epidemiological studies to determine the total Hg concentrations as biomarkers for MeHg exposure. In this context, hair samples were collected from 299 children (6-11 years) living in a urban area of South Italy (Taranto) and in 200 children (7 years) living in a urban area of North Italy (Trieste) to determine the levels of MeHg. Considering the neurotoxicity of MeHg, children were subjected to cognitive and neuropsychological tests.

Methods: To avoid external contamination due to environmental dirt and dust, sweat and desquamation of the skin, as well as detergents and cosmetic treatments, hair samples were submitted to adequate wash intervention in order to eliminate the external metals contamination. After drying, sub-aliquots of hair were used for the analysis of Hg by different methods by the direct Hg analyser (DMA-80) and the inductively coupled plasma mass spectrometry (ICP-MS).

Results: The hair values of MeHg in the children population groups were comparable with data reported in other international surveys. On the other hand, combining resulted of the neurological tests with MeHg levels, a possible relationship between MeHg and an increase of the errors average reported in the Motor Screening Test was noted.

Short discussion/conclusions: Although the MeHg levels were not elevated, a possible neurological influence in children, a population more susceptible than adults, might not be excluded.

HAIR ANALYSIS FOR BIOMONITORING OF HUMAN EXPOSURE TO ORGANIC POLLUTANTS

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Background: Even if urine and plasma are still the most used for the biomonitoring of exposure, increasing interest is currently observed in hair analysis for the assessment of human exposure to different organic pollutants (e.g. pesticides, PCBs, PAHs). Furthermore, it has been shown that biological fluids mainly provides information on the short-term exposure and may present a high level of variability regarding organic pollutants concentrations. Unlike urine and blood, hair analysis may provide information about chronic exposure that is averaged over several months depending on hair sample length.

Aim of the study: To evaluate the suitability of hair analysis for human biomonitoring and its further use within epidemiological studies intended to investigate the link between occupational or environmental exposure and associated adverse health effects.

Methods: Over the past few years, several specific aspects concerning hair analysis were addressed, such as hair decontamination, the detection of low levels of organic pollutants and the link between exposure and compounds concentration in hair. Thus, researches respectively focusing on analytical aspects, exposure simulation through animal models, and application of the developed methods to field samples collected from human volunteers were developed.

Results: The obtained results provided information for an adequate use of hair and its ability to accurately display human exposure. Optimized pre-analytical treatments and sampling procedure have been developed to avoid most of the bias responsible for the limitations previously associated with the use of hair. Moreover, these results will allow the development of standardized procedures for the sampling and treatment of hair samples used for the biomonitoring of human exposure to organic pollutants, like pesticides, PCBs and PAHs. Identifying the compounds (parent compounds and metabolites) detectable in hair, will help to direct towards the most suitable use of this matrix in epidemiological studies. Even more, linear correlations between the levels of exposure and the concentration detected in hair were demonstrated.

Conclusions: All these advances are strongly recommending hair analysis as a reliable tool for the biomonitoring of human exposure, and will benefit to epidemiological studies aimed to link exposure to health disorders onset.

ALTERNATIVE STRATEGIES FOR THE ANALYSIS OF NANOPARTICLE CORONA

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Background: Nanoparticles (NPs) entering biological systems instantly become coated with various biological molecules, influencing the effects and distribution of the particles *in vivo*. It is proposed that the biomolecules on the surface rather than the NPs themselves are the actual cause of numerous biological responses. Research in this field focuses on proteins to discover possible biomarker, even though NPs also harbors other organic molecules such as fatty acids, amino acids or carbohydrates (≈ organic corona). By analyzing the organic molecules (< 550 g/mol) alterations in the composition of the corona can be monitored, which might be related to toxicological event.

Aim of the study: This project is expected to facilitate the development of individual nanoparticle exposure biomonitoring tools and the analysis of early potential impact on health.

Method: The corona was prepared by incubation of particles (copper oxide (CuO), zinc oxide (ZnO), titanium oxide (TiO_2), zirconium oxide (ZrO_2), cerium oxide (CeO), gold (Au) and carbon black (CB)) under cell culture conditions for 24h. RPMI cell culture medium with 10 % fetal calf serum (FCS) or in simulated lung fluid containing physiologic concentrations of FCS (60 ppm) were used as biological fluids. After washing the NPs with phosphate buffer they were incubated 24h at 100°C in 6 M HCl to obtain the organic corona. The resulting mixture of organic molecules were then analyzed by gas chromatography-mass spectrometry.

Results: The analysis of the organic corona revealed over 200 organic substances bound to the tested nanoparticles. The substances can be grouped into different chemical classes, while alterations were observed depending of the utilized biological fluid. Statistical analysis of the obtained profiles highlighted a unique organic corona composition for each tested NP.

Discussion and Conclusions: We demonstrated the applicability of gas chromatography mass spectrometry for analysis of nanoparticle corona. While surface modifications had no effect on the composition of the corona, separation of the tested NPs was dependent on the type of particle material and tested biological fluid. The analyses of organic corona might be a useful approach for a better classification and characterization of NPs, which will help in biomarker discovery for various biomonitoring applications.

THE BIOLOGICAL EXPOSURE INDICES (BEIS®) OF ACGIH® – CONCEPT, FRAMEWORK AND PRACTICAL APPLICATION

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The Biological Exposure Indices (BEIs) of ACGIH are derived as guidance values for assessing human biomonitoring results. BEI values represent the levels of chemicals, or their metabolites, which are likely to be observed after inhalation exposure to these chemicals at the ACGIH Threshold Limit Value (TLV®). In this respect, they are health-based and should be protective against adverse toxic effects of the chemicals of interest. Altogether, more than 50 BEI values published in the 2017 ACGIH *TLVs® and BEIs®* book. Additionally, 25 feasibility assessments are available for chemical substances without adequate scientific data for the derivation of a BEI value.

The BEIs count among the strictly scientifically derived and toxicology-based assessment values for human biomonitoring, and they receive worldwide attention from scientific expert panels on the derivation of health-based assessment values for human biomonitoring as well as from health authorities. Hence, the aim of this presentation is to provide an overview of the fundamental principles of the BEIs and their derivation, such as the compilation of toxicological and analytical knowledge and the review of workplace studies as well as experimental studies. Furthermore, the rationales for additional notations (Nonspecific, Nonquantitative, Semi-quantitative, Background) and guidance on specimen collection and sampling times will be summarized. Similarities and differences to other existing assessment values will be highlighted and briefly discussed, as will be the use of BEIs for exposure assessment of individuals and groups of workers. Some specific procedures which ensure a transparent process, e.g. the communication of current developments and decisions in the Notice of Intended Changes (NIC) list will be explained.

A particular focus is set on the presentation of the recently introduced Population based ("Pop") notation, which responds to the demand for better interpretability of human biomonitoring results with respect to biomarker concentrations after occupational exposure and the background levels of the general population.

THE SCOEL APPROACH TO BIOLOGICAL LIMIT VALUES AND BIOLOGICAL GUIDANCE VALUES

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The Scientific Committee on Occupational Exposure Limits" (SCOEL), was established in 1995 to provide the European Commission with opinions relating to the effects of chemicals on workers' health, a major part being health-based proposals for occupational exposure limits (OELs). In parallel with the toxicological evaluations, SCOEL has developed its methodology as reflected by the recurrent updating of the "Key document" (Methodology for the Derivation of Occupational Exposure Limits. Key Documentation, latest published version (version 7) is from June 2013). The Key document discusses health-based biological limit values (BLVs) and biological guidance values in a five-page chapter. Biomonitoring offers several advantages, most importantly for chemicals that may enter the body via the skin or ingestion in addition to inhalation. Consequently, SCOEL decided that substances assigned a skin notation should as far as possible be assigned a BLV. According to SCOEL, BLVs can be derived in three ways:

The toxicokinetic and toxicodynamic parameters that determine or limit the sampling time should be considered. The sampling time is very important, especially for substances with short biological half times (of several hours or less). Accumulating substances (half times of days or longer) may not require a specific sampling time, instead steady-state conditions must have been reached after a certain exposure period. Sampling times may be standardized for practical reasons (end of shift, end of work-week, etc).

When the data cannot support a health-based (or equivalence-based) BLV, a biological guidance value (BGV) may be established. The BGV value typically represents the upper 90th or 95th percentile of the concentration found in a defined reference population. If background levels in unexposed people cannot be detected, the detection limit can be used as BGV. Unlike BLVs, BGVs are not health-based and therefore do not indicate absence or presence of adverse health effects.

SCOEL has so far proposed BLVs for aniline, benzene, cadmium, carbon disulphide, dimethylformamide, dinitro-o-cresol, hexachlorobenzene, methoxy- and ethoxyethanol and their acetates, hydrogen fluoride, lead and inorganic lead compounds, lead chromate, mercury and inorganic divalent mercury compounds, methylene chloride, NMP, phenol, propylene oxide, tetrachloroethylene and trichloroethylene. BGVs have been set for acrylamide, beryllium, MDA, MOCA, nickel and nickel compounds, PAHs containing BaP, and o-toluidine. Several more substances have been assigned skin notations but not, as yet, received a BLV or a BGV.

THE GERMAN APPROACH FOR THE RECOMMENDATION OF BIOLOGICAL LIMIT AND GUIDELINE VALUES

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In Germany biomonitoring is an essential occupational-medical instrument for assessing the risk of workers exposed to chemical agents. It is an integral part of preventive medical examinations as far as established analytical procedures and values for evaluating biomonitoring results are available. The DFG Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has published values for evaluating biomonitoring results. Those values are health related values such as the German Biological Tolerance Value (BAT), descriptive values such as the Biological Guidance Value (BGV) and Exposure Equivalents for Cancerous Substances (EKA), respectively.

Health-based threshold values are helpful as a criterion in risk assessment. However not for all substances a threshold can reliably be determined. For substances for which the concept of health-based threshold values is not applicable, the Working Group Setting of Threshold Limit Values in Biological Materials of the DFG Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has established Biological Guidance Values ("Biologische Arbeitsstoff-Referenzwerte" BAR, Biological Reference Values for Chemical Compounds in the Work Area) as an approach for evaluating biomonitoring data. The BAR represents the upper reference concentration of a biomarker in the general adult population without occupational exposure to the agent. Ideally, national environmental surveys including human biomonitoring results are used as basis for deriving BARs. The influence of age, sex, social status and life style factors on background exposure is considered in the evaluation of these values. Because tobacco smoking is the most frequent influencing factor, several BARs have been determined for non-smokers only. Establishing the BARs aims to facilitate the evaluation of human risk resulting from exposure to chemical compounds for which no health-based threshold values can be derived but an adequate assessment of exposure is required due to their toxicity. The application of BARs does not permit a toxicological evaluation, but does allow the occurrence and the extent of occupational exposure to hazardous substances to be proved.

DERIVATION OF OCCUPATIONAL BIOLOGICAL LIMIT VALUES AND BIOLOGICAL REFERENCE VALUES AT THE FRENCH AGENCY FOR FOOD, ENVIRONMENTAL AND OCCUPATIONAL HEALTH & SAFETY

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Disclaimer: the author is not an official representative of ANSES and opinions expressed here are his own and do not necessarily reflect those of the Agency.

One of the key advantages of Biological Monitoring resides in its ability to reflect the true workers' internal dose, namely taking into account skin exposure, breathing rate and use of personal protective equipment. However, collecting results on biomarkers concentrations is of little use for the prevention of intoxications without the existence of guideline values to help with their interpretation.

The development process of these guideline values within ANSES is a 2-step procedure. First, a dedicated Working Group on Biomarkers reviews the scientific literature and prepares extensive documents – often some 50 pages long – that contain specific recommendations on biological limit values (BLVs) and biological reference values (BRVs). The document is subsequently discussed and eventually approved by the Occupational Exposure Limits (OELs) Expert Committee that also makes recommendations on OELs as well as validated sampling and analytical methods for measuring airborne concentrations of occupational pollutants. The Working Group and Expert Committee are independent, multidisciplinary and include toxicologists, occupational physicians, hygienists, epidemiologists, and chemists. Whereas the main document is written in French, a detailed summary translation in English is also prepared. Before final adoption by the OEL Expert Committee and preparation by the Agency of its official opinion, these documents are made available for public comments on its website (https://www.anses.fr).

For threshold toxicants, the BLVs correspond either to exposure at the current French regulatory or recommended 8h-OEL or linked to a given adverse effect point of departure to which a series of adjustment factors is applied. For non-threshold carcinogens, the BLVs corresponding to risk levels of 10^{-4}, 10^{-5}, and 10^{-6} are provided when data allow this calculation. In addition, the 95th percentile of the selected biomarkers' concentration observed in the general population is established as BRV whenever available from large population studies.

BIOLOGICAL MONITORING WITHOUT LIMITS

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Background: Biological monitoring is a useful tool for assessing systemic exposure, particularly for substances absorbed through the skin or where control of exposure relies on respiratory protection. Many organizations propose health-based guidance values (BMGVs) but recognize that there are no 'bright lines' between safe and unsafe levels and avoid the word 'limits'. Where a health-based BMGV is not possible a non-occupational exposed background or reference value may be proposed. Exceeding such a reference value then indicates the likelihood of occupational exposure and possibly the need to review workplace controls. Great Britain (GB) has an alternative approach based on the 90th percentile value of biological monitoring data from workplaces following good occupational hygiene practice. It is not health-based and exceeding it simply indicates a need to review, and possibly improve controls.

Aim of the study: To compare recent biological monitoring results in GB with national and international guidance values.

Methods: In addition to workplace surveys HSE's laboratory also analyses biological samples from external occupational health professionals. The database was searched to produce 90th percentile values for substances with over 100 results per year for 3 years.

Results: Ninetieth percentile data for 15 substances show results generally below GB and international guidance values.

Short discussion/conclusions: The external samples are not representative of industry as a whole. The data may be biased upwards from workplaces experiencing difficulties controlling exposure or may be biased downwards if samples come from workplaces with good control and/or little exposures. The utility of the 90th percentile approach provides a pragmatic bridge between what is desirable (background/low level exposure) and what is achievable with current good practice. The 90th percentile value linking biological monitoring results to good occupational hygiene practice could also be adopted to develop 'in house' guidance values for substances without published guidance values. Appropriate use of this approach should drive sustainable reduction in harmful exposures over time.

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WHAT CAN METABOLIC PROFILING AND THE EXPOSOME TELL US ABOUT CHEMICAL RISKS?

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The complexity and metabolic regulation of the human ecosystem is partially controlled by environmental factors such as diet, lifestyle, toxic exposures and the gut microbiome, which interacts with the mammalian system at the level of genes, proteins and metabolism. Metabolic profiling of biofluids such as urine, plasma or fecal water encompassing high-resolution spectroscopic methods (NMR spectroscopy, LC-MS, GC-MS etc) in combination with multivariate statistical modeling tools, can provide a window for investigating the impact of toxins on human health since these metabolic profiles carry information relating both to genetic and environmental influences, including contributions from the microbiome, diet and xenobiotics [1]. Examples of urinary or faecal metabolites that are products of the metabolism of toxins or toxic/detoxification products of microbiota, or microbiota-host interactions include phenols, indoles, bile acids, short chain fatty acids and choline derivatives, all of which can be quantitatively profiled using spectroscopic technology.

The microbiome is highly metabolically active and has been shown to be capable of modulating toxins to either enhance or ameliorate the host response to toxicity. A range of examples taken from pre-clinical and clinical studies will be explored and the use of various models of microbial modulation discussed in the context of understanding drug metabolism and toxicity. Additionally the wider role of metabolic profiling in the context of biomonitoring applications is discussed.

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ETHICS AND BIOMONITORING: WHAT ARE THE INGREDIENTS FOR A SUCCESSFULL COMBINATION?

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Biomonitoring for a variety of exposure factors has been a major tool in worker's health surveillance in many countries. While environmental monitoring in assessing exposure may provide useful information on the effectiveness of preventive measures or on the lack of these, scientifically validated biomonitoring practices, if correctly executed and interpreted by competent occupational health professionals, may add considerably to prevention of work related ill health through a much more complete and reliable risk assessment relevant both at the collective level and at the level of the individual worker.

More precisely, that reliability might or does incite fear for selection practices based on biomonitoring results. Indeed, while biomonitoring can considerably contribute to prevention strategies, selection practices based on biomonitoring results are almost always scientifically irrelevant and therefore inadequate for preventing work related ill health.

Aiming at protecting both health and employment of every single worker is a basic requirement for scientifically and ethically sound biomonitoring practices. Ethical biomonitoring practices must therefore meet a series of requirements:

Validation of practices must be done for accuracy, relevance, need /necessity as well as its consequences at both the collective and the individual level. Execution must be done at the most relevant moment. Interpretation of results- especially at the individual level- can only be entrusted to competent OHPs, who are able to assess health in a holistic health protection approach.

Legal and other contextual elements must guarantee the independence of competent OHP's in their decision-making and in their unequivocal striving for protecting both health and employment.
This constitutes the basis for the necessary trust and confidence of workers in the OHP.

In such a context , not doing biomonitoring as part of health protection strategies when the tool is available and the added value undeniable, may be considered unethical.

BIOMARKERS OF EARLY GENOTOXICITY AND OXIDATIVE STRESS FOR OCCUPATIONAL RISK ASSESSMENT OF EXPOSURE TO STYRENE IN THE FIBREGLASS REINFORCED PLASTIC INDUSTRY

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Background: Styrene is used in the manufacture of fibreglass reinforced plastics, involving manual lay-up (open process) or compression (closed process) molding operations during which occupational exposure could occur. The main metabolic pathway of styrene is the formation of styrene-7,8-oxide, capable of binding with DNA potentially inducing genotoxic/oxidative effects.

Aim of the study: To identify sensitive and not invasive biomarkers of early genotoxic/oxidative effect of exposure to styrene in the fibreglass reinforced plastic manufacture.

Methods: We studied 11 workers of a plastic manufacture using open molding process (A), 16 workers of a manufacture using closed process (B) and 12 controls. We evaluated geno/cytotoxic effects by buccal micronucleus (MN) cytome assay and genotoxic/oxidative effects by Fpg-comet test on lymphocytes. On A workers we also evaluated, urinary concentrations of 8oxoGua, 8oxodGuo and 8oxoGuo to investigate oxidative stress induction. Personal inhalation exposure to styrene was monitored by passive sampling and GC/MS. Biological monitoring included urinary metabolites mandelic acid (MA) and phenylglyoxylic acid (PGA).

Results: Mean value of styrene exposure was 80.27 mg/m3 for A workers and 22.9 mg/m3 for B workers with 5/6 of subjects exceeding TLV value (85mg/m3) working at the manufacture A. The average MA+PGA end shift level was 282.16mg/g creat for A workers and 50.27mg/g creat for B workers vs 0.8mg/g creat of controls. Higher MN frequency in A workers (1.31‰) vs B workers (0.92‰) and controls (0.64‰) and higher karyolytic cell frequency (indicative of cytotoxicity) in both exposed populations vs controls were found. No induction of direct DNA damage and moderate oxidative DNA damage were found in exposed workers vs controls. High urinary 8oxoGua, 8oxodGuo and 8oxoGuo levels were found in the A workers exposed at high levels of styrene and a negative, even if not significant, correlation was found with oxidative DNA damage.

Short discussion/conclusions: The findings show higher styrene exposure in manufacture A using manual lay-up molding operations correlating with higher levels of urinary MA+PGA and MN induction. Fpg-comet assay and urinary oxidized guanine demonstrated to be sensitive biomarkers of oxidative stress while MN test on buccal cells represent a good not-invasive biomarker of cyto-genotoxicity at target organ. The study shows the usefulness of used biomarkers in biomonitoring of workers exposed to styrene.

EVALUATION OF SUGARCANE AND ORANGE VINASSES PHYTOTOXICITY BY MEANS OF GERMINATION AND ROOT GROWTH TESTS IN LETTUCE SEEDS

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Background: Vinasse is a byproduct of the processing of sugarcane and orange marc in alcohol by the sugar-alcohol and citrus industries. In view of the extensive fertirrigation of sugarcane monoculture by this byproduct, ecotoxicological studies that assess the effects of this byproduct on the organisms are necessary. Therefore, the germination test with lettuce seeds (Lactuca sativa) is used in worldwide to evaluate the toxicity of different substances.

Aim of the study: The objective of the present study was to evaluate the phytotoxicity of sugarcane and orange vinasses in L. sativa (verônica variety) seeds, which were obtained from a sugar-alcohol plant (sugarcane vinasse) and by means of the fermentation of the pear orange juice (orange vinasse).

Methods: Chemical analyses were performed for both vinasse samples. The bioassays were carried out in Petri dishes and filter paper, containing 30 seeds, submitted to germination in a BOD (22ºC±1). The seeds were germinated directly at six different dilutions (2.5, 5, 10, 20, 40 and 80%) of both vinasses. Negative control (CN) was performed with distilled water and positive (CP), with zinc sulfate heptahydrate (0.05 M). The assay was performed on five replicates randomly for 120 hours. After 8, 16, 24, 32, 40 and 48h of exposure, the germinated seeds were counted. After 120 h, the total number of germinated seeds was counted. The root lengths were measured using a digital caliper, and used in the regression curves (concentration x root growth). The germination data obtained were analyzed by the Anova/Tukey statistical test ($p < 0.05$).

Results: The dilution that inhibited 50% root growth (DI 50) relative to CN was 3.55 for sugarcane vinasse and 2.80 for orange vinasse. The germination speed was reduced statistically significantly in the dilutions of 40 and 80% of sugarcane vinasse and in the dilutions of 5, 10, 20, 40 and 80% of orange when compared to the CN. The germination rate decreased significantly in the 40 and 80% dilutions of sugarcane vinasse; and at the dilutions 20, 40 and 80% orange, when compared to the CN.

Short discussion/conclusions: High values of BOD, COD and organic carbon and low pH had a negative influence on the germination and development of lettuce rootlets. Although orange vinasse has been shown to be more toxic than sugarcane for the test organism employed, the final destination of these two wastes in the environment requires caution.

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EVALUATION OF THE CYTOTOXIC AND GENOTOTOXIC POTENTIAL OF THE CACTINEA NUTRACEA™ IN CULTURE OF LIVER HEPATOCELLULAR CARCINOMA HEPG2

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Background: Opuntia ficus-indica, a plant of the family Cactaceae of Mexican origin and popularly known as fig tree of India, is used in food and in folk medicine. From the fruits of Opuntia ficus-indica, the nutraceutical Cacti-Nea™, which has diuretic and antioxidant properties, is produced and is indicated for weight control and cell protection against oxidative damage caused by free radicals. Although such properties are described, the toxic potential of this compound is not known to humans.

Aim of the study: The aim of this work was to evaluate the cytotoxicity (MTT cytotoxicity test) and the genotoxicity (CBMN assay) of the nutraceutical Cacti-Nea™.

Methods: In this study the HepG2 cell line was used as a bioindicator. The cytotoxic potential was evaluated by the MTT (Tetrazolium assay - Thiazolyl Blue Tetrazolium Bromide) test, in which six concentrations of Cacti-Nea™ were analyzed (0.03; 0.01; 0.008; 0.006; 0.004 and 0.002 g/mL). The genotoxic potential was evaluated by the micronucleus test with blockade of cytokinesis (CBMN assay), in which the tested concentrations were 0.006, 0.004 and 0.002 g/mL.

Results: All tests were performed in triplicate and statistical analysis, after analysis of the data distribution pattern, was performed by the analysis of variance method (ANOVA), followed by multiple comparisons by the Tukey test. Values of $p<0.05$ were considered statistically significant. By the MTT test, it was observed that 0.03 and 0.01 g/mL concentrations were the only ones that generated a cellular viability lower than 80%, whereas by CBMN assay it was possible to observe that none of the concentrations evaluated induced significant frequencies of micronucleated cells.

Short discussion/conclusions: According to results obtained by the MTT test, only the highest concentrations of Cacti-Nea™ (0.03 and 0.01 g/mL) induced toxicity to human hepatic carcinoma (HepG2) cells. The concentrations that did not induce cytotoxicity had their genotoxic potential measured by the assay CBMN, for which it was possible to observe that they did not present genotoxic potential against the HepG2 cell line. Thus, it can be inferred that human consumption of Cacti-Nea™ is safe only in small concentrations, which are not capable of inducing cytotoxicity or genotoxicity.

ASSESSMENT OF OXIDATIVE DAMAGE IN WORKERS EXPOSED TO LOW-DOSE BENZENE

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Background: Benzene is an aromatic chemical compound classified in EU as Carc 1A and as a group 1 human carcinogen by the International Agency for Research on Cancer (IARC). Benzene exposure promotes oxidative stress through the production of reactive oxygen species (ROS), potentially harmful for biological structures. ROS can interact with biomolecules originating peroxidised derivatives including advanced oxidation protein products (AOPP), advanced glycation end-products (AGE) and 8-hydroxy-deoxyguanosine (8-OHdG), potential biomarkers of oxidant/antioxidant imbalance [1].

Aim: This study aims to investigate the relation between exposure to low-dose benzene and the occurrence of oxidative damage in gasoline station workers, identifying possible biomarkers of oxidative stress.

Methods: Study population consisted in a group of 80 men employed in gasoline stations and compared with a control group (n=63) of male office employees not exposed to benzene. Information regarding socio-demographic characteristics, lifestyle and job-related records were provided through a questionnaire. Urine levels of 8-OHdG were evaluated by competitive immunoassay. Urinary t,t-MA concentration was determined by HPLC for individual benzene exposure. Data were analyzed using Student's t test. Two-tailed Pearson test was used for correlation analysis.

Results: Significantly higher urinary t,t,-MA and 8-OHdG levels were observed in gasoline station attendants compared to subjects not exposed to benzene. Pearson's test demonstrated a strong correlation between benzene exposure level and 8-OHdG concentrations. 8-OHdG significantly correlated also with job seniority, whereas the relation with age resulted weaker.

Conclusions: These results indicate that chronic exposure to low-level benzene can determine oxidative DNA damage, as indicated by alteration of 8-OHdG which may represent a useful biomarker [2] for screening purposes in gasoline station workers and other subjects exposed to low-dose benzene. Identification of potential confounding factors and correlation with biomarkers of damage to other biological targets may support this assumption.

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OXIDATIVE DAMAGE AND URINARY MUTAGENICITY IN CHILDREN LIVING IN INDUSTRIAL CONTAMINATED SITES OF PRIOLO (ITALY)

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Background: The site of Priolo (Sicily, Italy) is characterized by a diffuse environmental contamination due to the presence of large industrial plants that in the last decades has caused a progressive contamination of the different environmental compartments. Exposure to environmental pollution is associated with inflammation and oxidative stress. Children are considered the most vulnerable part of the population.

Aim of the study: The aim of our study was to evaluate oxidative damage and urinary mutagenicity in children living in industrial contaminated sites of Priolo (Italy).

Methods: A cross-sectional study was carried out on 17 children living in Priolo and 17 children living in Grammichele (a rural area). The children's morning and evening urine was collected. Parents filled in a questionnaire on their child's habits and on the general health conditions. A medical check was made and personal data, as well as weight and height were recorded.
The following exposure biomarkers were dosed: 1-hydroxypyrene (1-OHP), s-phenylmercapturic and the urinary mutagens TA98 and TA100. Moreover, the effect biomarkers were dosed: isoprostanes, malondialdehyde, 8-Oxo-2'-deoxyguanosine (8-oxodGuo), 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and 8-Oxoguanine (8-oxoGua).

Results: The average age was 9.9 ±1.6 e 9.8 ±1.5 for Priolo and and Grammichele's children, respectively. Their BMI was 21.0 ±1.4 e 21.1 ±1.5. There is no statistically remarkable difference between 1-OHP and s-phenylmercapturic in morning urine. A statistically noticeable difference was detected in the evening urine. As to the urine mutagens, the TA98 was statistically significant both in the morning and the evening for both areas. As to the TA100 there was a statistically significant gap in the evening samples. From oxidative stress results, isoprostanes and malondialdehyde, there emerges a statistically significant difference between the industrial and the non-industrial area. The difference of 8-oxodGuo, 8-oxoGuo and 8-oxoGua appears statistically significant for industrial area children.

Short discussion/conclusions: In the present study, we observed that children living in industrial contaminated area have differences in the biomarkers level. But, as observed in other studies, the effects of contaminations of soil, air, water, etc. is a risk for DNA and RNA damages. It is necessary to implement a biomonitoring program for children in high-risk areas.

ANALYSIS OF THE TOXIC POTENTIAL OF TREATED VINASSE USING AN INTEGRATED TREATMENT SYSTEM WITH THE CWS: HISTOPATHOLOGY OF NILE TILAPIA *(OREOCHROMIS NILOTICUS)*

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Background: Vinasse is a byproduct of the alcoholic fermentation of sugarcane (*Saccharum* sp.). It is used as fertilizer due to its richness in organic matter and also because it improves soil fertility, since it favors the availability of some elements for plants. However, special attention should be given to the amount of vinasse used in the fertirrigation, once the soil retention capacity should not be exceeded and the dosages should be directed to the specific characteristics of each soil to be applied. When it used in unbalanced proportions can cause damages to soils and plants, in addition it being able to reach water bodies. In this sense, the use of constructed wetland systems (CWSs) has become interesting, as these are highly effective biogeochemical systems to treat wastewater from different sources. These CWSs have natural processes of aquatic macrophytes, which not only accumulate pollutants directly in their tissues but also it act as catalysts for purification reactions that usually occur in the rhizosphere of plants, an alternative treatment for vinasse. The integration of other systems such as biodegradation and filtration are complementary the CWSs, increasing the effectiveness of the treatment. Fish are excellent experimental models for aquatic toxicology studies because they warn about the potential danger of chemicals that reach the water bodies.

Aim of the study: Thus, this study aimed to verify the efficacy of sugarcane vinasse treatment in reducing its toxic potential through histological and histochemical tests in tilapia liver.

Methods: The animals were submitted to three different dilutions of the vinasse for 96 hours; after that period their livers were removed and submitted to histological routine.

Results: The analyzes of liver samples revealed that the liver cell pattern described for the species was not altered, thus attesting to the decreased toxicity of the treated vinasse.

Conclusions: In view of the presented results, it is concluded that the vinasse treated by the integrated system with the CWS had a reduction in its polluting potential, since the animals did not present histological alterations.

PON1 STATUS AND HDL SUBCLASSES AS CARDIOVASCULAR DISEASE BIOMARKERS

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Background: Serum ParaOxoNase 1 (PON1) is a High-Density Lipoprotein (HDL)-associated enzyme capable of hydrolyzing a wide spectrum of substrates, including oxidized lipids. Evidence indicates that phenotype and genotype of PON1, as well as HDL concentrations, and HDL subclasses could modulate the development of CardioVascular Diseases (CVD).

Aim of the study: To evaluate the relationship among genotype-phenotype, concentration of PON1, and lipid profile in three different populations: patients with CVD; with Cardiovascular Risk Factors (CRF), and in a healthy population.

Methods: A case-control study was conducted in 60 volunteers from an Institute of Health in Nayarit, Mexico. The study was approved by the local Institutional Ethics Committee. Written informed consent was obtained from each patient in compliance with Good Clinical Practices, and the investigation adhered to Declaration of Helsinki principles. Blood samples were obtained to evaluate lipid profile, HDL subclasses (by SDS-PAGE), PON1 genotype (A-162G, C-108T, L55M, and Q192R by TaqMan probes), phenotype (using dihydrocoumarin, paraoxon, phenylacetate, and 4-CMPA as PON1), and serum levels (by Enzyme-Linked Immunosorbent Assay [ELISA] kit).

Results: T-Cholesterol, LDL-C, and ApoB were higher in the healthy group. PON1 phenotype through LACtonase (LAC) (10.05; 8.30–11.15 U/mL), arylesterase (111 ± 28.03 U/mL), and paraoxonase (161.21 ± 58.32 U/L) activities were lower in the CVD group. No significant differences in CMPA activity were found. CDV group had lowest PON1 concentration (3.22; 2.78–4.00 µg/mL). PON1 genotype was similar in all groups. HDL subclasses HDL2a and -3a were in higher proportions in CVD and CRF vs. the healthy group, whereas HDL3b and -3c were found in lower proportions. The healthy group had HDL with smallest average diameter (8.53 ± 0.07 nm).

Discussion/conclusions: CVD are the main causes of death worldwide, and the participation of PON1 has gained importance. PON1 activity and concentration were lower in patients with CVD concomitantly with a lower proportion of small HDL. It was reported higher PON1 activity I is found in small HDL (3c). Not only HDL-C, but also the PON1 phenotype-genotype, together with the HDL subclasses, may predict susceptibility to develop CVD.

DNA REPAIR CAPACITY AND ITS ASSOCIATION WITH THE EXPRESSION OF DNA REPAIR GENES IN NEWBORNS FROM A POLLUTED URBAN CITY

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Background: Atmospheric pollution is a mixture of compounds including metals, polycyclic aromatic hydrocarbons (PAHs) and benzene, among others. The Metropolitan Area of Mexico City (MAMC) is one of the most populated and industrialized areas in Mexico, with levels of air contaminants such as particulate matter, metals and PAHs above national and international standards. The exposure to atmospheric pollutants has been associated with oxidative and genetic damage in newborns, however, there is little information about the expression of DNA-repair genes as well as the repair capacity.

Aim of the study: We evaluated the association between the expression of DNA repair genes and the genetic damage and repair capacity in umbilical cord blood cells from newborns of MAMC.

Materials and methods: A cross-sectional study was performed in 146 umbilical cord blood samples from newborns (mean gestational age of 39 ± 1 weeks). The expression of genes involved in DNA-repair (*PARP1*, *OGG1,* and *p53*) and antioxidant response (*Nrf2*) was evaluated by qRT-PCR and the genetic damage and DNA-repair capacity after 100 µM H_20_2 incubation were evaluated by the comet assay in cord blood cells, through the Olive tail moment parameter (OTM).

Results: The genetic damage showed a wide range (OTM= 0.22 – 2.85) with a significant difference by tertiles of damage ($p < 0.001$). We observed that 70 % of newborns (n=101) repaired the DNA damage and 30 % (n=44) were not able to repair the induced damage; we found a significant difference in the DNA damage by repair group ($p = 0.01$). Finally, our results showed a significant decrease in the expression of *p53* (p= 0.02) and a marginal decreased in *PARP1* (p= 0.05) in the non-repair group compared to the repair group.

Conclusions: Our results suggest that newborns from a highly polluted area such as MAMC show genetic damage, which was greater in children of the non-repair group; also, the difference in gene expression (*p53* and *PARP1)* in the repair groups suggests an alteration in the gene expression of newborns who did not repair the damage. This may represent a risk factor for developing DNA damage-related diseases later in life. This study was financially supported by Conacyt-Mexico (Grant #233710).

EFFECTS OF OCCUPATIONAL TOLUENE AND NOISE EXPOSURE ON HEARING LOSS

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Background: Organic solvents, such as toluene, have shown ototoxic effects and co-exposure with noise can have a significant aditive impact on the hearing loss. Workplace exposure to organic solvents is usually monitored through air sampling concentration meassurement, however bio-monitoring of the exposed workers is also important to prevent impairment of health. It can be performed through blood or urine samples analysis. Audiometry is necessary to evaluate the effects of mixed exposure to noise and toluene on hearing loss.

Aim of the study: The aim of this study is to investigate the effects of occupational exposure to organic solvents (toluene) and long term noise exposure on hearing impairment of workers in Industrial Rubber Products and Tyres plant in Kranj, Slovenia.

Methods: We investigated a group of 25 workers exposed to toluene and noise level 75-85 dB(A). Biomarkers of exposure hippuric acid and o-cresol were detected from urine samples. Detailed instructions on diet and fluid intake were given to all workers prior to the testing. Samples of urine were taken at the end of shift on 4th working day. Pure-tone audiometry were measured when they started work at plant and the same year as biomonitoring was performed. The pure-tone audiometry results were corrected for age and sex of an individual and Wilcoxon signed ranks test was used to assess weather the individuals experienced significant hearing loss.

Results: We found increased levels of urinary hippuric acid in four workers but the levels of o-cresol were within the normal limits in all subjects. The audiometry results showed no significant hearing loss in individual worker in any of the frequencies. There was also no significant change in hearing thresholds across frequencies before and after the exposure.

Short discussion/conclusions: The results of our investigation couldn't find significant hearing impairment at workers exposed to toluen and noise at work. The combined harmful effect of solvents and noise on hearing has been shown in some research; in our study the number of subjects investigated is small and therefore we suggest that the monitoring of these subjects should be performed further on.

COMPARING THE GENOTOXICITY OF A MULTIWALLED CARBON NANOTUBE AND CROCIDOLITE TOWARDS THE EVALUATION OF ITS POTENTIAL IMPACT ON THE WORKERS' HEALTH

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Background: Multiwalled carbon nanotubes (MWCNT) are one of the most promising and widespread class of manufactured nanomaterials, with several industrial and biomedical applications. However, their unique physicochemical properties may have detrimental effects on human health upon unintentional exposure by inhalation. Although there is still no sufficient epidemiological and toxicological data on most MWCNT, several professional and scientific organizations adopted a precautionary principle and considered MWCNT as an occupational hazard. *In vitro* toxicological studies can contribute to fulfil the gaps on the knowledge about their potential health adverse effects and to identify biomarkers for human biomonitoring, particularly in the workplace.

Aim- This study was aimed at characterizing the cytotoxic and genotoxic effects of NRCWE-006, a high aspect-ratio rigid MWCNT, comparatively to crocidolite, a well-known tumorigenic asbestos fiber causing mesothelioma, using a co-culture of alveolar epithelial cells (A549) and monocyte-derived macrophages (THP-1).

Methods: The MTT, the comet and the micronucleus assays were performed on a co-culture of A549 and differentiated THP-1 cells following exposure to a concentration- range of NRCWE-006 or crocidolite.

Results: Both NRCWE-006 and crocidolite revealed cytotoxicity by the MTT assay. NRCWE-006 did not induce a detectable level of DNA breaks under the comet assay conditions tested, while a significant increase in the micronucleus frequency was detected at 6.25 and 12.5 $\mu g/cm^2$. In contrast, crocidolite revealed a clear dose-dependent increase in the level of DNA strand breaks (comet assay) and induced a significant increase in the micronucleus frequency at the highest concentrations tested (10 and 20 $\mu g/cm^2$).

Discussion and Conclusions: Our results suggest that NRCWE-006 is less cytotoxic than crocidolite to alveolar cells grown in co-culture with monocyte-derived macrophages. As expected, crocidolite was clearly genotoxic, given that it was able to induce DNA and chromosome damage, probably due to its known potential of ROS production. On the other hand, even though NRCWE-006 did not cause DNA damage, it demonstrated aneugenic/clastogenic effects at the two lowest concentrations, which are closer to the ones that may represent a concern in terms of occupational exposure.

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HUMAN BIOMONITORING FOR EUROPE (HBM4EU): THE ROLE OF ITALY

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Background: The European Human Biomonitoring Initiative (HBM4EU) is a joint effort of 26 countries (including Italy) and the European Commission, co-funded by Horizon 2020. The main aim of the initiative is to coordinate and advance human biomonitoring in Europe.

Aim of the study: Italy will be involved in different work packages (WP7, WP8, WP10, WP11, WP13, WP14) with the following aims: harmonize tools and procedure for the HBM of chemical substances; addressing the lack of information concerning the exposure to chemicals and their interactions; understanding the health impacts of exposure to chemicals; find causal links between human exposure and adverse health effects.

Methods: Different chemical substances as Cd, Cr(VI), PAHs, Perfluorinated compounds, PCDDs, PCDFs, PCBs, and chemical mixtures will be studied. Various human cohorts (children, adolescents, mother-children pairs), occupational studies, hot spots, and other epidemiological studies will be used.

Results: The Italian contribution to the HBM initiative will concern: identification of existing national HBM studies and data gaps (WP7); comparability and alignment of national HBM surveys at EU level (WP8); statistical analysis of national HBM data in order to generate European reference values and integration of national data into IPChem database (WP10); provide the link between HBM levels and health data (WP11); support the relationship between a substance and health effects (WP13); selection of biomarkers of effects according to their utility (WP14).

Short discussion/conclusions: The HBM4EU initiative will help to better understand the consequences of human exposure to chemicals as a key aspect of environmental health; to bridge the gap between science and policy; to share existing experience in the EU; to generate better evidence for better regulation; to give better access to data by the IPChem database; to include aggregate exposure in the assessment of health risks.

ITALIAN REFERENCE VALUES: EVOLUTION AND INTERPRETATIVE CONTRIBUTION ALSO FOR THE EVALUATION OF EXPOSURES TO CARCINOGENIC/MUTAGENIC SUBSTANCES

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Background: The Italian Society of Reference Values (SIVR) currently defines biological reference values (RVs) in the environmental and occupational fields as estimates of concentrations of chemical substances or their products of transformation in biological materials from general population.

Objectives: SIVR intends to make its activities known to the scientific community in order to increase the number of laboratories involved in the definition of RVs for the Italian population.

Methods: SIVR RVs are produced using a procedure, described in previous published paper, that ensures standardisation, transparency and quality.

Results: SIVR defined in 2017 a new list of RVs, and the novelties introduced are here described. Examples of RVs, with particular reference to carcinogenic and mutagenic substances, are also presented in comparison with other produced internationally.

Discussion and Conclusions: In living and working environments Human Biological Monitoring is one of the ways to evaluate the magnitude and evolution of exposures to the potential pollutants present, with the advantage of identifying situations that could be resolved before they could give rise to real illnesses. Recently, the number of health surveys in populations resident near sources of environmental contamination has increased, as well as the concern of public opinion on the relationship between human health and the environment. In the occupational field, RVs are especially important because they represent the lowest possible limits for prevention purposes. They become fundamental for substances for which a toxicity threshold has not yet been defined (teratogens, mutagens and carcinogens) or in the absence of occupational limits (lack of available data), since for the purposes of prevention, occupational exposure involves an acceptable additional risk with respect to that associated with “normal” life. From this viewpoint, biological RVs and limit values proposed for working environments can be regarded as an integrated system of guideline values to orientate prevention in living and working environments

SMOKING HABIT, BIOMARKERS AND RISK PERCEPTION

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Background: A campaign to inform about health and environmental effects of tobacco and e-cig smoke has been undertaken during the European researcher's night hold in Milan in September 2015 and 2016.

Aim of the study: To evaluate the use of noninvasive biomarkers of exposure to tobacco and e-cig smoke and their association with smoking habit, and health risk perception among the participants of the fair.

Methods: A saliva sample for the determination of cotinine and nicotine was collected; carbon monoxide in exhaled air was assessed, a questionnaire and the Fagerström test for nicotine dependence were submitted to the visitors of the fair stand.

Results: Two hundred and forty subjects were included in the study, of which 161 smokers (commercial cig 61%, hand rolled cig 33%, e-cig 3%, cigar 2%, and narghile 1%) and 79 nonsmokers. In smokers, median salivary cotinine and nicotine levels were 230 and 218 µg/L, while CO in exhaled air was 10 mg/m^3. No difference in biomarkers was found comparing commercial and rolled cig. These biomarkers were positively correlated with the number of smoked cigarettes and with the Fagerström score. While smokers were well aware of the risks for health associated with smoking, they only in part supported the initiatives undertaken by the government to reduce smoking habit; interestingly, they evaluate as ineffective the increase of cig price; moreover no correlation between health risk perception and the daily consumption of cigarettes was found.

Conclusions: Saliva and exhaled air samples are convenient specimens, and salivary cotinine and nicotine are useful biomarkers suitable for large campaign for assessing smoking habit in the general population.

TIME-TRENDS OF THE GERMAN POPULATION EXPOSURE TO CONTAMINANTS USING THE PART FOR HUMAN SAMPLES OF THE GERMAN ENVIRONMENTAL SPECIMEN BANK (ESB)

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The German Environmental Specimen Bank (ESB) is a central element of the environmental monitoring system in Germany since the early 1980s. ESB do archive samples from different organisms which represent selected parts of different ecosystems but also human samples of young adults. Thus, the analysis of the samples of the ESB enables the investigation of time-trends of the population's exposure to various contaminants.

The presentation shall give an overview on time-trends of some prominent contaminants in human blood and urine during the last 2 decades.

Since 1997, blood and 24 h urine samples were taken each year from approx. 500 individuals (20-29 years, females and males) at four sampling sites in West, South, East and North Germany. Aliquots of these samples were analyzed in the year of sampling for selected parameters. The main part of the samples was stored in the ESB archive at temperatures of below -130°C making them available for retrospective time-trend analysis of additional contaminants. Metal analyses were performed by AAS and ICP-MS methods. Analyses of organic contamination and its metabolites were performed by GC-MS and LC-MS/MS. Time-trend data is available among others for the following parameters: lead in blood (Pb-B), mercury in urine (Hg-U), total arsenic in urine (As-U), hexachlorobenzene in plasma (HCH), pentachlorophenol in urine (PCP) and PCB congeners 138, 153 and 180 in plasma (PCB138, PCB153, PCB180).

The time-trend analysis revealed significant declines during the last 2 decades for all these parameters. Pb-B levels (geometric means) decreased within this period by a factor of 2, Hg-U by a factor of about 10 and As-U by a factor of 2. HCB decreased from 1997 to 2010 by a factor of 3, PCP by a factor of about10, PCB180 and PCB153 by a factor of 3 and PCB138 by a factor of 4. However, for almost all parameters the time-trends seem to reach a constant level in the last 5 years. Further analyses revealed different levels between females and males for Pb-B and moderate spatial differences for Hg-U.

The ESB supports the surveillance of regulated and emerging chemicals in the population and various environmental compartments. ESB results are therefore highly relevant for targeted and efficient action in German environmental health.

HUMAN BIOMONITORING – THE AUSTRIAN EXPERIENCE

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Background: Human biomonitoring (HBM) data reveal the individual body burden irrespective of sources and routes of uptake. Therefore HBM serves various administrative and scientific needs. In Austria we have a long history of applying HBM. However, until now only few systematic routines were implemented.

Aim of the study: This presentation demonstrates the history of HBM in Austria focusing on our medical experience.

Methods: We present examples of HBM surveys in Austria that either had a mostly scientific background or were triggered because of public concerns about environmental contamination. We also report about participation in European projects (COPHES, HBM4EU) and present our scientific journal *Biomonitoring* (De Gryter Open).

Results: Scientific questions are best addressed by either focusing on a specific population sub-group or by aiming for a representative sample of the total population. The former approach helps reducing confounding and can eventually guarantee a large variation in exposure. More recent examples for this type of study investigated synthetic musks (1) and occupational exposures (2). The latter is necessary when the aim is to establish reference values. Hot spot analyses in response to public concerns follow different rules. Interpretation is difficult in the absence of reliable reference values, as a recent example regarding a hotspot of HCB exposure demonstrates.

Short discussion/conclusions: HBM is a valuable method both for environmental health science and for public health issues. Depending on the specific problem different approaches are needed and different obstacles are present. Transparency from the conception of the study to the presentation of the results is a must in all cases. Public participation poses some difficulties but finally is the best guarantee of the desired public health impact.

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CYTOTOXICS IN MEDICAL CARE: QUESTIONS FROM OCCUPATIONAL PROFESSIONALS AND CAREGIVERS ABOUT BIOLOGICAL MONITORING

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Background: Health risks for caregivers handling cytotoxic drugs are now well known to occupational safety and health (OSH) professionals and researchers. However, the number of these treatments, their indications and the methods of administration continue to evolve. In addition, the cytotoxic drugs administered intravenously are not only used in hospitals but also under less controlled conditions (home hospitalisation, veterinaries).
Healthcare professionals handling cytotoxic drugs are exposed by dermal and digestive route and by inhalation. Numerous studies have examined the effects and ways of assessing cargivers' exposure. As part of our mission to provide assistance to occupational health services, we have noticed a significant number of questions in recent years.

Aim: The purpose of this communication is to review the requests received on cytotoxic drugs and the available answers; what type of recommendations can be proposed on what basis; what tools are available for information and training.

Methods: A survey of queries received in the Medical Studies and Assistance (EAM) division of INRS between 2012 and 2017 was carried out. All questions relating to the terms "cytotoxic", "cytostatic" and "chemotherapy" were included.

Results: A total of 50 queries from OSH professionals, but also directly from caregivers in contact with cytotoxics, have been identified. Of these, more than one-third focused on biological monitoring of occupational exposures: nature of the follow-up to be implemented, available dosages, time of measurement, interpretation of results... The questions also concerned the surface sampling or the preventive measures to be implemented. Several questions related to the risks to pregnant women handling cytotoxic drugs.

Discussion: The use of cytotoxic drugs is evolving and is not limited to oncology departments, while the level of information and protection of caregivers observed in the workplace and perceived through requests is extremely variable. There is also a need to focus on staff other than nurses and pharmacists (such as nursing assistants, domestic services staff) and non-hospital exposure conditions. Several tools and supports have been developed by the INRS to inform about the risks as well as the means of prevention: Biotox database, leaflets, articles, pamphlets. They are intended for use by OSH professionals as well as the concerned staff. As part of the risk assessment, new biological indicators and surface sample techniques should be developed for more cytotoxics.

A BIOBANK FOR STUDIES OF NORMAL VARIABILITY OF BIOMARKERS

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Background: Biomarkers of exposure or biomarkers of effects are often used in occupational health surveillance and in epidemiological research. For most biomarkers there is, however, limited information about normal day-to-day variability within individuals. For urinary biomarkers there may be diurnal variability making it important to fix the time of day the sampling is performed, but again there is very limited information in the literature about diurnal variability. Our aim was to establish a "variability biobank" of healthy individuals to make it possible for us and other researchers to examine normal variability of various biomarkers.

Methods: Blood and spot urine samples were collected from 60 healthy non-smoking study participants 18-65 years of age (29 men and 31 women; no diabetes, hypertension, or kidney disease). The spot urine samples were collected during two 24h periods about one week apart, and the study participants were instructed to urinate at six fixed times (first morning void, 9:30, 12:00, 14:30, 17:30, and 22:00). The samples were transferred to Minisorb tubes (NUNC) and kept at 4°C until analysis of proteins (Albumin and Alpha-1-microglobulin), creatinine and density within 3 days of collection. Blood samples were collected twice, one in each week. Aliquots of urine and blood (blood, plasma and erythrocytes) are stored frozen (-80°C).

Results: The biobank (more than 20,000 aliquots) is now open for researchers interested in examining normal variability of their favorite biomarker(s).

Conclusion: The biobank is especially suitable for analysis of diurnal variation in urine biomarkers (12 samples per subject), but could also be used for analyses of variability in biomarker concentrations in blood, plasma or red cells. Variability in certain biomarkers using a previous, smaller biobank have been published for cadmium and proteins (Akerstrom M et al., JEESE 2014), and 8-oxodG (Barregard L et al., ARS 2013).

OVERVIEW OF HUMAN BIOMONITORING INITIATIVES UNDER THE GOVERNMENT OF CANADA'S CHEMICALS MANAGEMENT PLAN (2007-2017)

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Background: In 2017, Canada's Chemicals Management Plan (CMP) is celebrating its 10th anniversary. It is the most comprehensive environmental agenda to ensure the safe management of chemicals in Canada. Under the CMP, multiple human biomonitoring (HBM) initiatives serve to inform chemical risk assessment and control.

Aims: The main objective of Health Canada's HBM initiatives is to establish national baseline measurements of chemicals in Canadians, including vulnerable populations. These initiatives provide baseline data to track trends over time. HBM is used increasingly along with other interpretation tools to quantify human exposure and to provide information for setting priorities and for taking action to protect public health.

Methods: HBM in Canada is performed through three major initiatives: 1) The Canadian Health Measures Survey (CHMS) is an ongoing survey that operates in two-year cycles in which approximately 6,000 Canadians are selected to participate 2) The Maternal-Infant Research on Environmental Chemicals follow 2,000 pregnant women-infant pairs across Canada, and 3) the Northern Contaminants Program supports biomonitoring studies in northern Canadian populations. In addition to conducting these broad national HBM initiatives, Health Canada is also developing tools to interpret and communicate HBM data such as Reference values (RV95), Biomonitoring Equivalents (BE) and tissue guidance values.

Results: Under the Canadian Health Measure Survey, in the past decade, over 250 chemicals (metals, persistent organic pollutants, environmental phenols, acrylamide, pesticides, phthalates) have been measured in 29,000 Canadians aged 3 to 79 years at 81 sites across Canada. HBM data generated have been used in over 350 scientific publications and are a key tool in assessing Canadians' exposure to environmental chemicals. RV95s derived from the CHMS, have been proposed for heavy metals, as well as persistent and non-persistent chemicals. BEs have been developed for over 90 chemicals.

Conclusions: HBM initiatives, under the CMP, have not only provided baseline concentrations in the Canadian population but it has, in turn, been used to inform regulatory risk assessment and improve evidence-based decision making in public policies aiming to reduce exposure to toxic chemicals and protect the health of Canadians.

TRADITIONAL REMEDIES AND RISK OF HEAVY METAL POISONING

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Background: Humans have used heavy metals for thousands of years. In Algeria, kohl (powder, often contains lead) is widely used as traditional topical remedy. Mercury is seldom used.

Aim of the study: This study aims to report two cases illustrating an unusual treatment that implicate a potential exposure to heavy metals by injection of kohl and ingestion of mercury.

Methods: It is a study of clinical data and toxicological analysis reported in the medical records of two patients admitted to medical neurology department. The toxicological analysis was preformed by AAS.

Results: Case 01: A 42-year-old man had injected kohl mixed with water repeatedly for 2 years to treat impotence. Consequently, he had surgery to evacuate the abscess formed in the sites of injection. *Four months later, he* had been *hospitalized for a severe polyneuropathy. Clinical findings revealed motor deficit, sensory disorder, pain, depression, insomnia, anorexia, weight loss, cough and constipation. A lead poisoning was suspected and confirmed by a* high blood lead level (BLL: 138.2 µg/dL), iron deficiency anemia, basophilic stippling of red blood cells and lead lines projecting the iliac bone. Chelating therapy by DMSA reduced blood lead levels to 15.5µg/dL. A rehabilitation program allows a gradual improvement of the clinical condition. The lead rate in a sample of offending kohl was 0.005%. **Case 02**: A 55-year-old man ingested elemental mercury to relieve a stomachache. Four days later, he had been hospitalized for motor dysfunction of the lower extremities. The abdominal X-rays showed evidence of mercury beads deposited in the intestine fields. However, there was no significant correlation between mercury toxicity and the clinical data. After elimination of a likely lead poisoning (BLL less than 1 µg/dL), further investigations revealed adenoma with lumbar spinal cord compression explaining the patient's motor deficit.

Discussion and Conclusions: The lead poisoning is definitely associated to use of kohl. Then, it is necessary to assess complications of this chronic exposure to lead and minerals as Antimony usually identified in kohl. Spite of the fact that elemental mercury is poorly absorbed by ingestion, an enema is suggested to clear it from the gut. According to these cases, an awareness programs in the population regarding possible metals exposure through use of kohl or mercury is highly recommended.

Keywords: Traditional remedies, Kohl, Lead poisoning, Blood lead level, Mercury.

ESTIMATION OF OCCUPATIONAL EXPOSURE TO METALS IN A PAINT FACTORY

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Background: Occupational hazards due to metal exposure raise serious health issues in many industrial sectors. A typical example is paint manufacturing as it involves a large number of processes handling metallic salts used as pigments. While solvent exposure has received wide attention, as can be attested by the number and depth of published studies, the real impact of metal hazards remains largely underestimated, in particular, when handling raw materials is not totally secured.

Aim of the study: The aim of this work is to estimate the levels of metal exposure of paint factory workers.

Methods: This study was undertaken among workers of western Algeria paint factory (SPOA). Analysis of the workers' activity is carried out by means of observations on the workplace, as well as by a specific information sheet identifying the professional activity of the subjects (tasks performed, seniority and rhythm of work etc.) Following INRS recommendations [(1)], urine samples were gathered at the end of the last weekly working day, whereas metals analysis was carried out using a multi-element method by inductively coupled plasma mass spectrometry (ICPMS).

Results & Discussion: We have developed and validated an ICPMS multi-element method for the analysis of lead, aluminum, chromium, copper, cobalt, arsenic and cadmium. Validation criteria were acceptable for these seven elements concerning linearity (R> 0.995), intermediate precision (RSD% between 2.42 and 5.43), and accuracy (recovery percentages obtained ranged between 89.33% and124.26%). Quantification limits are less than 1.4µg / l. The activity analysis revealed ten workers occupying risk-prone functions for whom a metal profile was made. Results exhibit higher levels of exposure than biological exposure indices for aluminum (133.03 ± 74.01) µg /g creatinine, and chromium (54.93 ± 32.26) µg/g. Both of these elements are present in metal salts used in paints. In addition, cadmium and arsenic levels for three workers were exceeding reference values of unexposed populations. This may result from a hidden exposure or previous impregnation.

Conclusion: Evidences of high levels of exposure faced by many workers bring to light the importance of periodic biological monitoring as a first step to ensure collective and individual protection in paint industry. Moreover, such a monitoring should be extended to other industries.

Key words: Metals, ICP-MS, occupational exposure, paints, industry

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A ROLE OF ENTEROBACTERIA IN ARSENIC INTAKE FROM SEAWEED

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Background: seaweeds consumed as food contain large amounts of various arsenic compounds, mostly arsenosugar (AsSug) and arsenolipid. The toxicity of arsenic compounds varies greatly depending on their chemical configurations. Assug and arsenolipid are metabolized into a possible carcinogen of dimethylarsinic acid (DMA). Intestinal bacteria are considered to play an important role in the carcinogenicity of DMA; however, the arsenic intake from seaweed in digestive system, particularly in the intestinal tract, is not yet understood.

Aim of the study: in order to clarify the role of enterobacteria in arsenic intake from seaweed, we examined the arsenic intake from wakame (Undaria pinnatifida), a typical seaweed, using an in vitro artificial gastrointestinal system.

Methods: dry wakame was incubated at 37ºc in gastric juice (NaCl 2 g, pepsin 100 mg/L, pH1.2) for 4 h, then in intestinal juice (bile extract 25 g, pancreatin 4 g/L of 0.1M $NaHCO_3$, pH7.2) for 0.5 h, and finally in enteric bacteria solution (prepared using fresh feces obtained from a healthy male adult after a 5-day restriction on seafood intake) for 24 h. Microbiota was analyzed by the next-generation sequence analysis. Changes of the arsenic compounds after artificial digestion were analyzed by HPLC-ICP-MS and HPLC-TOF-MS.

Results: the major arsenic compounds detected in wakame were arsenophospholipid1012, arsenohydrocarbon388, AsSug328, and AsSug482. Arsenic elution from wakame seaweed after 4 h of incubation in gastric juice, 0.5 h in intestinal juice, and 24 h in enteric bacteria solution were 13.0 ± 0.8% (n = 6), 18.6 ± 5.2% (n = 6), and 108.0 ± 2.9% (n = 3), respectively. Moreover, an increase was observed in polysaccharide-catabolizing bacteria. After artificial digestion, arsenophospholipid1012 and arsenohydrocarbon388 disappeared but several AsSugs and an unidentified arsenic were generated.

Discussion/conclusions: both artificial gastric and intestinal juice did not affect wakame digestion but enteric bacteria solution significantly digested it. DMA was not found during gastrointestinal digestion, it can be metabolized in liver.

OCCUPATIONAL EXPOSURE TO CHROMIUM AND NICKEL IN THERMAL SPRAYING WORKERS: PRELIMINARY BIOLOGICAL AND ATMOSPHERIC ASSESSMENTS

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Background: Exposure to metals during thermal spraying activities is a topical issue for occupational health.

Aim of the study: Thermal spraying is an industrial process in which a metal coating is applied at high temperature and high velocity on a metal object in order to produce a surface which is extremely resistant to corrosion or wear, or having particular conductive properties. Several processes exist, among which wire flame spray, powder flame spray, electric arc wire spray, high-velocity oxy-fuel (HVOF) and plasma spray. The present study is based on the observation that operators would be exposed differently depending on the processes involved. Its goal is to characterise the emissions and evaluate the levels of exposures from the different spraying processes in order to determine strategies for biological monitoring which could be used by occupational health services.

Methods: Sampling campaigns were undertaken in six plants by associating individual atmospheric samples with the urine samples of 29 volunteers, of which 14 thermal sprayers.

Results: Operators projecting chromium oxide by plasma spray process are exposed to Cr VI, with some concentrations exceeding the 8-hour time-weighted average (TWA) exposure limit of 1 $\mu g/m^3$. Urinary Cr levels remain relatively low, below 2 µg/g creatinine, remaining very distant from the American value applied to welders (25 µg/g creatinine). Whatever the process, urinary nickel (Ni) excretions are relatively high compared to non-exposed workers (11 and 3.8 µg/g of creatinine, respectively). Urinary levels of the latter are similar those observed in the general population.

Discussion/Conclusions: Urinary excretion levels of Cr and Ni observed during the field campaigns confirm an occupational exposure of operators to aerosols emitted during the thermal spraying processes and remind the importance of wearing an individual protection equipment adapted to the activity. A descriptive statistical analysis by processes seems to show that the wire flame spraying process lead to the highest excretion level. Nevertheless, these preliminary results encourage to continue the research undertaken in this activity sector.

OXIDATIVE DNA DAMAGE AND LIPIDS PEROXIDATION AS EFFECTS BIOMARKERS OF MERCURY-EXPOSED WORKERS

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Background: The toxic effects of elemental mercury vapor (Hg^0) associated with increased oxidative stress following long-term occupational exposure to Hg^0 affects increased oxidative DNA damage and lipids peroxidation.

Aim of the study: The aim of this study was to assess the differences in urinary levels of DNA oxidation marker 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and the thiobarbituric acid reactive substances (TBARS) concentration plasma as a marker of lipids peroxidation in these groups and theirs relationship with biomarkers of occupational exposure to Hg^0

Methods: Total Hg (THg) concentrations of urine and blood samples were determined using Thermal Decomposition Amalgamation Atomic Absorption Spectrometry method (TDA-AAS) using Direct Mercury Analyzer (DMA-80 by Milestone, Spectro-Lab, Poland). Plasma TBARS concentrations (TBARS-P) were measured using spectrofluorometric method and concentration of 8-oxodG in urine (8-oxodG-U) using liquid chromatography/tandem mass spectrometry (LC-MS/MS) method.

Results: Median (Me) urinary mercury (Hg-U) concentration was significantly higher ($p < 0.001$) in the whole exposed group 35.6 µg / g creat. (IQR - Interquartile range 13.2 – 88.7); than in control group Me: 0.2 µg / g creat. (IQR 0.1 – 0.2). The 8-oxodG-U concentration was significantly higher ($p < 0.001$) for workers than in control group Me: 41.2 µg / g creat. (IQR 28.1 – 61.1) and Me: 35.22 µg / g creat. (IQR 27.0 – 47.6), respectively as well as the median value of TBARS-P concentration was significantly higher ($p < 0.001$) in exposed group Me: 2.35 nmol / ml (IQR 2.0 – 2.8) than in control group Me: 2.3 nmol / ml (IQR 1.9 – 2.7).

Short discussion/conclusions: The exposure at relatively high concentrations of Hg^0 is associated with increased oxidative DNA damage and lipid peroxidation. Determination of 8-oxodG–U levels may give information about defense mechanism of cell in response to mercury-induced oxidative stress and could be useful for evaluation oxidative DNA lesions.

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EFFECT OF PERSONAL PROTECTIVE EQUIPMENT IN WORKERS' METAL EXPOSURES

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Biomass-fired power plant workers use respiratory protectors, coveralls, protective clothes, and protective gloves to protect themselves against metals of ash, but the usage and type of these protectors vary widely from site to site.

The aim of this study was to give recommendations to workers that which personal protective equipment's they should use in work task inside boilers to minimize their dermal and inhalation exposures to boiler ash metals.

Dermal body samples and hand-washing method were used in workers' dermal metal (body: As, Cd, Cu, Ni, and Pb; hands As, Cd, Cu, Ni, Pb, and Zn) exposure assessment, and biomonitoring methods of metals (Al, Cd, Pb, Mn, As, and Se) in total exposure assessment.

Results of this study showed that, long leather gloves that workers were using protected workers' hands 2.1 and 2.2 times better than short leather gloves and other gloves, respectively. Coveralls and hoods were 7.7 and 11 times better as a way to protect workers' bodies against these metals compared to open coveralls and coveralls without a hood, respectively. Average urine excretions of Al, Cd, Pb, and Se were smallest, 0.3 µmol/l1, 1 nmol/l, 0.6 µg/l, and 0.03 mg/g creat., respectively, when workers were using respirators with TM3-A2B2E2K2-P class filters. Urinary excretion of Al was 0.5 µmol/l1 when workers were using respirators with P2 or P3 class filters, and 1.5 µmol/l1 when respirators were not used. Average urine excretions of Cd were 2-3 nmol/l when workers were using respirators or when respirators was not used. Average urine excretions of Pb were 0.8-1.5 µg/l when workers were using P2 or P3 class respirators, and increased clearly (3.5 µg/l) when respirators were not used. As excretions of workers were almost at the same level (14.2-15.7 nmol/l) when workers were using respirators, and slightly higher (17.0 nmol/l) when respirators were not used.

Powered air respirators with TM3- A2B2E2K2-P cartridges, hooded one-piece coveralls, and over-wrist long leather protective gloves were recommended to biomass-fired power plant workers, especially those who work inside the power plant boilers or superheaters.

RECOMMENDATION OF BIOLOGICAL VALUES FOR HEXAVALENT CHROMIUM AND ITS COMPOUNDS FOR THE BIOMONITORING OF CHEMICALS AT WORPLACE

F. Lamkarkach[1], F. Sissoko[1], D. Brunet[1] and the Scientific Expert Committee on Occupational Exposure Limits[2]

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Background: The scientific Occupational Exposure Limits (OEL) committee of the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) recommends airborne and biological values for chemicals at the workplace. In 2009, the committee recommended OELs for hexavalent chromium (CrVI) and its compounds, by doing a quantitative risk assessment for lung cancer. On the basis of this expert appraisal, regulatory binding atmospheric values were established in France (8h-TWA of 1 $\mu g.m^{-3}$ and a STEL of 5 $\mu g.m^{-3}$).

Aim of the study: The OEL committee decided to supplement its expert appraisal on CrVI in order to recommend biological limit values (BLV) in addition to the atmospheric OELs.

Methods: The first step was to identify dose-effect relationship between concentrations of urinary chromium (CrU), the selected biomarker, and carcinogenicity or renal toxicity but bibliographic data were insufficient. Therefore studies carried out at workplace and describing correlations between atmospheric concentrations of CrVI and concentrations of CrU were analyzed. Derivation of a BLV for CrU based on the exposure to the regulatory atmospheric 8h-TWA of CrVI (i.e. 1 $\mu g.m^{-3}$) was considered to be the most relevant approach.

Results: Two studies, carried out in the chromium-platine sector, were selected because of a high level of analytical capability making it possible to extrapolate urine levels while remaining within the scope of validity of the methods used. Thereby, based on the correlation between atmospheric concentrations of CrVI and concentrations of CrU, the OEL committee recommended a BLV of 2.5 $\mu g.L^{-1}$ (1.8 $\mu g.g^{-1}$ of creatinine).

Discussion and Conclusions: Only red blood cell chromium is specific to exposure of CrVI. Due to a lack of information, CrU was proposed for the biological occupational monitoring of exposure to CrVI, so in cases of joint exposure to trivalent and hexavalent chromium, results should be interpreted in the light of the atmospheric concentrations of the different chromium compounds.

CHRONIC KIDNEY DISEASE OF UNKNOWN ORIGIN IN SUGARCANE INDUSTRY: METALS ANALYSIS IN BIOLOGICAL SAMPLES

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Introduction: In Central America, there is an epidemic of Chronic Kidney Disease of unknown cause (CKDu), also referred as Mesoamerican Nephropathy (MeN) [1]. There it has been estimated that CKDu has caused in the last 15 years, the premature death of approximately 20,000 individuals, mainly young males, who work in physical intensive manual labour, hot climate agriculture such as sugarcane cultivation and sugar production [4,5]. Sufferers reach end stage renal disease before the age of fifty. Over the last two decades there has been a progressive increase in CKDu and it has been observed across Central America including in Nicaragua, El Salvador, Costa Rica and Guatemala. The work presented here is part of a study investigating different possible causative factors.

Method: Following site visits to different locations in Nicaragua blood and urine samples were collected from 330 workers. This includes workers who do and do not exhibit kidney function decline, as well as from two different geographical locations. The blood samples were taken at the start of the harvest season and the urine samples were collected at both the start and end of the harvest season. In this part of the project a number of metals, including cadmium and mercury, were analysed in the blood and urine samples by ICP-MS at the UK's Health and Safety Laboratory.

Results: The initial results from the metals analysis showed elevated urinary cadmium, aluminium and manganese levels in different groups. The urinary cadmium results show median results of more than forty times higher levels, aluminium median results are approximately seven times higher and manganese more than fifteen times higher than a UK population. The initial analysis of the blood samples showed that there a few samples with cadmium concentrations up to 3000 nmol/L.

Discussion: As yet there is no clear evidence as to what is the leading cause of kidney failure and multiple pathways have been proposed. The levels of elements determined here provide a suggestion of metals exposures however this is not in every worker and more work is needed to understand this further. The aetiology of CKDu is likely multifactorial, potentially involving a combination of causal and susceptibility factors. The examination of a limited kidney biopsies of patients with MeN suggest a tubular injury rather than a glomerular one, which may indicate toxic or metabolic aetiology that ultimately leads to kidney dysfunction.

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METALS BIOMONITORING IN HAIR, BLOOD AND URINE IN THE NORTHWEST TERRITORIES, CANADA

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Background: Mercury, lead, arsenic, and other metals may be naturally present at elevated levels in the environment and Northern First Nations communities in Canada sometimes face high contaminants exposures due to several factors (local geology, long-range transport, local sources). To monitor metal exposures, urine and/or blood are usually the preferred matrices. However, the collection of blood and urine for biomonitoring in remote locations can be challenged by logistical constraints (e.g. lack of local expertise for collection, shipping costs, sample refrigeration).

Aim: A biomonitoring project, supported by the Northern Contaminants Program, is underway to assess metals exposure among Indigenous communities in the Northwest Territories, Canada. The results provide 1) an exposure baseline for participating communities and 2) an estimate of the correlations among matrices used for biological monitoring of metals. This abstract presents the results from the first of three years of sample collection.

Methods: Essential metals and toxic metals were measured in blood, urine and hair samples collected in one pilot community in 2015-2016. Samples were digested and/or diluted prior to analysis by ICP-MS. Univariate methods were used to quantify the correlations among matrices.

Results: 21 participants volunteered to provide at least one biological sample; a total of 20 hair, 13 blood and 10 urine samples were collected. All samples fell below the health-based guidelines established for mercury, cadmium, and lead. Generally, metal exposures were similar to those seen in other biomonitoring studies in Canada; however, a few essential metals (e.g. manganese, selenium and zinc) appeared to be above those usually seen in the Canadian population. Particularly strong inter-matrix correlations were noted for mercury (hair-blood) and, to a lesser extent, iron (hair-urine).

Conclusions: Overall, this biomonitoring project will improve knowledge on the baseline levels and the risks from metals in First Nations communities in the Northwest Territories. Results presented herein represent only the first pilot year of sample collection; further work is required to understand the generalizability of the results. However, as hair samples do not require specialised training, expensive equipment, or refrigeration during storage/shipment, their use may empower remote First Nations communities to undertake biological monitoring over time.

ASSOCIATIONS OF BLOOD LEAD LEVELS WITH NEUROPSYCHOLOGICAL SYMPTOMS AND DELTA-AMINOLEVULINIC ACID DEHYDRATASE GENOTYPE IN GLASS CUTTERS

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Background: Lead inhibits delta aminolevulinic acid dehydrase (ALA-D) and deficiency of ALA-D elevates levels of 5-aminolevulinic acid (5-ALA), which is neurotoxic in hepatic porphyrias and this may also be in the case of lead exposure. ALA-D polymorphism is expressed with two co-dominantly alleles, ALAD1 and ALAD2. The results of study concerning genetic susceptibility towards lead are inconsistent.

The aims of study are: To search the relationship between blood lead levels (BLL) and neuropsychological symptoms and the association between ALAD gene polymorphism Lys59Asn with cumulative blood lead index (CBLI) in glass cutters workers.

Methods: We were following 100 glass cutters (29 male and 71 female) who had been exposed to inorganic lead in 2009. We did biological monitoring BLL (atomic absorption spectrometry), and then used Finish Institute of Occupational Health Questionnaire about neuropsychological symptoms. We calculated CBLI by the method used by Howard Hu and others. And then we applied the method of real time polymerase chain reaction with specific TaqMan® probes for Lys59Asn polymorphism. In statistics we used Spearman correlation coefficient and linear regression method.

Results: The average age of the glass cutters was 41 years for both sex, duration of the employment at the working place was 22 years. The average of the BLL in females was 168 µg/L and in males 253 µg/L and CBLI was 631 µg/L in all workers. Correlation coefficient between BLL and ALA-D was -0,52 , $p<0,001$. Females had more neuropsychological symptoms such as sleeping, memory and concentration disturbance, fatigue, emotional lability and somatic complaints than males. BLL is associated only with sleeping disturbances $p=0,022$ and emotional lability $p=0,055$. ALA-D polymorphism was not associated with CBLI (gender and duration of employment were confounders).

Discussion/Conclusion: We found association between BLL and some neuropsychological symptoms, but maybe a more exact instrument is needed for the estimation in neurotoxicity of lead and not only by questionnaire. We could not confirm any relationship between ALAD gene polymorphism and accumulation of lead in blood. We suggest to use an additional genotype of ALAD polymorphism in further search.

RECOMMENDATION OF BIOLOGICAL VALUES FOR BERYLLIUM AND ITS COMPOUNDS FOR THE BIOMONITORING OF CHEMICALS AT WORKPLACE

F. Sissoko[1], F. Lamkarkach[1], D. Brunet[1] and the ANSES Scientific Expert Committee on Occupational Exposure Limits[2]

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Background: The Occupational Exposure Limits (OEL) Committee of the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) has been assigned by the French labour ministry to recommend airborne and biological values for chemicals at the workplace. The Committee has developed its own guidelines for the selection of biomarkers and the recommendation of 2 types of biological values: Biological Limit Values (BLVs) and Biological Reference Values (BRVs).

Aim: Anses OEL Committee recently studied beryllium and its compounds in order to recommend biological values for this heavy metal.

Methods: In a first step, literature has been reviewed in order to identify relevant biomarkers and for assessing biomonitoring data for beryllium. For non-threshold-effect carcinogenic substances like beryllium, if available data allow a quantitative risk assessment, BLVs should be expressed as concentrations corresponding to 3 individual excess of cancer-risk of 10^{-4}, $10^{-}5$ and 10^{-6}. Failing that, a pragmatic BLV can be proposed (based on another health effect). Pragmatic values do not aim at preventing from cancer but can nevertheless be useful tools to limit occupational exposure on workplaces. Otherwise, when possible, BRVs based on concentrations of biomarkers found in a general population of adults or in a non-occupationally exposed population will be recommended.

Results: Urinary beryllium was selected as suitable biomarker. It was not possible to link health effects (carcinogenic effects and chronic beryllium disease) with urinary concentrations of beryllium. The derivation of a BLV based on the exposure to the atmospheric concentration of 0.01 µg/m3 (8h-OEL recommended by Anses OEL Committee) was also explored.
Finally, Anses OEL Committee turned towards the recommendation of BRV based on urinary concentrations of beryllium in a general population of adults whose characteristics are similar to those of the French population.

Conclusion: A BRV below 7 ng/L for urinary beryllium was recommended for the biological monitoring of occupational exposure to beryllium.

NRF2, KEAP1 PROMOTERS AND DNA METHYLATION AS EARLY EFFECTS BIOMARKERS OF INORGANIC ARSENIC EXPOSURE

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Background: The factors present in the human environment significantly affect the integrity of the genetic material. Many of them have mutagenic and even carcinogenic effects. An important group of compounds with carcinogenic effect, which occur both in the living and occupational environment, are metals such as arsenic. The epigenetic effects of arsenic have been an area of intense research interest.

Aim of the study: The aim of the study was to investigate the association between iAs level in a population with occupational exposure to As and the link between iAs levels and epigenetic modification.

Methods: Global DNA methylation levels were assessed by colorimetric ELISA method using Methylflash Methylated DNA Quantification Kit (Epigentek, Farmingdale, NY, USA), according to manufacturer's instructions. DNA methylation of *NRF2* and *KEAP1* was analyzed using quantitative methylation-specific real-time PCR assay (qMSP) with FastStart Essential SYBR Green Master (Roche, Basel, Switzerland).

Results: The occupationally exposed group showed a significantly higher degree of *NRF2* methylation (Me 0.47% (IQR 0.29% -0.59%) vs Me 0.27% (IQR 0.13% -0.47%), p=0.0017), *KEAP1* methylation (Me 0.13% (IQR 0.03% - 0.20%) vs Me 0.04% (IQR 0.001% -0.18%), p=0.0238) and overall 5-mC content (Me 1.41% (IQR 0.95% -1.81%) vs Me 0.85% (IQR 0.59% - 1.26%) p<0.0001) than the control group.

Short discussion/conclusions: The results of this study indicate that arsenic occupational exposure is positively associated with global DNA methylation, but changes in gene methylation status need to be presented in the context of gene expression patterns.

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SIMULTANEOUS DETERMINATION OF URINARY S-PHENYLMERCAPTURIC AND TRANS, TRANS MUCONIC ACIDS BY SOLID-PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY

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Background: Benzene is a recognized carcinogen for man. For the two urinary metabolites considered by the ACGIH, the BEI established in 2016 were considered. These substances show different concentrations in urine and, thus no single assay is currently capable of quantitating the S-phenylmercapturic acid (SPMA) and trans,trans-muconic acid (MA) with its precursor, trans,trans-sorbic acid (SA).

Aim of the study: This paper reports the first contribution to the simultaneous determination of the SPMA, MA and SA directly in the urine by automated head space/solid-phase micro extraction (SPME) and gas chromatography (GC)/mass spectrometry (MS) analysis previous in-sample derivatization by trimethyloxonium tetrafluoroborate (TMO).

Methods: Two mL of diluted urine were transferred into a 20 mL vial with a magnetic stirring bar and mixed with 40 µL of the internal standard cyclopentanecarboxylic acid, water solution (50 µg/mL). The conversion of the pre-SPMA to SPMA was performed upon treatment of urine at pH 1.1. To convert the MA and SA into their methyl esters, and the SPMA as methylphenylsulfide, reaction with TMO was performed at room temperature in two steps. While stirring about 150 mg of sodium carbonate were added and within 6 minutes approximately 30 mg of solid TMO were added in two aliquots. After 1 minute the solution was neutralized with about 15 mg of sodium bicarbonate. This procedure was repeated again. Finally, sodium chloride (0.5 g) was added and the vials were processed with xyz autosampler. The SPME unit was equipped with 85 µm P fiber. Analysis was performed with a Varian Saturn 2200 Ion Trap-MS equipped with VF-5 analytical column.

Results: In MS/MS-PCI mode we selected methanol as the reactive solvent with the formation of a m/z 171 MA-methyl ester precursor ion (product ion m/z 111). For all the other compounds we operated in EI mode, full-scan. The limits of quantification for these benzene exposure biomarkers were 0.1 (m/z 124), 10 and 3.2 (m/z 111) µg/L for SPMA, MA and SA, respectively.

Short discussion/conclusions: Urine is a complex sample and hence the acquisition method required specific GC/MS instrumentation capable of supporting the changeover, fully automated during a single chromatographic separation, from MS to MS/MS and both PCI and EI modes. The treatment with TMO of SPMA leads to the formation of its sulfonium derivative, which is instable in aqueous medium and readily hydrolized producing methylphenylsulfide.

EVALUATION OF THE PROFESSIONAL CARBON MONOXIDE EXPOSURE

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Background: The workplace is an important setting for carbon monoxide (CO) exposures. These exposures could cause cardiovascular, pulmonary or cerebral effects.

Aims of the study: The purpose of this study is to evaluate the exposure of CO in motor vehicle exhaust at automotive workers in the City of Oran.

Methods: We realized a cross-sectional study covering a period of 3 months (January to March 2017). The information was collected using a questionnaire. The exposure to CO was evaluated by the spectrophotometric assay of carboxyhemoglobin (HbCO) by the method of Beutler and West.

Results & Discussion: On the whole, the study covered 32 workers exerting in mechanics, washing and guarding of carparks.
These workers are all male and without particular pathological histories. 65% of the cases are smokers, 36% consume cannabis and 18% alcohol.
The HbCO assay reveals impregnation with CO at 34% of the population with rates > 2.5% (a rate not to be exceeded according to WHO recommendations). Besides, there are many gaps in worker's knowledge of CO, in particular as regards sources of exposure and symptoms of poisoning.

Conclusion: It emerges from this work that it is essential to make a thorough sensitization about the poisoning by the CO among these workers and to improve the working conditions by the ventilation of premises, the installation of CO's detectors and the compulsory wearing of personal protective equipments. These workers also require regular biomonitoring by measuring the exhaled CO or carboxyhemoglobinemia.

Keywords: Carbon monoxide; Carboxyhemoglobin; Chronic exposure; workplace; automotive workers.

RESULTS OF A 1-VINYL-2-PYRROLIDONE METABOLISM STUDY IN SPRAGUE-DAWLEY RATS

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Background: 1-Vinyl-2-pyrrolidone (VP) is produced in industrial sale and is classified as skin penetrating and as carcinogenic substance category 4 by the German Research Foundation. The VP metabolism is not fully understood yet.

Aim of the study: We postulated the formation of a VP specific mercapturic acid (VPMA) as well as six potential VP oxidation products in order to establish a human biomonitoring method.

Methods: 20 female SD rats were divided into four groups and exposed inhalative (5 ppm VP for 6 h) or dermal (40 µL VP/kg rat). Each exposure pathway comprised an additional control group. Urine was collected over 24 h in 6 h intervals. A LC/MS/MS and a GC/MS/MS was established and validated for the target molecules. Three internal isotope labeled internal standards for best analytical results are available.

Results: Four of the seven target molecules could be identified as metabolites of VP. These are VPMA, N-2-Hydroxyethyl-2-pyrrolidone (N-2-HE-2-P), (2-Oxo-1-pyrrolidinyl) acetic acid (OPAA) and Hydroxy-(2-oxo-1-pyrrolidinyl) acetic acid (H-OPAA). Mean molar fractions of the dose applied were 6 ppm (2 ppm) for VPMA, 0.7 ‰ (0.3 ‰) N-2-HE-2-P, 4.8 ‰ (2.4 ‰) OPAA and 0.2 ‰ (0.3 ‰) H-OPAA after inhalative (dermal) exposure. Half life times were determined to be 8.6 h (11.5 h) for VPMA, 5.9 h (8.9 h) for N-2-HE-2-P, 3.5 h (7.3 h) for OPAA and 14.4 h (7.5 h) for H-OPAA.

Short discussion/conclusions: All parameters excluding H-OPAA were measured in control urine samples. Concentration changes during the day were observed in control urines with unknown cause, most likely metabolic processes. The significant changes observed, leave no doubt that the four target molecules are VP metabolites. None of them is a major metabolite. An accumulation of the metabolites over the work week is possible due to the half life times derived. VPMA and N-2-HE-2-P seem to be suitable for establishing a human biomonitoring method. The method has yet to be applied to a collective of workers exposed to VP.

A REVIEW ON HUMAN BIOMONITORING FOLLOWING EXPOSURE TO SOLID WASTE INCINERATOR EMISSIONS

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Background: Solid waste incinerators (SWI) emit several toxic pollutants among which polychlorodibenzodioxins and furans (PCDD/F), polychlorobiphenyls, metals, monocyclic and polycyclic aromatic hydrocarbons (PAHs). Health effects following human exposure to SWI have been of concern since a long time. Starting from the '80s several studies measured biomarkers of exposure and/or early biological effects in individuals from the general population and in workers exposed to SWI emissions.

Aim of the study: The results of a systematic review of peer-reviewed literature of biological monitoring studies on SWI exposure is presented to illustrate the state of the art, to highlight strength and deficiencies of these studies, and to draw conclusion useful to drive future research in the field.

Methods: Relevant studies were selected through three steps: 1) a literature search was performed in the Medline, CAplus, and Embase database, through PubMed and Scifinder Scholar interfaces (filter: English or Italian language, time limit: 1900-13th January 2017; 2) the retrieved abstracts/titles were screened by four independent reviewers using defined inclusion criteria; 3) the full text of the relevant studies was examined and scored using a modified version of the STROBE-ME statement.

Results: A total of 128 papers were finally included in this review. The studies were focused on the general population (n = 63), SWI workers (n = 51), or a mix of the two groups (n = 14). The countries with the highest number of studies were Spain (23 studies), Korea (19), Japan (14) and Portugal (12). Biomarkers were assessed in blood (109), urine (37), breast milk (14) and hair (5); the most frequently investigated biomarkers were PCDD/F in blood (84). The median (25th-75th) score was 18 (13.9-21.5). Some earlier studies showed an increase of blood levels of PCDD/F, lead, and PAHs in individuals (mainly workers) exposed to emissions from old SWIs; the latest studies (from the year 2000), investigating exposure to modern SWI, showed no clear increase for most biomarkers, moreover decreasing levels were observed in prospective studies.

Conclusions: Limited evidence of the impact of SWI on exposure and effect biomarkers was found, with age of the plant as the most relevant factor in determining the increase of biomarkers. Most studies presented several limitations, such as a scarce exposure characterization, the poor information on possible confounders, and the lack of statistical power; future research needs to tackle with these issues.

DECLINE IN BREATH ETHANOL AFTER INHALATION OF ETHANOL VAPORS AND USE OF MOUTH WASH

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Breath analyzers are commonly used to estimate the blood alcohol concentration (BAC). Breath analyzers have several advantages: they are portable, lightweight, non-invasive and give fast results. These instruments estimate the BAC indirectly, based on a stable partitioning of alcohol between blood and breath (BAC:BrAC = 2100). However, local exposure to ethanol, eg inhalation of vapors, use of mouth wash etc, may increase the BrAC without affecting the BAC (since the systemic intake is minimal) and thereby overestimate the true BAC.

The aim of the study was to study the decline in breath ethanol levels following local exposure (inhalation and mouth wash) to ethanol.

The study was approved by the regional ethical committee and performed after informed consent by the volunteers. Ten healthy subjects (5 men, 5 women) were exposed by inhalation to 1000 mg/m^3 (the Swedish 8-h occupational exposure limit) ethanol vapor for 15 min in an exposure chamber. Thereafter, breaths were sampled in 1-L Tedlar bags every 10 s for two minutes. After a break of 45 min, the subjects rinsed their mouth for 30 seconds with 20 ml of a typical mouth-rinse containing 21% ethanol. Post-exposure breaths were collected every 2 min for 19 min. Capillary blood was sampled from the fingertip before and after each exposure. Ethanol in breath and blood was analyzed by gas chromatography.

No or negligible levels (less than 0.0016 mg/g) of ethanol were detected in blood before and after the exposures. The decline in breath was monoexponential after both exposures. The average half-life of ethanol after inhalation exposure was 37 (range 28-46) s, while that after mouth wash was longer, 96 (64-162) s. The concentration of ethanol in breath corresponded to 0.2 (0.1-0.3) mg/g and 9 (6-13) mg/g blood in the first breath samples after the inhalation and mouthwash exposure, respectively. On average, it took 22 (14-34) s and 12 (8-16) min, respectively, for the estimated BAC to fall below the Swedish statutory limit of 0.20 mg/g blood.

In conclusion, the results from breath analysis should not be a problem even if the subject would have inhaled ethanol vapors prior to the test. However, use of mouth wash may severely overestimate the true BAC for several minutes.

INHALATIONAL AND DERMAL EXPOSURE OF DEUTERIUM-LABELLED BIS (2-ETHYLHEXYL) PHTHALATE [DEHP] AND DIETHYL PHTHALATE [DEP] AND SUBSEQUENT BIOMONITORING IN HUMAN URINE

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Phthalates are ubiquitous contaminants in indoor environments. They are found in a wide range of applications from where they can easily leach into the environment, causing constant daily exposure. There are large concerns that phthalates might have impact on human health due to disruption of the endocrine system.

The aim of this study was to compare the uptake of the two phthalate esters bis(2-ethylhexyl) phthalate [DEHP] and diethyl phthalate [DEP] into the human body after inhalational as well as dermal exposure. To avoid background contamination the deuterium labelled phthalate analogues were used.

16 participants were exposed to elevated air concentrations of D_4-DEHP or D_4-DEP, either with or without wearing a hood supplying fresh air. The participants spent three hours in an exposure chamber filled with air concentrations of 100 µg/m^3 of phthalate esters, as quantified by online high resolution time of flight aerosol mass spectrometer (HR-ToF-AMS) and gas chromatography tandem mass spectrometry (GC-MS). Urine samples were collected every hour after the exposure for the next 24 hours. Liquid chromatrography tandem mass spectormetry (LC-MS/MS) was used to analyze the urine samples for the metabolites of D_4-DEP and D_4-DEHP, namely D_4 labelled mono-ethyl phthalate (MEP), mono-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (5-OH-MEHP), mono-(2-ethyl-5-oxo-hexyl) phthalate (5-oxo-MEHP), mono-(2-ethyl-5-carboxypentyl) phthalate (5-cx-MEPP) and mono-[2-(carboxymethyl) hexyl] phthalate (2-cx-MMHP).

First analysis results point to a high uptake of both, D_4-DEP and D_4-DEHP, through inhalation, while dermal uptake is low (D_4-DEP) or not detectable (D_4-DEHP). In this human biomonitoring study phthalate metabolites were assessed as biomarkers for inhalational and dermal exposure of the phthalate esters DEP and DEHP. Knowledge on metabolism, uptake and biodistribution of phthalate esters is crucial for estimating our daily exposure as well as for correct risk assessment in humans.

CAN SKELLEFTEÅ MODEL REDUCE FIREFIGHTERS' EXPOSURE TO CHEMICAL AGENTS IN OPERATIVE WORK?

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At the Skellefteå fire brigade was done the study where firefighters' risks to get into the exposure situation or contact with harmful chemicals was mapped by risk evaluation methods during normal operative work. The most critical work tasks was evaluated and firefighters was re-educated to behave more safely way than before. After intervention was noticed that firefighters' risk to get contact with carcinogenic agents, was reduced [1].

The aim of the our study was to evaluate can Skellefteå model also reduce firefighters' exposure levels compared to the conventional model and what were firefighters' exposure increasing factors in operative work.

Firefighters' (n=24) exposure to chemical agents through different exposure routes was measured by hand washing samples, by dermal exposure samples under firefighting garments and their total exposure was measured by biomonitoring of polycyclic aromatic hydrocarbons, benzene, hydrogen cyanide and metals when they followed Skellefteå and conventional models. In addition to that, firefighters' inflammation and stress markers were measured and total exposure increasing factors were recorded by questionnaire.

Firefighters who followed the Skellefteå model had lower total exposure and dermal exposure to the polycyclic aromatic hydrocarbons reflecting also lower risk to be exposed through the gastrointestinal route than firefighters who followed the conventional model. Also firefighters who followed Skellefteå model had lower levels of inflammation markers. The smoke diving, cleaning of fire site and maintenance of equipment were the most important factors, which increased firefighters' total exposure.

Skellefteå model seems to reduce firefighters' exposure, but the model must be updated according to the needs of Finnish rescue departments. For example, for the residential fires, a "protection zone" way of thinking is required. Also maintenance was one of the most important factor affecting firefighters' total exposure and due to that recommendation for the protection level and cleaning technology used for the fire equipment must be linked to the fire class in which the equipment has been contaminated. Firefighters' health condition have to monitor more intensively and their recovery and exposure to carcinogenic agents have to follow more consistently.

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PERFLUORINATED COMPOUNDS BIOMONITORING IN SERUM OF ITALIAN CHILDREN

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Background: Perfluorinated compounds (PFCs) have been widely used in industrial applications and consumer products since the 1950s. Their persistent nature and potential health impacts are of concern. PFCs are currently found at detectable levels in human as well as animal blood worldwide; children often reveal higher serum concentrations than adults.

Aim of the study: The aim of this biomonitoring study is to determine PFC levels in children (6 to 12 years) living in Italy.

Methods: Serum levels of 11 PFCs were measured in 56 children. Medical records, socio-demographic data and diet habits were collected. Quantities of perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), perfluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDeA), perfluorooctane sulfonamide (PFOSA), 2-(N-methyl-perfluorooctane sulfonamide) acetic acid (Me-PFOSA-AcOH), 2-(N-ethyl-perfluorooctane sulfonamide) acetic acid (Et-PFOSA-AcOH) perfluoroundecanoic acid (PFUA) and perfluorododecanoic acid (PFDoA) were measured by HPLC interfaced to a tandem mass spectrometry after an on-line solid-phase sample extraction.

Results: The mean of ∑PFCs serum concentrations was 18.30 ng/mL. PFOS and PFOA were detected in more than 80% of the samples, PFNA and PFHxS in more than 40%, other PFCs in less than 20%. The mean values for PFOS and PFOA concentrations were 5.86 ng/mL and 6.14 ng/mL, respectively. PFCs concentration in serum from children was not significantly different with age and no differences were observed between boys and girls. ∑PFCs serum concentrations showed significant negative correlations with BMI ($r = -0.199$, $p < 0.05$).

Short discussion/conclusions: The children's serum analyzed in this study contained comparatively lower concentrations of PFCs than those found in previous studies on children in other countries including Australia, Germany and the USA. A similar correlation between PFCs and BMI was found in a Korean biomonitoring.
Human biomonitoring is an important tool in environmental medicine to evaluate the level of internal exposure of general population to environmental pollutants.

URINARY BIOMARKERS FOR EXPOSURE TO DIESEL EXHAUST

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Background: Emissions from diesel engines are a significant source of pollution in the urban environment and have been linked to cardiovascular and respiratory health effects. The development of a reliable, specific, exposure biomarker will help to characterise and potentially control exposure to diesel exhaust (DE) in both the occupational and environmental setting.

Aim of the study: To quantify levels of 1-aminopyrene (AP) (a metabolite of 1-nitropyrene) and a suite of metals associated with DE in urine samples obtained from workers at motor vehicle repair garages.

Methods: Employees (n=19) were recruited from two garages. Pre- and post-shift urine samples were obtained for five consecutive days (not all employees provided samples for 5 days). Environmental sampling (elemental (EC) and organic carbon plus nitrogen oxides) was also conducted on day one. AP was quantified using liquid chromatography with fluorescence detection. Elemental analysis used inductively coupled plasma – mass spectrometry.

Results: In total, 154 urine samples were collected. Over half (55%) of samples contained detectable levels of 1-AP (>20 ng/l; 92 pmol/l). Omitting smokers, median (90th percentile; CV(%)) values were 17.4 nmol AP/ mol creatinine (78.2; 136%) and 7.9 (112; 212%) for pre- and post-shift samples respectively (63 pairs). Platinum and cerium levels were marginally above general population levels while other measured elements were within normal population background ranges. Environmental analysis found quantifiable but low levels of EC and nitrogen dioxide.

Short discussion/conclusions: AP results were highly variable, as has been previously reported elsewhere. Initial analysis of pre- and post-shift samples showed no distinct pattern. No correlation with environmental exposure was found. However, AP levels reported here are within previously reported non-occupationally exposed ranges. Further investigation is required to establish whether AP can be considered a reliable biomarker for DE. Further analytical work is planned to investigate other candidate biomarkers for DE.

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HBM4EU –SCIENCE AND POLICY FOR A HEALTHY FUTURE

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HBM4EU initiative is a joint effort of 26 countries, the European Environment Agency and the European Commission, co-funded under Horizon 2020.

The main aim of the initiative is to coordinate and advance human biomonitoring in Europe. HBM4EU will provide better evidence of the actual exposure of citizens to chemicals and the possible health effects to support policy making. Specific key objectives include 1) Harmonizing procedures for human biomonitoring across the 26 participating countries, to provide policy makers with comparable data on human internal exposure to chemicals and mixtures of chemicals at EU level 2) Linking data on internal exposure to chemicals to aggregate external exposure and identifying exposure pathways and upstream sources; 3) Generating scientific evidence on the causal links between human exposure to chemicals and health outcomes; 4) Providing the most relevant tools to detect emerging chemicals and to identify the chemical mixtures of highest concern; 5) Adapting chemical risk assessment methodologies to use human biomonitoring data and account for the contribution of multiple external exposure pathways to the total chemical body burden and 6) Feeding information on exposure pathways into the design of targeted policy measures to reduce exposure.

HBM4EU will run for five years, from 2017 to 2021. In developing priorities for HBM4EU under the first annual work plan, the consortium conducted a prioritisation exercise to identify those substances to be the focus of activities. Prioritized substances include phthalates and their substitutes (e.g. DINCH), bisphenols, per-/polyfluorinated compounds, flame retardants, cadmium and chromium, polycyclic aromatic hydrocarbons and air pollutants, aniline compounds, chemical mixtures (e.g. pesticide mixtures) and emerging chemicals. Additional rounds of prioritisation will be carried out during the project lifespan to ensure that our research responds dynamically to policy needs.

The HBM4EU initiative contributes directly to the improvement of health and well-being for all citizens, by investigating how exposure to chemicals affects the health of different vulnerable groups, such as children, pregnant women and workers.

Data used and produced under HBM4EU will be made accessible via IPCHEM – the Information Platform for Chemical Monitoring. IPCHEM is the European Commission's reference access point for searching, accessing and retrieving chemical occurrence data collected and managed in Europe.

The activities developed to deliver these objectives are organised into work packages clustered under three pillars. These pillars are I) Science to policy, II) European human biomonitoring platform and III) Chemical exposure and health.

The HBM4EU Project is coordinated by the German Environment Agency (UBA). Flemish Institute for Technological Research (VITO) is the co-coordinator of the project.

OCCUPATIONAL EXPOSURE TO MYCOTOXINS A REALITY IN PORTUGUESE BAKERIES?

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Background: Exposure to flour dust in bakeries is largely reported and higher exposure occurs during mixing and kneading, dough-making and bread-forming, as well as cleaning, since these are the dustiest tasks. Flour dust may contain besides the cereals several other non-cereal components, so called dough-improvers, such as a variety of enzymes, chemical ingredients, flavorings, spices, and other additives as well as contaminants such as storage-related mites, microbes or fungi. Fungi are responsible for the production of mycotoxins that are toxic secondary metabolites. Both, fungi and mycotoxins can be present in the flour dust and are likely to be inhaled by workers. In this way, exposure can occur by inhalation when performing tasks that involve exposure to dust.

Aim of the study: Occupational exposure to mycotoxins in Portuguese bakeries was investigated in order to clarify if contaminated dust should be considered as additional risk of mycotoxin exposure by inhalation.

Methods: Twenty four urine samples of workers from several bakeries located in Portugal were analyzed. An improved "dilute and shoot" LC-MS/MS multi-mycotoxin approach was used to monitor urinary excretion of 23 mycotoxins.[1]

Results: Only deoxynivalenol-3-glucuronide (DON-3-GlcA) was found in three urine samples in quantifiable amounts (results in ng/ml: 56,02; 76,2; 105,46).

Discussion/Conclusion: This is a *Fusarium* mycotoxin commonly found in maize and wheat. The few results can be explained by the low fungal contamination found in the bakeries environments and flour dust when comparing with other Portuguese occupational settings already studied. However, this entire scenario can be changed with different climacteric conditions that can influence fungal contamination on cereal crops.

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MONITORING THE EXPOSURE OF THE POPULATION TO METAL-NANOPARTICLES: A NEW ANALYTICAL CHALLENGE

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Background: The European Commission published its recommendation on the common definition of the term "nanomaterial" and several European Regulations (like cosmetic and food) already use this definition for certain types of nanomaterials to ensure safe use. Thus, reliable measurement techniques are needed to assess the levels of nanoparticles in consumer products and in the general and occupationally exposed population.

Aim of the study: To determine particles number, size distribution and chemical composition, different measurement techniques were compared to detect specific metal-nanoparticles (Ag, Au, TiO_2, ZnO) and applied to different consumer products and human matrices.

Methods: Counting methods as Single Particle Inductively Coupled Plasma Mass Spectrometry (SP-ICP-MS) and fractionation methods like Field Flow Fractionation (FFF) coupled to ICP-MS were compared with practical examples on human samples as urine, serum and blood and consumer products like cosmetics and tattoo inks.

Results: The two different analytical methods seems to be appropriate for polydisperse samples and to detect and count particle at the lower size range of the definition (smaller than 10 nm). The methods showed sufficient reliability to assess the exposure of the population to metal-nanoparticles and whether a product contains nanomaterials although it is not labelled as such.

Short discussion/conclusions: The analytical methods can be used to evaluate the fate of metal-nanoparticles in the population and to manage their potential risks.

ANALYSIS OF GENE POLYMORPHISMS IN URINARY CELLS

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Background: In the occupational biomonitoring of exposures to carcinogens, dose and susceptibility biomarker detection is carried out on two biological matrices: urine for metabolite measurement and blood for gene polymorphism analysis. Blood is the preferred matrix in genomic studies although the collection procedure is invasive and requires trained medical personnel undergoing potential infection risk.

Aim of the study: As alternative to blood we propose urine as single biological matrix for simultaneous detection of dose and susceptibility biomarkers.

Methods: The proposed methodological approach allows genomic DNA isolation by protein salting out and alcohol precipitation from urine sediment. It is fast and inexpensive, avoiding the use of toxic reagents comprised in some common DNA extraction kits. Polymorphic gene analysis is carried out by Polymerase Chain Reaction (PCR), with or without the need of Restriction Fragment Length Polymorphism (RFLP), depending on the gene polymorphism type. This method allowed to detect positive and null polymorphic variants of Gluthatione-S-Transferase enzymes, namely GST-T1 and GST-M1 which are involved in the biotransformation of benzene.

Results: Urine of twenty volunteers was used to evaluate the method efficacy and feasibility before extending to large cohorts of workers. Despite low number of subjects, inter-individual and gender variability in DNA recovery, the collection of multiple urine samples per subject at different time-points allowed to increase the DNA yield.

Conclusions: In large epidemiologic and occupational studies, when blood is not available, urine exfoliate is advised as source of genomic DNA for susceptibility biomarkers detection.

SALIVA AS A MATRIX FOR OTOTOXIC SOLVENTS ABSORPTION

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Background: Exposure to styrene mainly occurs in the production of polystyrene. Short-term exposure to styrene in humans results in mucous membrane, eye irritation, gastrointestinal effects. Chronic exposure affects the central nervous system and causes depression, hearing loss, and peripheral neuropathy. IARC classified styrene in Group 2B, possibly carcinogenic to humans. The ototoxic effect is particularly interesting in occupational setting due to the synergistic effect of noise exposure.

Aim of the study: Measurements in workplaces are performed by styrene air monitoring and biological monitoring of mandelic plus phenylglyoxylic acids in the end shift urine of the workers. Saliva is an alternative matrix for measuring solvent absorption: its meaning is comparable to the blood concentration (ACGIH BEI 0.2 mg/l) but sample collection is less invasive. Due to its aqueous nature, saliva is an excellent candidate to the static headspace technique. For these purposes, we developed and validated in field a method allowing measurement of low level of styrene in saliva.

Methods: After collection 1ml of saliva is transferred in 20 mL headspace vial and saturated with of sodium sulphate; samples can be stored at 4°C until use. The method is based on static headspace (SHS) gas chromatography–mass spectrometry(GC–MS). The limit of detection (LOD) and the lower limit of quantification (LLOQ) of the method are 0.19 ng/mL and 0.54 ng/mL respectively. The same method could be used for different organic solvents.

Results: The feasibility study of saliva as a matrix for biological monitoring was tested in workers exposure of fiberglass industry. Results reflected the potential workers exposure: the highest styrene values referred to workers engaged in molding that requires manual preparation of the resin, making easy the styrene vapour dispersion in air. Not detectable values of salivary styrene were determined in workers exposed to very slight styrene sources. The habit of smoking does not seem to affect the final results.

Short discussion/conclusions: Results of biological monitoring in saliva confirmed the existence of a good relationship between the salivary levels of styrene and the degree of exposure. In conclusion, measuring unchanged styrene in saliva matrix seems to be a useful method for biological monitoring, because sampling is not invasive, samples are stable under definite conditions and analysis is fast, easy and not affected from matrix contribution.

BIOLOGICAL MONITORING OF BISPHENOL S IN URINE OF OCCUPATIONALLY NON-EXPOSED GERMAN ADULTS

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Bisphenol S (4,4'-sulfonyldiphenol, BPS) is a chemical compound used as a reagent in polymer production, e.g. polyethersulfones, and as a developer in thermal paper. The aim of the present study was the development of an analytical method for the determination of BPS in human urine. Initially, this method was applied to identify background concentrations in urine of occupationally non-exposed adults.

A method was developed and validated for the determination of total BPS (free and conjugated) in human urine. Sample preparation involves enzymatic hydrolysis and dispersive liquid-liquid microextraction. The analyses are carried out by liquid chromatography with tandem mass spectrometry. In total, 146 urine samples of 146 employees of a chemical company without occupational exposure to BPS (67 % male, 33 % female) in the age range between 21 – 64 years were collected and analyzed. Each employee gave a written informed consent to participate in the study.

The analytical method enables the reliable determination of BPS with a limit of quantification (LOQ) of 0.05 µg/L. The imprecision is 2.1 – 10.0 % within series and 4.6 – 14.5 % between series. The relative recovery ranges between 95 - 106 %. BPS was detected in 86 % of the analyzed samples with a median concentration of 0.10 µg/L (range: <LOQ – 2.75 µg/L; 95th percentile: 0.64 µg/L) and 0.10 µg/g creatinine (range: <LOQ – 7.43 µg/g creatinine; 95th percentile: 0.49 µg/g creatinine), respectively. No significant differences in median concentrations between men and women (each 0.10 µg/g creatinine) or regarding the sampling time (morning: 0.11 µg/g creatinine; afternoon: 0.09 µg/g creatinine) were observed. The present results are in accordance with other investigations of BPS in urine samples from the USA and 7 Asian countries, with detection rates between 42 - 100 % and median concentrations in the range of <LOQ up to 1.04 µg/L (Liao et al. 2012).

In conclusion, an analytical method for the quantification of BPS was developed and applied to urine samples of German adults without occupational exposure to the substance. The sensitivity of the method enables the detection of background BPS concentration in most samples. Additionally, the analytical method can be applied to monitor occupational exposure to BPS.

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GENOTOXICITY ASSESSMENT OF MOBILE PHONE RADIATION IN EXFOLIATED BUCCAL CELLS IN HUMAN SAMPLES

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The International Agency for Research on Cancer (IARC) has classified electromagnetic fields (EMF) as "possibly carcinogenic", and this uncertainty is likely to remain unresolved while a consensus on the subject continues to elude the scientific community. However, with an immense proportion of the world population now carrying their own EMF sources, such as mobile phones, additional insights into this matter are increasingly in demand. Several publications establish associations between EMF exposure and DNA damage, with a consequential increase in risk of occurrence of certain tumours. However, many other studies affirm there is no tangible relation between these two factors.

DNA anomalies can be detected by, amongst other methods, screening of cytomorphological changes, such as micronuclei (MN), which are indicative of genomic instability and are thus presently considered useful biomarkers of genotoxicity, especially in human biomonitoring.

The aim of this study was to investigate the possible genotoxic effects of mobile phone-associated EMF exposure by the measurement of MN in buccal exfoliated cells, which were chosen because they are the direct target cells available by the least invasive sampling procedure.

Cells smears were obtained from the left and right inner cheeks of 86 healthy mobile phone users aged 18-30, who also completed a characterization survey. Cell smears were stained by Feulgen's method and screened for MN by recommended parameters for this assay. MN frequency was tested against potential confounding factors, duration of mobile phone use and preferential side of mobile phone use.

No relationship was observed between MN frequency and duration of mobile phone use in calls. Similarly, cells ipsilateral to mobile phone use presented no statistically significant higher frequency for this biomarker, when compared to cells contralateral to exposure. A highly statistically significant ($p < 0.0001$) increase in MN frequency was found in subjects exposed to known genotoxic agents.

In conclusion, our results suggest mobile phone-associated electromagnetic radiation at the observed exposure levels is unable to induce micronuclei formation in buccal cells.

Keywords: Electromagnetic fields, mobile phones, genotoxicity, micronuclei, exfoliated buccal cells.

DEVELOPMENT OF BIOLOGICAL REFERENCE MATERIAL FOR PROFICIENCY TEST PROGRAM - URINARY TOTAL ARSENIC AND PHENOL

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Background: Reference material for biological monitoring in industrial health field is essential element that confirms accuracy of analysis for assessing level of workers' exposure to hazardous compound. Thus, it is important to develop and verify new reference materials in biological monitoring continuously.

Aim of the study: This study is performed to develop new urine reference materials to enhance analytical ability of laboratories in industrial health field in Korea. We aim to apply these materials for proficiency test on analysis of biological markers of exposure to arsenic and phenol.

Methods: Urine samples of 3 concentration levels including criteria of exposure of arsenic and phenol were prepared. In case of total arsenic, arsenic metal ion, monomethylarsonic acid, dimethylarsinic acid and arsenobetaine were added to make similar condition of real urine sample. Likewise, phenol was added to prepare reference sample. Stability test for 30 days at 4 different temperatures and homogeneity test were performed for these samples. Analytical methods used in this study were graphite-AAS for total arsenic and gas chromatography/mass selective detection for phenol. These reference samples were provided to laboratories in industrial health field in Korea for proficiency test of biological monitoring.

Results: As results of analysis in our laboratories, prepared samples showed good recovery of total arsenic and phenol after 30 days at room temperature. All samples were homogeneous at confidence level of 95%, so they were verified as adequate reference materials for quality assurance program. In proficiency test of biological monitoring, 2 laboratories were participated in proficiency test for total arsenic in 2016 and 1 laboratory was participated in proficiency test for phenol in 2017. These laboratories were all certificated as adequate for analysis of total arsenic and phenol in urine.

Conclusions: The stability and homogeneity of total arsenic and phenol in urine reference materials were confirmed. These samples were utilized as reference samples for Korean quality assurance program for proficiency test of analytical ability of laboratories working in industrial health field. From the results of proficiency test, they were also confirmed practically as adequate reference materials for biological monitoring in industrial health field.

DETERMINATION OF N-(2-HYDROXYETHYL)VALINE IN GLOBIN OF ETHYLENE OXIDE-EXPOSED WORKERS USING TOTAL ACID HYDROLYSIS AND HPLC/MS/MS

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Background: Ethylene oxide (EO), an important industrial chemical and a gaseous sterilant for medical devices, is a suspected human carcinogen known to bind to DNA and proteins. Since 1980´s, the dominant analytical strategy used in biomonitoring of exposure to EO consists in the conversion ("modified Edman degradation") of 2-hydroxyethyl adduct at the N-terminal valine (HEV) in globin to a specific pentafluorophenylthiohydantoin accessible to GC/MS analysis. Though highly sensitive, the method is laborious and, at least in our hands, not sufficiently robust.

Aim of the study: To evaluate an alternative strategy of HEV determination based on direct analysis of acid-hydrolysed globin.

Methods: Globin isolated from human blood and spiked with HEV-d_4 as an internal standard was subject to total acidic hydrolysis (12 N HCl, 100 °C, 16 h). The acid was evaporated and the reconstituted residue analyzed by HPLC/PESI-HRMS/MS to monitor product ions at *m/z* 116.1070 and 120.1321 from HEV and HEV-d_4, respectively.

Results: For 5 mg globin samples, calibration was linear up to 25 ng HEV/sample ~ 30 nmol/g globin, and a limit of quantitation was ca. 0.02 ng HEV/sample ~ 25 pmol/g globin. Three batches of globin samples from EO-exposed workers (n=50, HEV 0.2-27.2 nmol/g globin), each analyzed at least twice in 2011-2016 by modified Edman degradation, were reanalysed at least twice by a new method. Results of both methods correlated well with each other (R^2>0.95). However, reproducibility of the new method was much better than that of modified Edman degradation, which occasionally provided markedly deviated results. Accuracy of the new method was confirmed by successful passing through German interlaboratory comparison programme G-EQUAS.

Conclusions: The new method for HEV determination in globin proved to be superior to modified Edman degradation being similarly sensitive, much less laborious and, in our hands, providing significantly more accurate and reproducible results.

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METABOLOMIC FINDINGS IN YOUNG OBESE MEN AND CONTROLS

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Background: Obesity, defined as body mass index (BMI) ≥ 30kg /m^2, is one of the common diseases related to diet, lifestyle and comorbidities. It is an important risk factor of increased morbidity and mortality.

Aim of the study: The aim of the study was to assess early metabolic changes in obese men without any concomitant diseases.

Methods: A comprehensive metabolic profiling for 99 serum spectra of young (range 18-35 years old) healthy men based on the ^{1}H NMR spectroscopy in combination with principal component and partial least square discriminant analysis was performed. In this study we compared metabolic profiles of the obese (BMI≥30) male group (n = 35) with a normal body weight group (n = 64, BMI - 18.5-25) .

Results: Our analysis revealed metabolic differences between obese vs normal weight men for tyrosine, glutamine, adenosine, leucine, creatine, arginine and isoleucine. These 6 metabolites were significantly altered in obese individuals compared to normal weight men. In the obesity group we observed significantly depleted level of metabolites involved in protein synthesis (glutamine, arginine), oxydation of glucose (arginine) and skeletal muscle atrophy (creatine) contrary to control group. In obese patients, in contrast to control group, an increased level of metabolites involved in modulation of insulin signaling (leucine, isoleucine), energy transport and self-regulation of circulation (adenosine) and protein amino acid metabolism (tyrosine, leucine) was observed.

Conclusions: High blood levels of branched chain amino acids (leucine and isoleucine) have recently been postulated as biomarkers of the early phase of insulin resistance. Taken together, our study revealed that metabolic changes in healthy young adult men might be associated with insulin associated pathways.

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NEW PERSPECTIVES ON THE PROPERTIES OF THE PROTEIN CORONA FROM SEQUENTIAL ELUTION USING DETERGENTS

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Background: After entering a biological environment, nanoparticles (NPs) are rapidly covered with biomolecules forming a corona. This nanoparticle corona is the major contact with the cell, triggering uptake of the NPs and the cellular response to them. Therefore, it is of interest to elucidate the structure and composition of the NP corona. The corona itself is discussed to be multilayered and it is hypothesized that on top of the tightly and nearly irreversibly bound biomolecules ("hard" corona) an outer layer of loosely associated biomolecules ("soft" corona) exist.

Aim of study: The standard method for corona analysis involves harsh detergent and heat treatment, which often results in loss of NP-protein interaction information. In an effort to overcome this problem, we optimized a method for sequential retraction of proteins from the corona by utilizing detergents of different strength. A thorough understanding of the protein corona, formed around nanoparticles, is crucial for the identification of potential biomarkers.

Method: The protein corona was formed by incubation of zinc oxide or gold NPs in RPMI culture medium containing 10 % fetal calf serum. After removal of loosely bound proteins by washing, the particles were titrated with various concentration of detergents (Urea, CHAPS, Triton X-100 and SDS) to elute proteins. The eluted proteins were separated by 1d-gel electrophoresis, identified by mass spectrometry and further analyzed by gas chromatography-mass spectrometry (GC-MS).

Results: The obtained 1d-gels showed unique pattern of eluted proteins depending on the utilized detergents, and type of particle material. Further analysis using GC-MS of early and late eluting serum albumin showed strong variations in the amount of carbohydrates, fatty acids, and lipids.

Discussion and Conclusion: The method enabled us to distinguish the protein corona into two forms, a peelable and a non-peelable one. The GC-MS analysis highlighted changes of functional residues in sequentially eluted proteins, which changed from hydrophobic to lipophilic with increasing detergent concentration. The current method for the first time enabled the analysis of the protein corona according to physicochemical and biochemical aspects, providing the information which is lost while its analysis via standard method.

A METHOD TO ASSESS MERCAPTURIC ACIDS IN URINE AS BIOMARKERS OF EXPOSURE TO ELECTROPHILIC CHEMICALS IN TOBACCO SMOKE

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Background: Mercapturic acids (MAs) are metabolic end-products formed by the conjugation of glutathione with electrophilic compounds. MAs are, therefore, suitable biomarkers of exposure to toxicants, such as the over 3,000 chemicals present in cigarette smoke.

Aim of the study: Aim of this work was to develop a liquid chromatography tandem mass spectrometry (LC-MS/MS) methods which allow the rapid and sensitive determination of 8 MAs derived from acrolein, benzene, 1,3-butadiene, 4-nitrochlorobenzene, propylene oxide, toluene, and ethylating agents.

Methods: Urinary samples were obtained from 10 subjects, 5 non-smokers and 5 smokers. Samples were heated at 50°C for 10 minutes to dissolve sediments. Subsequently samples were vortexed and an aliquot of 0.5 mL of urine was diluted with 0.5 mL of water, added with a solution containing deuterated MAs analogs as internal standards, and filtered. 2 µL of sample were directly injected in LC-MS/MS equipped with a UPLC column, and the analytes were eluted with a gradient of methanol and 5 mM aqueous solution of ammonium formate. Signals of MAs and their internal standards were acquired in negative ionization mode using scheduled MRM method, to maximize sensitivity. To assess active and passive tobacco smoke exposure, the presence of cotinine and nicotine was quantified by LC-MS/MS.

Results: The developed method is accurate and sensitive, and it has proved to be simple and quick, with a minimum sample preparation. The median concentrations ranged from 1.1 µg/L to 243.7 µg/L for the metabolite of ethylating agents and acrolein, respectively. Cotinine show a positive correlation with almost all analytes (seven out of eight MAs). Significant increase of MAs was observed in the urine of smokers compared to those of non-smokers subject, and a dose dependence was obtained when subjects were categorized in non-smokers, mild smokers, and heavy smokers.

Conclusions: In conclusion, the developed method seems to represent a powerful tool for the fast quantification of 8 MAs. A first application suggests several suitable biomarkers for seven toxicants present in tobacco smoke.

DETERMINATION OF N-HEPTANE METABOLITES IN URINE BY HEADSPACE-SOLID PHASE DYNAMIC EXTRACTION-GAS CHROMATOGRAPHY/MASS SPECTROMETRY (HS-SPDE-GC/MS)

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Background: n-Heptane is an ingredient of many industrial products such as coatings, glues or cleaning and degreasing agents. Inhalation exposure of workers may occur under unfavorable working conditions due to the high vapor pressure of the compound. Oxidative urinary metabolites such as heptanols, heptanones or 2,5-heptandione have been suggested as markers for biomonitoring of n-heptane exposure in the past [Csanády 2012].

Aim of the study: To develop an analytical method for simultaneous determination of eight potential n-heptane metabolites, namely heptane-1-ol, -2-ol, 3-ol, 4-ol, -2-one, 3-one, 4-one and -2,5-dione in urine. The method was validated to prove the its suitability for analysis of samples collected from volunteers after experimental inhalation exposure to n-heptane.

Methods: 2 ml aliquots of urine were spiked with isotopically labelled internal standards in 20-ml-headspace vials and after buffering with acetate enzymatically hydrolyzed. Then, sodium sulfate was added to promote the partition of analytes into the headspace. Automated extraction of 2 ml-aliquots of the vapor phase of the pre-treated samples was done with the help of a syringe. During the repeated extractions, enrichment of the analytes in a sorbent, applied as a coating on the inner wall of the syringe cannula, took place (SPDE). After desorption in the hot injection port of the GC-system, the analytes were chromatographically separated and detected using single ion monitoring after positive chemical ionization with methane as a reagent gas.

Results: Method validation resulted in limits of detection between 0.4 (-2-on) and 1.4 µg/l (-1-ol) dependent on the particular analyte. A wide linear calibration range up to heptanol concentrations of 12 mg/l in spiked pool urine was found. Intra and inter assay coefficients of variation ranged from 1.8 to 14.5% and relative recovery from 79 to 102 % for all analytes except 4-one (concentrations of 12 and 200 µg/l, n= 8). Fluctuating amounts of 4-one in blank urine samples impaired the analytical reliability of this parameter.

Short discussion/conclusions: The method proved suitable for the quantification of seven urinary heptane metabolites after exposure to n-heptane. Besides the expected main metabolites such as -2-ol, a simultaneous determination of the minor, but in terms of neurotoxicity relevant metabolite -2,5-dione is possible with a minimum of efforts concerning sample preparation.

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USE OF BUCCAL MICRONUCLEUS CYTOME ASSAY TO EVALUATE GENOTOXIC AND CYTOTOXIC EFFECTS OF ANTINEOPLASTIC DRUGS IN WORKERS OF DIFFERENT ITALIAN HOSPITALS

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Background: Nurses and pharmacy technicians who work in oncology units are at risk of exposure to antineoplastic drugs, a heterogeneous group of chemicals that inhibit tumor growth by disrupting cell division and killing growing cells.

Aim of the study: To evaluate, by using buccal micronucleus cytome (BMCyt) assay, genotoxic and cytotoxic risk in workers who handle antineoplastic drugs in oncology units of three Italian hospitals.

Methods: In the three hospitals (A,B,C) we studied 17 pharmacy technicians/nurses who prepare antineoplastics, 25 nurses who administer them and 53 controls. Workplace monitoring of 5-fluorouracil (5FU) and gemcitabine (GEM) was performed by HPLC-UV on wipes and swabs collected in areas of pharmacy and administering wards (350 samples). Personal exposure to 5FU and GEM was monitored by pads placed on the protective clothes. Absorption of 5FU was assessed by measuring its urinary metabolite α-fluoro-β-alanine (AFBA) by LC-MS-MS. Total amount of handled drugs was also calculated. BMCyt assay was used to measure biomarkers of DNA damage (micronuclei MN and nuclear buds NB), cytokinetic defects (binucleated cells BN) and cell death (condensed chromatin CC, karyorrhexis, picnotic and karyolitic cells).

Results: Drug contamination was found only in the 30% of wipe and swab samples, with GEM more frequently present than 5FU. In the different areas contamination ranged from 0.02-1443 µg/m2 for GEM and from 0.02-200 µg/m2 for 5FU, with no statistically significant difference among the hospitals. Only GEM deposition (0.18-15.21 µg) was found on workers' pads (93% of samples). No AFBA was found in urine samples. Total amount of handled drugs was higher in hospital B. In A, we found the highest frequencies of MN (4.3‰) and CC (6.7‰) in nurses administering drugs and the highest frequency of NB (4.0‰) in pharmacy technicians. In B, pharmacy nurses showed higher frequencies of MN and NB (2.0 and 1.8‰) vs controls (1.1 and 0.3‰). In C, nurses administrating drugs showed higher frequencies of MN and CC (3.5 and 2.7‰) vs controls (1.1 and 1.0‰).

Short discussion/conclusions: In pharmacy nurses of hospital B, where the highest quantity of drugs is prepared, we observed a higher frequency of DNA damage; in the administrators of A and C we found a higher frequency of genotoxicity and cytotoxicity biomarkers. This demonstrates the suitability of BMCyt assay as sensitive and no invasive biomarker of early effect for occupational antineoplastic exposure.

BUTYRYLCHOLINESTERASE ACTIVITY AND LIPIDS PARAMETERS IN WORKERS OCCUPATIONALLY EXPOSED TO PESTICIDES

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Background: Pesticides are compounds used to prevent, destroy, or control pests and vectors. Particularly, Organo Phosphate (OP) pesticide exposure has been associated with inhibition of the cholinesterase enzymatic activity, such as Butyrylcholinesterase (BuChE). On the other hand, changes in BuChE activity have been associated with obesity, diabetes, uremia, hyperthyroidism, and metabolic syndrome. However, few studies have evaluated the effects of pesticides on BuChE and lipid parameters.

Aim: To evaluate lipid parameters in urban sprayers and their association with BuChE activity.

Methods: A cross-sectional, descriptive, and analytical study was conducted in workers exposed to pesticides. BuChE activity was determined spectrophotometrically in serum and lipid parameters were determined at a certificated laboratory. Information regarding anthropometric characteristics, lifestyle, symptoms related to pesticide exposure, frequency of exposure, and others was obtained from a structured questionnaire.

Results: The mean value of BuChE activity was 5,550.8 U/L (5,334.3–5,767.3). Lipid parameters by level of pesticide exposure and Body Mass Index (BMI) showed differences in cholesterol, Low-Density Lipoproteins (LDL), and atherogenic index in the group with overweight. Furthermore, positive correlations between BuChE activity and lipid parameters were observed, these effects dependent on the BMI of the individuals as follows: triglycerides, Very-Low-Density Lipoproteins (VLDL), and total lipids in the group with normal weight; in the group with overweight with cholesterol, atherogenic index, triglycerides, LDL, VLDL, and total lipids.

Discussion/conclusions: The results suggested an association between serum BuChE activity and lipid parameters. Some authors have proposed that BuChE could interact with lipoproteins thorough its polar phosphorylcholine group and that BuChE might play a role in lipid metabolism directly or through synergistic action with cholesterol esterase. Thus, exposure to OP might disrupt lipid metabolism, causing lipid parameter alteration.

CHOLINESTERASE ACTIVITY IN INDIGENOUS MEXICAN FARMWORKERS EXPOSED TO PESTICIDES

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Background: Mexico has one of the largest populations of agricultural workers in the world, among which are indigenous groups**.** Each year, 40% of indigenous Huicholes leave their communities to work in agricultural fields of Nayarit state. According to the government, pest control of these agricultural fields is accomplished with mainly Organophosphorus (OP) pesticides. Work activity, living conditions, and cultural and social factors ensure that these populations are vulnerable and at risk for the effects of pesticides.

Aim of the study: To evaluate Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE) activities as well as the general health status in indigenous Mexican farmworkers exposed to pesticides.

Methods: A transversal study was carried out in Huichol farmworkers from three communities in Nayarit, Mexico. Information on anthropometric and socioeconomic characteristics, exposure to pesticides, use of personal protective equipment, and symptoms related to pesticide exposure were obtained from a structured questionnaire. Blood samples were obtained to evaluate clinical analysis and toxicological biomarkers, such as evaluation of AChE and BuChE.

Results: The study population includes females (57%) and males (43%) with an average age of 40 years. The participants worked 6 hours daily on average, 6 days a week; 76% had headache, 73% excessive thirsty, and 69% dizziness during the working day, and 81% of these farmworkers have used some type of pesticides, the most used being phenoxyacetic acid, bipyridyls, and OP. The geometric mean of AChE activity was 25 U/g Hb in males and 26 U/g Hb in females. Regarding BuChE, the geometric mean was 4,151 U/L in males and 4,043 U/L in females with normal values. There were no differences in AChE and BuChE activities by gender (p >0.05). Average erythrocyte content in males was 4.7 (4.52–5.9) mL/mm^3 and in females, 4.2 (4.1–5.3) mL/mm^3. Likewise, average hemoglobin, leukocyte, and platelet values were found in the normal range for Mexican population.

Short discussion/conclusions: The enzymatic activity observed in this study was similar to that reported in a pilot study conducted by the working group, who found BuChE activity to be lower in Huichol farmworkers. The preliminary results suggest that serum BuChE activity could be more sensitive and could comprise a more adequate indicator of mixed pesticides exposures than AChE.

BIOTOXICOLOGICAL ASSESSMENT OF OCCUPATIONAL EXPOSURE TO ORGANOPHOSPHORUS PESTICIDES AMONG EMPLOYEES OF A NATIONAL PESTICIDE PRODUCTION COMPANY BY THE DETERMINATION OF PLASMA CHOLINESTERASE ACTIVITY

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Background: The question of the health effects of pesticides is an important issue for all professionals involved in handling throughout their careers many substances, some of which are known to be very toxic, such as organophosphates (OPs). The biomonitoring of occupational exposures to OPs is based on exposure biomarkers (dosage of the product itself or its metabolites) and/or markers indicating a toxic effect (cholinesterase assay).

Aim of the study: The objective of the study is to assess exposure to organophosphorus pesticides in occupational settings.

Methods: This is a cross-sectional descriptive study carried out among the employees of an Algerian company producing phytosanitary products. Data on exposure to pesticides were collected through an information sheet. Assays of plasma cholinesterase activity (BChE) were carried out by spectrophotometric method which is based on the Ellman method.

Results: In total, the study was performed in 44 employees with a sex ratio of 10 and an average age of 37 years. These workers are divided among operators, maintenance agents, handlers, administrative staff, etc. Dichlorvos is the only OP pesticide found in the list of raw materials used. All the BChEs belong to the usual values with an average of 7833.14±1293.96 IU/L. Some employees complained about symptoms at the time of exposure (headache, hypersecretions, allergies, breathing difficulties, dizziness, etc.)

Discussion: BChE assays are very disparate and have not revealed pathological levels. This could be explained by the discontinuity of workers' exposure and/or the speed of BChE regeneration. The presence of evocative exposure symptoms could be explained by the neglect of wearing personal protective equipment.

Conclusion: The large inter-individual variability of BChE levels imposes the provision of a so-called pre-exposure baseline. It would be complementary to determine the activity of the globular cholinesterases (good index of the cholinesterase activity of the other tissues in particular nervous) or even urinary metabolites, dialkylphosphates (more sensitive biomarker).

A BIOMONITORING, DERMAL AND INADVERTENT INGESTION SAMPLING STUDY OF SMALL QUANTITY PESTICIDE USERS IN THE HORTICULTURAL AND AMENITY GARDENING SECTOR

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Background: A pilot occupational biomonitoring study to assess pesticide exposure among amenity horticulturists to glyphosate and fluroxypyr was completed during the gardening season of 2015. A spot urine sampling strategy was followed, which involved collecting urine samples before the work task involving pesticides and within one hour of completing the work task. Study results of post tasks samples had a geometric means (geometric standard deviation) of 0.66 (1.11) $\mu g\ L^{-1}$ for glyphosate and 0.29 (1.69) $\mu g\ L^{-1}$ for fluroxypyr, which showed an exposure potential among amenity horticultural workers and warranted further investigation.

Aim of the study: To conduct a more comprehensive occupational biomonitoring study, using 24 hour urine sampling to evaluate total exposures to glyphosate and fluroxypyr among amenity horticulturists. To investigate the contribution of dermal and inadvertent ingestion to total exposure, a dermal and an inadvertent ingestion assessment study will be completed concurrent with the biomonitoring study.

Methods: Workers will be grouped into four similar exposure groups based on the pesticide application method and active ingredient used, glyphosate or fluroxypyr. Twenty four hour biomonitoring samples will be collected pre-exposure (before the work task begins), post-exposure sample (within one hour of the task completion) and a following morning void. Gloves and dermal wipes of the hands and perioral region will be collected before and after the work task. Detailed contextual information regarding the worker, task and the environmental conditions will be collected by the researcher to support all samples collected.

Results: Data collection and analysis will be completed by August 2017. Urinary and wipes sample pesticide concentrations will be analysed using descriptive statistics and mixed effects modelling analysis to evaluate total exposure, the contribution of dermal and inadvertent ingestion exposure routes, and different work factors to exposure.

Short discussion/conclusions: This study will provide a comprehensive biomonitoring dataset describing amenity and horticultural worker exposure to glyphosate and fluroxypyr and the contribution of dermal and inadvertent ingestion routes total body burden of pesticides.

EVALUATION OF HEALTH STATUS AND RISK PERCEPTION BY THE USE AND HANDLING OF PESTICIDES IN URBAN SPRAYERS

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Background: Pesticides are compounds widely used for pest control. Urban sprayers are one of the populations with higher risk of exposure to these xenobiotics. There are studies in the literature that have evaluated the health outcomes caused by occupational exposure to pesticides, including hematological, biochemical, and hepatic parameters.

Aim of the study: To evaluate clinical, hematological and biochemical parameters and risk perception by the use and handling of pesticides in urban sprayers.

Methods: A transversal, descriptive, and analytical study was conducted on 208 pesticide sprayers. Information related with pesticide exposure and risk perception was evaluated through a structured questionnaire. Clinical parameters were determined in a certified clinical analysis laboratory. Biochemical parameters were conducted by spectrophotometric methods.

Results: A total 63.2% of the study population mixes and applies pesticides, and more than 85% does not use proper personal protective equipment. Organophosphorus, followed by pyrethroids and carbamates were the most used pesticides. A total of 11.05% of sprayers had been poisoned and 33.3% does not consider pesticide-spraying activity as a hazardous occupation. Alterations were observed in some clinical and hematological parameters, such as glucose, lipid profile, transaminases, and white blood cell count. The results showed a geometric mean of paraoxonase activity (PONase) of 233.63 U/L, arylesterase of 96.72 U/mL, CMPAase of 19.62 U/mL, AChE of 19.65 U/gr Hb and butirylcholinesterase (BuChE) of 5283.1 U/L. BuChE and arylesterase activities were related with self-reported pesticide exposure grade but not AChE activity.

Short discussion/conclusions: Occupational exposure to pesticides might cause biochemical parameters alteration and more training conducted to improve the risk perception is needed in the study population.

IMPROVING EXPOSURE ASSESSMENT METHODOLOGIES FOR EPIDEMIOLOGICAL STUDIES ON PLANT PROTECTION PRODUCTS

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Background: The presentation describes a comprehensive study to better understand the reliability of assessment of human exposure to plant protection products (PPP) in previous occupational epidemiological investigations, and to use this information to recommend improvements in scientific practice for future studies. Further, it aims to assess the reliability and external validity of surrogate measures used in these studies to assign exposure to individuals or groups of individuals on the basis of self-reported information, job/crop exposure matrices and exposure algorithms. The project will also evaluate the size and the effects of recall bias on the misclassification of exposure to PPPs and associated health effects.

Methods: Previously collected biological monitoring exposure data from several existing epidemiological studies and historical records, along with new studies in various working populations in Europe, Africa and Asia will be used to examine the performance of exposure assessment approaches. Urinary metabolites of PPPs will be selected based on the extent of use within the study populations, validity of biomonitoring methods and knowledge of toxicokinetic parameters. Initial information suggests that active ingredients such as glyphosate, chlormequat, chlorpyrifos and cyhalothrin are regularly used within the cohorts; these are all amenable to specific urinary biomonitoring. Within existing epidemiological studies, the performance of various (combinations of) exposure assessment methods will be compared and contrasted to show the effect of improved exposure assessment in studies on health effects of PPP.

Results: The main outcome will be the validation of widely accepted and easily adaptable semi-quantitative individual-based exposure assessment methods against measured levels of urinary PPP metabolites in a broad range of settings. Additionally, the study will compare the reliability and performance of several grouped - and individual-based exposure assessment methods in the frame of existing epidemiological studies.

Short discussion/conclusions: The project is due to commence shortly. We propose to present the study protocols and rationale.

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BANANA PLANTATION WORKERS: OCCUPATIONAL EXPOSURE TO PESTICIDES AND HEALTH EFFECTS IN ECUADOR

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Background: The working conditions and the health of farm workers in the countries of the Global South have become more of an issue during the last years. Farm workers often complain about health issues caused by pesticide exposure (e.g., [1]). Intensive agrochemical application in banana production has been documented in Ecuador, world's largest exporter of bananas.

Aim of the study: This study assessed working conditions, wellbeing and health of male farmworkers in conventional farming using biocides and in organic farming.

Methods: In a cross-sectional epidemiological study exposed and non-exposed male farmworkers were interviewed based on standardized questionnaires about, inter alia, exposure history, pesticide application practices, health and wellbeing. Furthermore, swab samples of buccal cells were taken (Buccal Micronucleus Cytome Assay, BMCA), fixed, stained and later in the laboratory blindly evaluated for nuclear anomalies indicative of cytotoxic and genotoxic effects, according to standard protocols (2).

Results: In total, 68 farmworker participated (provinces Los Rios, El Oro). 87% resp. 78% of the pesticide exposed respondents did not use masks/gloves at all; 10% resp. 19% used masks/gloves all the time. Pesticide workers (n=31) showed significantly more often symptoms such as dizziness (OR=4.80), nausea/vomiting (OR=7.50), diarrhoea (OR=6.43), burning eyes (OR=4.10), skin irritation (OR=3.58). Furthermore, eight out of nine biomarkers of the BMCA were significantly more frequent among exposed workers ($p<0.001$) (micronucleated cells: OR=2.55; total micronuclei: OR=2.45; nuclear buds: OR=1.84; binucleated cells: OR=1.33; condensed chromatin: OR=1.38; karyorrhectic cells: OR=1.30; karyolytic cells: OR=1.19; broken eggs: OR=1.20).

Short discussion/conclusions: BMCA is a sensitive, standardized tool for bioeffect monitoring that proved feasible also for difficult field studies. Our findings indicate that the impact of pesticide use is not restricted to acute effects on health and wellbeing, but also point to long-term health risks. BMCA results suggest that pesticide users have a higher risk of developing cancer. There is an urgent need for safety training and minimizing application of pesticides.

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STRESS PROTEIN AND HISTOPATHOLOGY EVALUATION OF TWO METALLIC-INSECTICIDES IN THE MIDGUT OF THE MILLIPEDE *RHINOCRICUS PADBERGI*

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Background: In order to protect soil fauna, many countries have banned the marketing of some pesticides, as sulfluramid, used to combat leaf-cutting ants. As an effective and safe alternative to the use of sulfluramid, researchers have developed metallic-insecticides complexes, which are formed by a flavonoid (hesperetin) bonded with metals.

Aim of the study: In this sense, this study used the millipede *Rhinocricus padbergi*, a bioindicator organism of the edaphic fauna, to evaluate morphophysiological and heat shock protein (HSP70) alterations caused by two metallic-insecticides, [Mg(5-methyl-phen)(hesperitin)$(H_2O)_2$](CH_3COO) and [Mg(5-Cl-phen)(hesperitin)$(H_2O)_2$](CH_3COO).

Methods: Ten specimens were exposed in terrariums containing different concentrations of the complexes (0.5 mg/mL, 1.0 mg/mL and 2.0 mg/mL). After periods of 21 and 90 days, three individuals from each terrarium were dissected in order to remove the midgut, the organ where absorption of toxic substances occurs. Fragments of the midgut were submitted to histological and immunohistochemical routine. The slides obtained were stained with hematoxylin-eosin and hystochemical techniques were used to detect calcium, lipids, proteins and polysaccharides; immunolabeling was performed with primary and secondary antibody conjugated to alkaline phosphatase to detect HSP70 stress protein.

Results: The results showed that the complexes may increase HSP70 labeling, although not at all concentrations and periods of exposure. Histopathological and hystochemical alterations were not significant at any concentration.

Short discussion/conclusions: The metallic-insecticides were capable of inducing stress to midgut cells; however this stress could be repaired by the cytoptotective action of HSP70. Major damage to the organism, such as morphological alterations, was prevented by amplified expression of this stress protein. Thus, metallic-insecticides showed no significant toxicity to millipedes. As severe morphological alterations were not observed, these metallic-insecticides should not present population level effects.

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PESTICIDE RESIDUE ANALYSIS IN HAIR AND NAILS

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Background: Humans are exposed to pesticides through multiple routes, i.e. food, use of home and garden pesticides/biocides, and the environment (air, dust, especially in rural areas due to nearby agricultural activities). Human biomonitoring allows to assess the overall exposure. Urine is the most frequently used matrix but most modern pesticides are rapidly metabolised and excreted, which results in two limitations: *i)* urine provides only information on exposure in the preceding day(s); *ii)* human biomarkers are not always known and/or analytical reference standards are not readily available. For many pesticides, the parent compound is incorporated in hair and nails. Determination of the pesticides in these keratinous matrices can provide information on long term exposure.

Aim of the study: a) to develop and validate a multi-residue method for pesticides in human hair and nails at pg/mg level, and b) to conduct a brief survey to gain insight in the presence of pesticides in hair and nails.

Methods: A multi-residue method with emphasis on 20 frequently used pesticides was developed based LC-MS/MS. The final method involved washing/drying (water/dichloromethane) followed by pulverization. The sample was extracted with acetonitrile and cleaned using cartridge filters. The method was validated at the 1 and 5 pg/mg level following guideline SANTE/11945/2015. Hair and nail samples were collected from approx. 25 subjects and analyzed. For several subjects both hair and nail samples were available, in some cases both finger and toe nails.

Results: Several extraction methods were compared. Using the final method, adequate recoveries (70-120%) and repeatabilities (<20%) were obtained for most pesticides down to the 1 pg/mg level, and for all at the 5 pg/mg level. Such levels were detected in hair and to a lesser extend in nails. The most frequently detected pesticides included azoxystrobin, boscalid, imazalil, imidacloprid, and thiabendazole.

Short discussion/conclusions: Most of the pesticides detected in hair and/or nails belonged to the group of pesticides that are found most frequently in fruit and vegetables, which might indeed indicate a relationship between (dietary) exposure and residues in the keratinous matrices. However, at this stage this relationship is rather qualitative and more research on factors affecting incorporation and toxicokinetics is needed.

RELATIONSHIP BETWEEN MICRONUCLEI FREQUENCY AND ANTIOXIDANT ENZYME ACTIVITIES IN WORKERS OCCUPATIONALLY EXPOSED TO PESTICIDES

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Background: Oxidative stress (OS) cause DNA damage and genome instability, and eventually chromosomal rearrangements and nuclear anomalies such as micronuclei (MN). Antioxidant systems involved in protection against intracellular OS include among others superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR). Exposure to pesticides can induce OS by increased production of free radicals that can accumulate in the cell and damage biological macromolecules or alter antioxidant defense mechanisms, including detoxification and scavenger enzymes.

Aim of the study: The aim of this study was to evaluate the antioxidant enzyme activities and their relationship with the MN frequency in workers occupationally exposed to pesticides.

Methods: A cross-sectional, descriptive, and analytical study was conducted in 139 males and 69 females dedicated to the spraying of houses, schools, and other areas. The cytokinesis-block micronucleus assay (CBMN) was conducted in cultures from whole blood samples and the activities of SOD, CAT, GPx and GR were determined by spectrophotometry.

Results: The geometric mean for the antioxidant enzymes were: 94.78 U/mL for SOD, 69.77 U/g Hb for CAT, 198.68 U/mL for GPx and 38.96 U/g Hb for GR activity. Results showed statistically significant differences according to gender in MN frequency, males had lower MN than females and higher Nuclear Index. In addition, age displayed an influence on the MN frequency, observing an increase after the 35 years old. MN frequency was not associated with occupational pesticide exposure. Our data showed a negative correlation between the MN frequency and the GPx activity but a positive correlation between MN frequency and GR activity.

Short discussion/conclusions: The results obtained in this study suggest an important role of GPx enzyme in the MN frequency.

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