

SECOND DRAFT

Proposal for pollutants /biomarkers in regard to EU Human Biomonitoring Project

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Table of content	page
1. Introduction	2
2. Objectives	2
3. Pollutants / Biomarker	3
3.1. Biomarker of Scenario 1	3
3.1.1. Lead in Blood	4
3.1.2. Cadmium in Urine	5
3.1.3. Mercury in Hair	7
3.1.4. Cotinine in Urine	9
3.2. Biomarker of Scenario 2	10
3.2.1. Metabolites of Phthalates in Urine	10
3.2.2. Metabolites of Polycyclic Aromatic Hydrocarbons (PAHs) in Urine	12
3.2.3. Metabolites of Pyrethroids in Urine	15
3.2.4. Metabolites of Organophosphate-Insecticides in Urine	17
3.2.5. Perfluorinated Chemicals in Blood	19
3.2.6. Polybrominated Flame Retardants in Blood	21
4. Summary and Recommendation	24
5. References	25

1. Introduction

The first draft of this paper served as a contribution for ESBIO in general, and was initially presented at the workshop of WP 6 in Lodz, Poland on 30-31 March 2006.

Since it is also subject of the deliverable D2.2 ("Proposal for pollutants and biomarkers including justification of recommendations to be discussed with Member States based on the results elaborated under the BiPro Project") of WP 2, this second draft is meant to reflect the step-wise progress on the elaboration of suggested biomarkers for the EU-HBM Pilot Project as incorporated into the "3rd Recommendations on HBM" and presented at the meeting of the "Consultative Forum" in Luxemburg on 30 November 2006.

The first and second draft "Utility and sensitivity of biomarkers" compiled by ESBIO WP 6 and received on 2 March and 29 August 2006, respectively is hereby acknowledged. In linkage to this WP 6 deliverable, this draft intends to provide additional information in regard to the proposed basic biomarkers of "Scenario 1" and furthermore specific information on the currently discussed chemicals of "Scenario 2" for the first time. It aims to reflect both the German expertise gathered in 20 years of German Environmental Surveys (GerES), particularly the recent Survey on children (GerES IV, 2003-2006), and the embedded scientific activities of the German Human Biomonitoring Commission.

2. Objectives

- Scientific support for ESBIO, in particular for WP 1, 3, 6 and 8
- Provision of recommendations regarding pollutants/biomarkers in order to facilitate an environmental health-related monitoring scheme at the European Union Member State level
- Contribution to the Implementation Group on HBM (IG) for its preparation of the "Recommendations on Human Biomonitoring"
- Facilitating the establishment of collaboration networks and the sharing of methodologies as adopted in the objectives of the ESBIO Project
- Dedication of WP 2 towards Deliverable D2.2 and Milestone 2 of the ESBIO-contract

3. Pollutants / Biomarker

In the following, current knowledge regarding the proposed biomarkers for the Pilot Project will be presented in a clear and brief manner.

According to the "3rd IG Recommendations on Human Biomonitoring" the compounds will be arranged in two groups, representing the two scenarios of basic/obligate and extended/facultative biomarkers for the Pilot Project.

It is noteworthy that all compounds addressed are regarded as biomarkers of exposure, since only those are appraised as suitable candidates for the Pilot Project.

Beside the health related aspects of the individual pollutants, a brief rationale for the selected type of specimen and proposed biomarkers will be provided.

3.1. Biomarker of Scenario 1

Scenario 1 includes three heavy metal pollutants and one biomarker of ETS-exposure, all of which are of public health concern due to their largely recognized toxicity and toxicological assessments available (e.g. action level, HBM-values, PTWI).

On top of this, validated analytical methodologies for sufficiently sensitive determination are readily available and assumed to be established in the Member States.

Out of the four proposed biomarkers, at least one of those shall find general approval in order to be measured in all Member States participating in the EU Pilot Project.

The determination of (preliminary) reference values is proposed as a study objective; thereto related implications on the study design are beyond the scope of this document.

D2.2 Proposal for pollutants and biomarkers

3.1.1. Lead in Blood

Environmental lead is still an element of large health concern, though its ubiquitous dispersal and human exposure seems on the decline in most western countries.

Nevertheless, further reduction of the environmental occurrence of lead is worthwhile, particularly in regard to its neurotoxic effects onto children's development even at low concentration levels.

New toxicological findings have been discussed recently during a toxicology workshop of NTOXMET, a joint event of several scientific committees of the International Commission on Occupational Health (ICOH) in Brescia, Italy.

It was emphasized that the development of more sensitive and sophisticated analytical instrumentation has led to the recognition of sub-clinical toxicity and developmental neurotoxicity of lead at progressively lower levels of exposure, resulting in the knowledge that the extent of toxicity is much greater and the size of the affected population much larger than initially appreciated.

Though it was acknowledged that the removal of organic lead from gasoline has produced declines of >90% in population mean blood lead levels in industrially developed nations, the experts claimed that the current exposure standard for lead (100 µg/l) needs urgently to be reduced. It was postulated that the action level, which triggers community prevention efforts to reduce exposure sources, should be immediately reduced to a blood lead concentration of 50 µg/l for children and women of reproductive age. The latter were explicitly addressed, because lead passes freely across the placenta from the maternal to the foetal circulation to enter the developing brain where it causes prenatal brain injury.

The so called "Declaration of Brescia" was issued after the workshop and its proceedings published subsequently (Landrigan et al., 2006).

The major exposure pathway of lead is still the uptake via food, but also tobacco smoke and drinking water in case of leaded water pipes can contribute significantly to the corporal burden (German HBM Commission, 1996).

The results of GerES demonstrate that the concentrations of lead in blood decreased continuously since the ban of leaded gasoline. The current lead level of 16 µg/l determined in GerES IV is the lowest mean concentration reported from German studies on children so far (Becker et al., 2006b; Schulz et al., 2007) and will entail a revision of reference values for children in the near future.

D2.2 Proposal for pollutants and biomarkers

The currently valid German reference values for lead read 50 µg/l for children of 6-12 years, 70 µg/l for women and 90 µg/l for men, both 18-69 years (German HBM Commission, 2002).

Analytical target:

Lead in blood

Justification:

The determination of lead in whole blood is regarded as most suitable HBM parameter for the assessment of environmental exposure to lead. Since about 95% of lead is bound to the membranes of erythrocytes, the collection of venous blood is preferable to capillary blood due to a less varying cellular content and a lesser likelihood of contamination.

This recommendation attributes to children as well.

The sampled blood (volume ~ 2 ml) is subjected to anti-coagulation/refrigeration or freezing for transport and storage. Effects onto the stability of such blood lead samples have not been observed (German HBM Commission, 1996).

Chemical analysis and expected LoQ:

Quantification by means of graphite-furnace AAS, ICP-(MS)_x or ICP-AES.

LoQ of 2 - 5 µg/l should be achieved.

3.1.2. Cadmium in Urine

Environmental exposure to cadmium can cause kidney dysfunction and osteoporosis. Some data indicate that cadmium could induce lung cancer (WHO/ICPS, 1992 and IARC, 1993) or act as an endocrine disruptor with effects on reproduction, development and the immune system (Schoeters et al., 2006).

Resorption of cadmium into the blood stream leads to its complexation with metal-thioneine in the liver, followed by transport and successive accumulation in kidney cortex and adrenal. Especially in combination with a low iron status cadmium tends to being accumulated in the kidneys of females to a higher degree than observed with males. Due to this cumulative deposition, the urinary excretion level of cadmium depends on the nephritic load (age, smoking habit and pattern) and lately on the magnitude of renal dysfunction (German HBM Commission, 1998).

D2.2 Proposal for pollutants and biomarkers

The presence of environmental cadmium is due to its natural origin (soil) and the ease of its soil-plant-transfer accounts for the major exposure pathways: the ingestion via food and inhalation of tobacco smoke.

The Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA, 2003) established a PTWI¹ of 7 µg/kg bodyweight and it has been postulated that for the general population the urinary cadmium levels should be below 2.4 µg (♂) and 3.3 µg (♀) per gram creatinine, respectively (Kobayashi et al., 2006).

Urinary excretion of early biomarkers of kidney dysfunction (mainly low-molecular proteins and certain catabolic enzymes) can be increased starting at urinary cadmium levels of 2.0 µg/g creatinine (Noonan et al, 2002).

GerES IV provided a mean Cd-U value of 0.07 µg/l for children aged 3 - 14, while the total adult population of GerES III (aged 25 - 69) showed a geometric mean of 0.24 µg/l of urinary cadmium which was different for non-smokers with 0.21 µg/l than smokers with 0.33 µg/l (Schulz et al., 2007).

The currently valid German reference values for cadmium read 0.5 µg/l for children of 6-12 years of age and 0.8 µg/l for non-smoking adults aged 18-69 years (German HBM Commission, 2005).

Analytical target:

Cadmium in morning urine

Justification:

The cadmium concentrations in urine are statistically associated with the corporal burden, in particular with the concentrations stored in kidneys, and is therefore regarded as an indicator of long-term exposure. In contrast to cadmium blood level which reflects the acute exposure the urinary level indicates the chronic exposure to cadmium.

The statistical evaluation of results requires the assessment of smoking status and habit.

The collection of morning urine is not difficult and an acceptable alternative to the slightly more valid 24-h urine.

Chemical analysis and expected LoQ:

Quantification by means of graphite-furnace AAS or ICP-(MS)_x; a LoQ of 0.05 µg/l is achievable.

¹ Provisionally Tolerable Weekly Intake

3.1.3. Mercury in Hair

Mercury in its elemental, vaporous form is released into the atmosphere from a number of natural sources (degassing of the earth's crust, emissions from volcanoes and water reservoirs). Man-made emissions, primarily from combustion of fossil fuels, account for about 70% of the total emissions to the atmosphere (Mason et al., 1994) with subsequent dispersal on local, regional and global scales, but meanwhile a declining trend of emission have been observed for Europe (Meili et al., 2003).

While mercury is mainly deposited in elemental or inorganic forms, various micro organisms in soil and water but also in the intestines of animal species including fish convert ionic mercury into highly toxic methyl-mercuric (CH_3Hg^+) compounds (Sverdrup, 2003).

Due to these biomagnifications in the aquatic food chain, fish and seafood products are the dominant sources of methyl-mercury, contributing about 80-95% to the total mercury load in fish and shellfish (WHO/IPCS, 1990). The use of fish meal as feed for poultry and other productive livestock may lead to increased methyl-mercury levels in those animals and thus to an additional human exposure.

Ingested methyl-mercury is absorbed almost completely in the gastro-intestinal tract and is distributed via the bloodstream to all tissues of the body. Methyl-mercury is known to cross the blood-brain and placental barrier. In the foetus it tends to accumulate especially in the brain. In general, the kidneys retain the highest tissue concentration. The brain /blood concentration ratio for methyl-mercury is about 5:1 whereas the hair /blood ratio stands at approximately 250:1 (ATSDR, 1999).

Mercury levels in urine mainly reflect the exposure to elemental and inorganic mercury but more toxic organic forms of mercury are usually determined in blood or hair.

The effects of methyl-mercury on adults differ both quantitatively and qualitatively from the effects observed after prenatal and - possibly - postnatal exposure. The critical organ is the nervous system and effects include neurological developmental abnormalities in infants and paraesthesia in adults. Since foetuses bear the particular risk of developing neurological disorders following prenatal methyl-mercury exposure, health risk assessments focussed also on the observed mother-child-transfer.

Benchmark dose calculations by the U.S. EPA (IRIS, 2001) provided benchmark levels of 11 $\mu\text{g/g}$ maternal hair and 44 $\mu\text{g/l}$ maternal blood or a daily intake of 1.1 $\mu\text{g/kg}$ b.w./day.

By applying the common uncertainty factor a daily methyl-mercury intake of 0.1 $\mu\text{g/kg}$ b.w./day represents the current U.S. EPA RfD², corresponding to 1.1 $\mu\text{g/g}$ hair.

² Reference Dose for chronic oral exposure

D2.2 Proposal for pollutants and biomarkers

The average daily intake of methyl-mercury by the general population (not occupationally exposed to mercury) was estimated to be 2.4 µg (WHO/IPCS, 1990).

Recent studies on Swedish women with higher fish consumption revealed mercury concentrations in the range of 0.08 - 6.6 µg/g hair, indicating that 20% of the women actually exceeded the US EPA RfD (Bjornberg et al., 2005).

The European Food Safety Authority's (EFSA) Scientific Panel on Contaminants in the Food Chain (CONTAM) published an opinion regarding the possible risks to human health associated with the consumption of foods contaminated with mercury (EFSA, 2004).

In that context the EU Parliament in its Resolution on the Community Mercury Strategy called upon the Commission to "*ensure that mercury especially in vulnerable populations is included in the biomonitoring programme foreseen in the European Environment and Health Action Plan 2004-2010*" (EUPARL, 2005).

Analytical target:

Mercury (total) in scalp hair

Justification:

Methyl-mercury exposure is preferentially determined in blood or hair. Scalp hair is the non-invasive specimen of choice for assessing the long-term exposure to mercury. It is widely acknowledged that organic species account for more than 80% of the total mercury detectable in hair (Berglund et al., 2005).

Chemical analysis and expected LoQ:

Quantification as total mercury by means of cold-vapour AAS or ICP-methodology, LoQ range 0.005 - 0.05 µg/g should be obtained.

3.1.4. Cotinine in Urine

Tobacco smoke represents a major source of exposure to a vast number of chemicals. Within the complex mixture of around 4.000 compounds generated during combustion more than 50 human carcinogens contained in tobacco smoke could be identified (IARC, 2004).

Due to this undisputed health risk EU legislations focus on the protection of non-smokers by imposing restrictions on tobacco smoking in public places.

Nicotine is a psycho-active alkaloid of the tobacco-plant and can be found in processed products in mean ranges of 10-18 mg/g (Malson et al., 2001).

The determination of nicotine and cotinine concentrations in urine enables a differentiation not only among smokers and non-smokers but also for non-smokers with ETS exposure.

From those values obtained, objective and more reliable criteria for assessing the extent of ETS exposure and associated findings are provided, compared to the subjective statements obtained through questionnaire-based evaluations.

On top of this, both active and grave passive exposure to tobacco smoke always marks an important confounder in regard to adverse health effects and contributes to the corporal burden with heavy metals (notably cadmium) and PAHs.

GerES IV revealed that around 50 % of the German children are living in households with at least one smoker. The cotinine levels measured in children indicate an ascending degree of children's exposure to ETS if compared to previous GerES and confirm the ongoing importance to reduce this involuntary (in-door) exposure (Becker et al., 2006b; Schulz et al., 2007).

Analytical target:

Cotinine in morning urine

Justification:

Both nicotine and cotinine can be measured principally in blood, urine, saliva and hair.

Unfortunately, nicotine has a short half-life of about two hours, unfavourable for the determination of back dated exposure and magnitude.

Cotinine as main metabolite of nicotine is currently regarded as the best biomarker in active smokers and in non-smokers exposed to ETS. Its half-life is about 20-70 h, whereby prolonged in non-smokers and children (US EPA, 1992). Furthermore, external contaminations like those observed with nicotine are much less likely.

Since cotinine levels of moderate smokers and non-smokers exposed to ETS are very low in saliva and hair, the desired differentiations are restricted by the analytical LoQ.

D2.2 Proposal for pollutants and biomarkers

Therefore, urine is the most often selected specimen for the intended purpose.

Chemical analysis and expected LoQ:

Quantification by means of HPLC/DAD, LC/LC-(MS)_x or ELISA.

LoQ of 0.5 - 2.0 µg/l is expectable.

3.2. Biomarker of Scenario 2

Following the 3. Recommendation of the "Implementation Group on Human-Biomonitoring" (IG, 2006), the additionally proposed biomarkers of an extended HBM-programme offer the eligible opportunity to include current (emerging) pollutants of concern into the framework of the Pilot Project at Member State level.

Keeping the study objective of data comparability in mind, the IG suggestion that at least 5 Member States should address the same biomarker(s) of Scenario 2 was valued a scientific recommendation but the minimum of three Member States deemed an operational condition for the Pilot Project.

A list of potential candidates, e.g. PAHs, phthalates, perfluorinated and polybrominated chemicals and certain classes of pesticides, has been proposed for Scenario 2 and shall be presented here.

3.2.1. Metabolites of Phthalates in Urine

Phthalates comprise a number of industrially used compounds and are utilized for a range of desired properties. Phthalates serve as plasticizers, modifiers, emulsifiers, repellents and as substrate fluids in biocide formulations and in cosmetics, perfumes, etc. Accordingly, they are common constituents of synthetics, paints, vinyl tiles, food packaging, insecticides, pharmaceuticals and personal care products (Calafat & McKee, 2006).

Annual production figures in Western Europe amount to one million metric tonnes of phthalates of which more than 90 percent are destined for PVC-production. The most commonly used phthalates are Di(2-ethylhexyl)phthalate (DEHP), Dibutylphthalate (DBP), Benzylbutylphthalate (BBP), Di-isononyl-phthalat (DINP) and Di-isodecyl-phthalate (DIDP). Of particular concern proved DEHP, meanwhile largely substituted by DINP and DIDP as plasticizers for PVC-production. Because phthalates are not chemically bound to the plastics, they get released (by evaporation, solubilisation and abrasion) as the products are used and

D2.2 Proposal for pollutants and biomarkers

disposed. An annual amount of 1 – 5 % of the total DEHP produced is estimated to become released directly into the environment.

Regarding food packing, phthalates are known to migrate into the surrounding food matrix, so that uptake via phthalate-contaminated food may play a major role in exposure.

While the US Food and Drug Administration (FDA) still permits the use of DEHP in food contact applications (e.g., can coatings, adhesives, defoaming agent in paper manufacture, in cellophane used for food packaging - concentration must not exceed 5% - and as a surface lubricant in the processing of metal foil), it is not clear if industry currently uses DEHP in these applications.

Thus, the uncertainty associated with current concentrations in food makes quantifying intakes via food speculative. This might be especially true given the recent activity in substituting or eliminating phthalates from some consumer products (ATSDR, 2002).

The major route of human exposure for most phthalates is ingestion; exposure by inhalation, through drinking water, and via dermal contact tends to be limited (Clark et al., 2003). After ingestion, phthalates are metabolized to their corresponding hydrolytic monoesters and may further metabolize to more hydrophilic oxidative products. These metabolites can be excreted unchanged or undergo Phase II biotransformation to glucuronide conjugates (ATSDR, 2002). These metabolites and not the parent di-esters are likely to be the bioactive species (Stroheker et al., 2005).

There is an increasing number of scientists who consider phthalates in general and DEHP in particular as endocrine disruptors of the non-classic type, interfering with the endocrine system by dysregulation of hormone synthesis and hormone metabolism rather than by means of an inherent hormonal activity. Such harmful mechanism of action (reproductive toxicity) is assigned not only to DEHP but also to DBP and BBP, while Di-isononylphthalate (DINP) and Di-isodecyl-phthalate (DIDP), both used as substitutes, have not been assessed as harmful (EU/JRC, 2005).

Human biomonitoring data from the NHANES (National Health and Nutrition Examination Survey) conducted in the USA (Blount et al., 2000; CDC, 2003 and 2005) as well as data from Germany (Koch et al., 2004) including data from GerES (Becker et al., 2004) have demonstrated that the general population is ubiquitously exposed to DEHP among other plasticizers. Several studies were conducted in a selected cohort in southern Germany and among kindergarten children (Koch et al., 2003a, 2003b and 2004) on the excretion of DEHP metabolites in urine.

D2.2 Proposal for pollutants and biomarkers

These studies suggest that both the TDI (tolerable daily intake; 37 µg/kg bw/day) and the RfD (reference dose for chronic exposure, 20 µg/kg bw/day) levels are exceeded in a considerable part of the population. In addition, children being the most vulnerable group appear to be subject to a higher exposure than adults (Koch et al., 2004).

Reference values of the German general population³ read 150 µg/l for 5-oxo-MEHP and 220 µg/l for 5-hydroxy-MEHP, respectively. Both metabolites derive from DEHP.

Analytical targets:

Secondary metabolites of phthalates, e.g. 5OH- and 5oxo-MEHP (2-ethyl-5-hydroxy-hexylphthalate and 2-ethyl-5-oxo-hexylphthalate) of DEHP.

Justification:

The secondary phthalate metabolites in urine (see above) constitute biomarkers which are extremely reliable and provide much better evidence of phthalate exposure than the formerly used primary metabolites (e.g. MEHP, mono(2-ethylhexyl)phthalate of DEHP) or measurements of the parent phthalates and external exposure, respectively (Koch et al., 2004).

Chemical analysis and expected LoQ:

Sophisticated instrumentation and laboratory performance is required (Silva et al., 2003) for quantification by means of GC/ or LC-(MS)_x.

LoQs of 1.0-1.5 µg/l for the individual metabolites are achievable

3.2.2. Metabolites of Polycyclic Aromatic Hydrocarbons (PAHs) in Urine

PAHs are formed during incomplete combustion of organic matter, but are also present in liquid and solid products, where their emergence is due to heating of raw materials (i.e. crude oil distillates, used engine oils and tar/bitumen) in absence of atmospheric oxygen.

PAHs are present almost everywhere. In Europe the lowest range of aerial concentration amounts to 0.1-0.5 ng/m³. The higher ranges of 0.5-6 ng/m³ are generally connected with areas of high emission (WHO, 2003).

While raw food normally does not contain higher levels of PAHs (vegetables may get contaminated by the deposition of airborne particulates) they can be formed during

³ http://www.umweltdaten.de/daten-e/monitor/DEHP-2005_engl.pdf

D2.2 Proposal for pollutants and biomarkers

processing, roasting, backing or frying.. The levels of individual PAHs in different kinds of food can range between 0.01-10 µg/kg. Concentrations of over 100 µg/kg were found in smoked meat and up to 96 µg/kg in smoked fish. The median exposure via inhalation of ambient air was estimated to be 0.16 µg/day (range 0.02–3 µg/day).

Potential indoor sources of PAHs include non-vented space heaters and food preparations (WHO, 2003).

Known exposure sources are mainly the nutritional pattern (smoked and grilled foodstuff), active smoking and passive smoking in regard to children (Chuang et al., 1999, Siwinska et al., 1998). Smokers of approximately 10 cigarettes a day exhibit markedly higher concentrations of PAH metabolites than non-smokers.

It has been shown that the carcinogenic potential of PAHs is strictly associated with the metabolic activation to form reactive bay region diol epoxides which can bind covalently to DNA (Szeliga & Dipple, 1998).

There are clues to children's elevated susceptibility to PAHs and an age-dependent carcinogenesis (Schneider, 1999). According to assessments on risk of cancer and air pollution in urban centres, the general population carries a high burden of PAH, benzene and diesel-engine emissions.

Therefore, a European population survey on current PAH exposure is of highest interest and besides enables the research on the relationship between PAH exposure and their metabolites concentration in urine (Li et al., 2004).

1-Hydroxypyrene (1-HP) was introduced as a biomarker of exposure to PAHs some twenty years ago (Jongeneelen & Anzion, 1988) and has been widely applied since then. Its suitability covers the facts that pyrene is present in all PAHs mixtures in relatively high concentrations, that it is metabolized predominantly to 1-HP which is excreted mostly via urine. (Contrary to higher molecular PAHs metabolites which are excreted mainly via faeces.) For assessment of the temporal trend between 1990/92 (GerES II) and 1998 (GerES III) in Germany, a random sample of 150 stored urine samples from GerES II never-smokers was analysed retrospectively in 1998. Results revealed a decrease of the mean urinary 1-HP concentration by approx. ½, but back in 1992 children showed higher 1-HP concentrations than none-smoking adults. The GerES IV Pilot Study data underscored the decreasing trend since 1990/92 (Seiwert et al., 2005).

This reduction in PAH burden is probably a result of the continuing changeover to modern heating systems. This assumption is indirectly substantiated by the fact that people who live in

D2.2 Proposal for pollutants and biomarkers

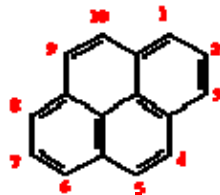
properties without central heating (generally using coal-burning stoves) demonstrate higher levels of PAH metabolites.

Alongside all advances in emission reductions, smoking of tobacco remains the most dominant source of PAH exposure and metabolites can be detected in passive smokers, too. In contrast, the influence of road traffic and the consumption of grilled and smoked food (both factors usually covered by the accompanying HBM-questionnaire and frequently mentioned in literature) could not be confirmed by data of GerES II and III (Federal Environment Agency, 2006).

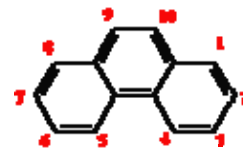
Because the environmental health database currently available is insufficient, the German HBM Commission has not yet been able to derive toxicology-based HBM values for biomarkers of PAH exposure.

Analytical targets:

1-Hydroxypyrene, 1-, 2/9-, 3- and 4-Hydroxyphenanthrene and 1, 2, 3,4 -tetra-Hydroxyphenanthrene ("Tetrol") in morning urine.



Pyrene



Phenanthrene

Justification:

The fate of higher condensed PAHs is their excretion mainly via faeces, while the lighter PAHs and their metabolites undergo renal excretion (Li et al., 2004). In order to cover the detection of higher molecular PAHs in urine, highly sensitive methodologies are conditional. Validated urinary biomarkers are 1-Hydroxypyrene and several isomers of Hydroxyphenanthrene. The newly investigated Phenanthrene-tetrol (Seidel et al., 2006) should be considered for a validation exercise, too

Phenanthrene is the simplest PAH with a bay region and though generally considered to be non-carcinogenic, its metabolisms through the diol epoxide pathway closely mimics the most potent PAH carcinogens like Benzo(a)pyrene (Hecht et al., 2003).

D2.2 Proposal for pollutants and biomarkers

Chemical analysis and expected LoQ:

Established quantification methods by means of LC-(MS)_x or GC-(MS)_x after derivatization.

LoQ of 0.004 - 0.02 µg/l, depending on the individual urinary metabolite.

3.2.3. Metabolites of Pyrethroids in Urine

Due to their increased domestic degree of utilization, derivatives of pyrethrins and pyrethroids⁴ have gained in importance in respect to health-related concerns. Pyrethroids are neurotoxins and a large number of symptoms following pyrethroid poisoning have been reported, even though the discussion was controversial (Mersch-Sundermann, 1999; Butte et al., 1998). Pyrethroid insecticides are subject for review as potential developmental neurotoxicants because of their mode of action on voltage-sensitive sodium channels (Shafer et al., 2005). In addition, permethrin, the most widely used pyrethroid insecticide, is suspected to be an endocrine-disrupting chemical and, along with fenvalerate, has been classified as a potential carcinogen at high exposure levels (US EPA, 2006a). Toxicological studies have also suggested that pyrethroids have a suppressive effect on the immune system and may cause lymph node and spleen damage. Unlike organophosphate or carbamate pesticides, pyrethroids do not appear to exhibit a single common toxicologic mechanism in humans (Lu et al., 2006). While numerous toxicity studies on this class of insecticides have been conducted, validated findings regarding the general population's exposure are scarcely available up to now. However, the US EPA has recommended daily oral exposure limits for 10 different pyrethroids ranging from 5.0 - 50.0 µg/kg b.w./day (ATSDR, 2003). First German steps entailed the measurement of house-dust samples during the German Environmental Survey (GerES II) of 1990/92 (Friedrich et al., 1998). Results provided a significant rise of the permethrin content between 1985/86 (GerES I) and 1990/92. Furthermore, concentrations of pyrethroid metabolites were on a higher level when people specified the domestic use of pest control products (Leng et al., 2003). Due to the rapid advancement in analytical instrumentation, even small amounts of pyrethroid metabolites in urine can be detected and quantified nowadays (Heudorf et al., 2004). The Pilot Study of GerES IV provided evidence that exposure of German children is of the same magnitude as in the US (CDC, 2005). It was evaluated that the exposure is influenced

⁴ In this deliverable, the term "pyrethrins" refers to the natural insecticides derived from chrysanthemum flowers; "pyrethroids" are the synthetic chemicals, and "pyrethrum" is a general name covering both compounds.

D2.2 Proposal for pollutants and biomarkers

by age, sampling location, consumption of boiled vegetables and the domestic application of biocides (Becker et al., 2006).

In a different study (Lu et al., 2006) it was concluded however, that in-door use of insecticides is the primary source of children's pyrethroid exposure.

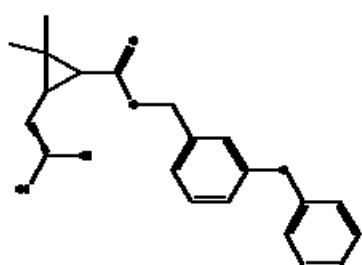
Analytical targets:

Metabolites of pyrethroids in morning urine.

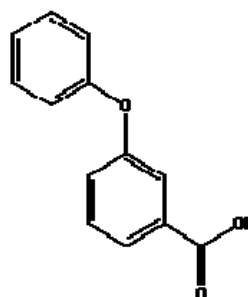
The targeted metabolites include 3-phenoxybenzoic acid (PBA), 4-fluoro-3-phenoxybenzoic acid (FPBA), *cis*-2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid (*cis*-Cl₂CA), *trans*-2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid (*trans*-Cl₂CA), and *cis*-2,2-(dibromo)-2-dimethylvinylcyclopropane carboxylic acid (Br₂CA).

Kuhn et al. (1999) have demonstrated the relationship of chemical structures between common pyrethroids and their urinary metabolites, compiled in the table following.

<i>cis</i> -Cl ₂ CA	permethrin, cypermethrin and cyfluthrin
<i>trans</i> -Cl ₂ CA	
Br ₂ CA	deltamethrin
PBA	most pyrethroid insecticides
FPBA	cyfluthrin



permethrin



3-phenoxybenzoic acid (PBA)

Justification:

Due to their short half-time in blood, pyrethroids can only be detected reliably following acute intoxication. In case of the more common chronic exposure, detection of their metabolites in urine represents a viable approach (Hoppe & Köster, 1994) which is commonly applied.

Chemical analysis and expected LoQ:

Quantification by means of LC-(MS)_x or GC-(MS)_x after derivatization.

LoQ of *cis*-Cl₂CA, Br₂CA and 3-PBA should reach 0.1 µg/l urine, while

LoQ of *trans*-Cl₂CA and F-PBA should stand at 0.4 µg/l urine.

3.2.4. Metabolites of Organophosphate-Insecticides in Urine

Organophosphates (OPs) belong to the most highly utilized group of pesticides in today's agriculture and account for half of the insecticides used worldwide. Chemically they can be divided into esters of phosphoric acid, thiophosphoric- and dithiophosphoric acid.

Organophosphates affect the nervous system by reducing the ability of cholinesterase, an enzyme, to function properly in regulating the neurotransmitter acetylcholine. Acetylcholine helps transfer nerve impulses from a nerve cell to a muscle cell or another nerve cell. If acetylcholine is not properly controlled by cholinesterase, the nerve impulses or neurons remain active longer than they should, over stimulating the nerves and muscles and causing symptoms such as weakness or paralysis of the muscles. This paralyzing effect is the wanted mode of action in utilizing OPs as insecticides.

Following legal requirements, the US EPA conducted an extensive cumulative risk assessment (2001 - 2006), which evaluated OP exposures based on a common mechanism of toxicity, evaluating the risk from food, drinking water, residential and other non-occupational exposures resulting from the registered use of over 30 OP pesticides. The Agency was aware that a number of OP pesticides can be transformed in the environment to oxons and that data suggested those oxons to be more toxic than the parent OP. With respect to the exposure estimates from food, the PDP (Pesticide Data Program) tested for several oxon metabolites of OP pesticides in food commodities. The majority of these oxons could not be detected except of one (omethoate, the dimethoate oxon) that was consistently found in PDP samples.

Ultimately, EPA summarizes all investigations in its final report by stating that *"the assessment shows that most organophosphate pesticides degrade rapidly and that people are exposed to levels that are not toxic or dangerous"* (US EPA, 2006b).

However, this assessment does not meet unanimous approval.

Since also chronic exposure to low levels of OP compounds impairs the acetylcholine degradation it was discussed that OPs may produce lasting neurotoxicity affecting cognitive function in humans (Prendergast et al., 1998; MRC, 1998). Low-level exposure to OPs was linked to hyperactivity, behaviour disorders, learning disabilities, developmental delays and motor dysfunction (Winrow et al., 2003).

As stated by the U.S. Department of Health and Human Services, OP pesticide metabolites are now found in the urine of 95% of Americans tested and levels are nearly twice as high in urine samples of children than adults (CDC, 2005).

D2.2 Proposal for pollutants and biomarkers

Studies on occupationally exposed persons and their children have also been published (Curl et al., 2002; Davies & Peterson, 1997). Adults may show remarkably high levels of corporal burden (Heudorf, 2000) presumable due to ingestion.

Results of a former German study on children depicted that urinary OP metabolite concentrations were higher in children up to the age of six compared to adults surveyed at the same time (Heudorf & Angerer, 2001). The results of the GerES IV Pilot Study provided concentrations of the same range. The exposure was mainly influenced by age, consumption of fresh fruits and fruit juice, living in an urban area, and season (Becker et al., 2006a). This is in line with findings provided by others (Lu et al., 2005), who demonstrated that the metabolites of OP insecticides can be formed already in fruit juices.

With regard to data on exposure of children in other countries, a literature search provided data from the USA (CDC, 2005) and Italy (Aprea et al., 2000).

Analytical targets:

Metabolites of various organophosphates (compiled in the table below) in morning urine.

metabolite	name	sum formula
DMP	Dimethyl-phosphate	C ₂ H ₇ O ₄ P
DMTP	Dimethyl-thio-phosphate	C ₂ H ₇ O ₃ PS
DMDTP	Dimethyl-dithio-phosphate	C ₂ H ₇ O ₂ PS ₂
DEP	Diethyl-phosphate	C ₄ H ₁₁ O ₄ P
DETP	Diethyl-thio-phosphate	C ₄ H ₁₁ O ₃ PS
DEDTP	Diethyl-dithio-phosphate	C ₄ H ₁₁ O ₂ PS ₂

Justification:

The metabolism of organophosphate pesticides entails at least one of the above stated six compounds, beside a few exceptions (Hardt & Angerer, 2000). Due to their short physiological half-time and accordingly low concentrations in blood, it is common practice to determine these metabolites in urine instead (Heudorf et al., 2004).

Chemical analysis and expected LoQ:

Quantification by means of LC-(MS)_x.

LoQ for DMP at 3.0 - 5.0 µg/l and remaining others at 0.5 - 1.0 µg/l.

3.2.5. Perfluorinated Chemicals in Blood

Perfluorinated chemicals (PFCs) are characterized by chains of carbon atoms of varying lengths, to which fluorine atoms are strongly bonded, yielding essentially indestructible chemicals that were thought to be biologically inert.

PFCs are used in a vast array of industrial products and processes. They can be contained in non-stick pans, furniture, cosmetics, household cleaners, clothing and packaged food containers. PFCs are mostly used in products designed to repel soil, grease, and water, including carpet and furniture treatments, food wraps, sprays for leather, shoes and other clothing, paints and cleaning products, including shampoo and floor wax where PFCs are used as surfactants. To a minor degree they are also used in chromium plating, photography and in hydraulic fluids for aviation (EA, 2004).

About 96 PFC products are made of fluoropolymers and telomer alcohols that break down predominantly into PFOA (perfluorooctanoic acid), PFOS (perfluorooctane sulfonate) and - to a minor extent - into other related chemicals in the environment and inside biota.

A number of disturbing scientific findings since the late 1990s have elevated PFCs to the gallery of toxic and persistent chemicals that contaminate humans and wildlife the world over. As more studies pour in, scientists get concerned because several root compounds of the PFC family virtually never degrade in the environment (EWG, 2007).

In 2000 the U.S. EPA, sensitized by study reports of PFC-manufacturer 3M (3M, 1999 and 2000), forced PFOS off the market and 3M voluntarily ceased its worldwide production. PFOS was the active ingredient used for decades in the original formulation of 3M's popular *Scotchgard*[®] stain and water repellent. Shortly thereafter, 3M also stopped manufacture of PFOA that is currently under regulatory pressure as well (U.S. EPA, 2007a).

The OECD meanwhile alerted, too, carried out a hazard assessment of PFOS (OECD, 2002) and two consecutive surveys on production, importation and use of PFOS, PFAS, PFOA, their related substances and products/mixtures containing these substances (OECD, 2005 and 2006). The assessment reads "*PFOS is persistent, bioaccumulative and toxic to mammalian species. There are species differences in the elimination half-life of PFOS; the half-life is 100 days in rats, 200 days in monkeys, and years in humans. The toxicity profile of PFOS is similar among rats and monkeys. Repeated exposure results in hepatotoxicity and mortality; the dose-response curve is very steep for mortality. This occurs in animals of all ages, although the neonate may be more sensitive. [] The perfluorinated compounds represent a very unique chemistry whose toxicological properties are presently not well understood and clearly the presence of different length (perfluorinated) carbon chains and functional groups*

D2.2 Proposal for pollutants and biomarkers

are likely to influence toxicity. It is not clear at this time whether the hazard concerns of PFOS can be extrapolated to other perfluorinated compounds except under circumstances where the compound may degrade to PFOS" (OECD, 2002)

A PBT⁵ assessment has been carried out at a later stage as part of a European risk evaluation in 2004 and revealed that PFOS meets the vP, B and T criteria and hence is considered a PBT substance (EA, 2004).

European legislation reacted onto these evaluations; initiated by the Commission proposing restrictions on the marketing and use of PFOS (EU COM, 2005) the European Parliament and Council endorsed the protective measure and resolved upon Directive 2006/122/EC in December 2006 (EUPARL, 2006).

Section 7 of this Directive reads: "*Perfluorooctanoic acid (PFOA) and its salts are suspected to have a similar risk profile to PFOS, and consequently there is a need to keep under review the ongoing risk assessment activities and the availability of safer alternatives and to define what kind of risk reduction measures, including restrictions on marketing and use, if appropriate, should be applied within the European Union*".

In parallel to European activities on PFOS, the U.S. EPA started to review several PFCs, including PFOA, in 2000 to determine whether they might present concerns similar to those associated with PFOS. The preliminary draft risk assessment, released in April 2003, focused only on developmental toxicity and identified considerable uncertainties in the assessment. Since that time, new data have become available that are suited to refine the understanding of the potential development toxicity risks. EPA also broadened the analysis to consider the full range of potential human health effects, including carcinogenicity and systemic toxicity. This revised PFOA risk assessment is anticipated to be completed within 2007 (U.S. EPA, 2007a).

Regarding the human exposure several facts are evident: the common degradation products of PFCs feature a long serum half-life in humans (8.7 years for PFOS and 1 – 3.5 years for PFOA) and are subject to distribution among liver, blood serum and kidneys. Due to their amphiphilic nature they tend to bind to macromolecular proteins, e.g. serum albumins, and yielded mean serum concentration ranges of 17–53 µg/l (PFOS) and 3–17 µg/l (PFOA) in non-occupational exposed humans (EA, 2004 and U.S. EPA, 2007a).

Based on compiled toxicological and biomonitoring data available, the German Federal Institute of Risk Assessment (BfR) published a preliminary TDI of 0.1 µg PFOS/kg bw/day (BfR, 2006).

⁵ Persistent, Bioaccumulative and Toxic

Analytical targets:

PFOS and PFOA in blood (serum).

Justification:

For many years, PFOS has been measured in the serum of workers occupationally exposed to PFOS. PFOS and PFOA serum values have been also determined in epidemiological and toxicological studies and are assumed to represent steady state levels which allow for comparison of data. A blood volume of 3-5 ml is recommended.

Chemical analysis and expected LoQ:

Recent methods applied solid phase extraction prior to separation by HPLC and quantification by tandem MS (ES, negative ion mode).

LoQ of approximately 0.01-2 µg/l (compound dependant).

3.2.6. Brominated Flame Retardants in Blood

There are more than 175 different types of flame retardants, which are generally divided into classes that include the halogenated organic (usually brominated or chlorinated), phosphorus-containing, nitrogen-containing, and inorganic flame retardants.

Brominated flame retardants (BFRs) is the trivial name of a broader class of polybrominated organic chemicals indicating their use as flame retardants in furniture foam, plastics for consumer electronics and small appliances, wire insulation, back coatings for draperies and upholstery. Beside the low costs of their manufacture, the obvious benefit of these chemicals is their ability to slow ignition and rate of fire growth (U.S. EPA, 2007b).

BFRs comprise of five major classes: brominated bisphenols, diphenyl ethers, cyclododecanes, phenols, and phthalic acid derivatives, whereby the first three classes represent the highest production volumes.

Out of those, the five majors are tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCD), and three commercial mixtures of polybrominated diphenyl ethers (PBDEs), namely decabromodiphenyl ether (DBDE), octabromodiphenyl ether (OBDE), and (pentaBDE) pentabromodiphenyl ether (BSEF, 2001).

The structures of these chemicals (from: Birnbaum & Staskal, 2004) are shown overleaf.

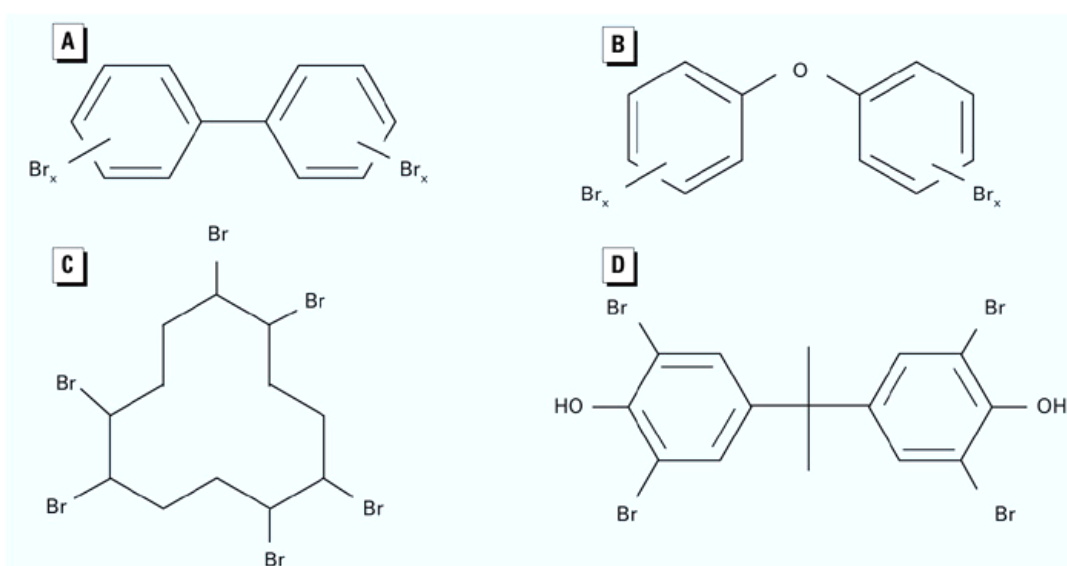
D2.2 Proposal for pollutants and biomarkers

Figure 1. Chemical structures of (A) PBBs, (B) PBDEs, (C) HBCD, and (D) TBBPA.

Many reports have demonstrated that BFRs occur in the environment far from the locations where they are produced and/or used, and that the concentrations of some of the BFRs, both in the environment and in humans, are rapidly increasing (Alaee & Wenning, 2002).

There is undisputed evidence that PBDEs persist in the environment and accumulate in living organisms, as well as toxicological testing indicates these chemicals can cause liver toxicity, thyroid and neurodevelopmental toxicity. Several studies using *in vitro* models have also suggested that PBDEs could have endocrine disrupting effects (Birnbaum & Staskal, 2004). Environmental monitoring programs all over the globe have detected several PBDEs in human breast milk and blood, fish, aquatic birds, polar bears and elsewhere.

Particular PBDE congeners, tetra- to hexabrominated diphenylethers, are the forms most frequently detected in wildlife and humans. The mechanisms or pathways through which PBDEs get into the environment and humans are not yet established satisfactory, but could include releases from manufacturing or processing of the chemicals into products like plastics or textiles, aging and wear of the end consumer products, and direct exposure during use, e.g. from furniture (U.S. EPA, 2007b).

The congener-specific breakdown of PBDEs shows large regional differences. DBDE is the most widely used PBDE globally, with equal use in America and Asia. PentaBDE - still in use in America - and Octaforms of PBDEs were banned for marketing and use in the European Union in August 2004, and a separate directive putting a ban on all PBDEs in electronics took effect across Europe in July 2006 (BSEF, 2003).

D2.2 Proposal for pollutants and biomarkers

Given that PBDEs are absorbed and partly metabolized and given the increasing production and use of these chemicals (in North America), attention to the status of humans is escalating. A study carried out by the German Federal Institute of Risk Assessment (BfR) on behalf of the Federal Environment Agency (UBA) showed that mean PBDE levels in breast milk in Germany (2.5 ng/g milk fat) were in the lower range compared to other European countries (BfR, 2005) and compared to the US where PBDE concentrations in breast milk were 10 to 70 times higher (She et al., 2004).

The major source of PBDEs exposure is foods of animal origin (BfR, 2005). The U.S. EPA⁶ set the RfD (for Chronic Oral Exposure) of DBDE at 10 µg/kg b.w./ day.

For the total PBDE level an ADI of 1 mg/kg b.w./d was estimated (Darnerud et al., 2001) which has been used for further (German, Swedish, etc) assessments.

HBM studies revealed a wide range of PBDE blood serum levels. The cause of this is difficult to correlate with different factors such as diet, age or employment situation.

An overview of some European findings is presented in the following table.

Region	Year	Sample	Median (range)		
			Σ BDE	Octa-BDE 183	Deca-BDE 209
Great Britain	2003	Serum	4.6 (0.52-420)	0.59 (0.19-1.8) ^a	83 (35-240) ^b
London & Lancaster	2001-2003	Milk	6.6 (0.3-69) **		
Sweden	2001/2002	Blood	4.9* ^c		
Finland	1994-1998	Milk	2.1*		
Germany ^d	1985	Blood	3.1*		
	1990		3.6*		
	1995		3.7*		
	1999		3.9*		
Sweden	1972	Milk	0.07*		
	1980		0.45*		
	1990		1.2*		
	2000		2.6*		
Norway	1977	Serum	0.44*		
	1986		1.1*		
	1995		3.1*		
	1999		3.1*		
Sweden	1997	Serum		11 (3.0-25) E	4,8 (<0.29-9.5) E
	1999			1.2 (0.23) C	1,5 (<0.96-6.8) C
	2000			< 1.9 G	34 (6.7-280) G

Σ BDE = BDE 47, 99, 100, 153, 154; a = only samples with a positive result for BDE (n=85); b = only samples with a positive result for BDE (n=11); c = Σ BDE 47, 99, 153; d = BDE 47; * = arithmetic mean; ** = 95 percentile; volunteers: E = electronic scrap disassembly workers, C = computer technicians, G = rubber cable production workers.

Concentrations are expressed in ng/g fat basis (taken from: FoEE, 2005)

⁶ See: <http://www.epa.gov/iris/subst/0035.htm#reforal>

Analytical targets:

PBDEs (DBDE and octa-, hexa, penta, tetra-brominated congeners) in blood (serum).

Justification:

PBDE serum levels have been determined in epidemiological and environmental studies, as well as in occupational assessments, and allow for comparison of data.

Chemical analysis and expected LoQ:

Separation and quantification by HRGC-(MS)_x; validated methods (for products: UBA, 2005) are available (U.S. EPA, 2007b).

LoQ of approximately 0.01-2 µg/l (compound dependant).

4. Summary and Recommendation

A rationale for the consideration of biomarkers (Scenario I and II) to be measured in a European Human Biomonitoring Pilot-Study has been provided. Validated analytical methods are available for all compounds presented. Certified reference material is available for biomarkers of Scenario I; analytical standards (of certified purity) are commercially available for all biomarkers suggested.

Laboratories capable to measure the compounds with the required specificity and sensitivity have to be identified and selected at national level (see: deliverable 2.5).

Regarding the type of specimen material, urine, blood and human hair are proposed.

Urine is regarded superior in ranking to blood (which is collected by an invasive method requiring authorized personnel, entailing more ethical concern, problem of compliance in respect to younger children). However, the determination of lead, PFCs and BFRs requires sampling and analyzing of blood.

Validated utilization of scalp hair is limited to only a few biomarkers, to which the proposed methyl-mercury belongs.

Guidelines for sampling, handling, transport and storage of specimen are presented in deliverable 2.3.1 and 2.4, respectively.

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