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Human biomonitoring and environmental health

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Editorial

I have a dream – a European Biomonitoring Programme

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There are many arguments against investing in a European human biomonitoring (HBM) programme:
- HBM is not applicable for many chemicals of interest;
- it reflects exposures and daily intakes differently for different chemicals;
- biological guideline values are available for only a limited set of chemicals;
- obstacles along the path from the multitude of national programmes into one harmonised European programme are enormous;
- privacy issues may narrow the usability of the data; and
- a European HBM programme, sufficiently large to be useful, would be expensive – in the order of 10 to 20 M €/y.

So, why pursue the idea against these obstacles? The European Conference on Human Biomonitoring[1], 4-5 November 2008 in Paris, was organised by the French Presidency of the EU to find answers. The first indirect answer, however, can be drawn from the fact that the two countries which have developed national HBM programmes, Germany (GerES I - IV, 1986 – 2006) and the US (NHANES/CDC, 1971 – 2008), have repeated and expanded their programmes several times. WHO has surveyed human milk internationally for dioxins and PCBs already since 1997. Canada and several European countries have also started HBM programmes, ranging from narrowly focused to population based.

Biomonitoring connects environmental monitoring to human health. It integrates the contributions of all exposure media, from all sources, every contact pathway and route of entry, all locations, activities and consumer products.

What should we expect from a European HBM Programme ? We would start by generating European level distributions of chemicals in humans by areas (countries, urban/rural, proximity to sources), socio-demographically (age, gender, socioeconomic status, ethnicity). With more data accumulating we would begin to generate exposure maps and graphics for the interests of the public and decision makers. After some years we would be analysing time trends. The results would lead to new and better targeted risk assessments and risk management actions. Past examples include:
- association of blood lead with lead in petrol (US NHANES, 1971 – 1990) paved the way out of leaded petrol also in Europe;
- linking of blood mercury and dioxin to fish diet resulted in consumer information campaigns worldwide;
- high blood mercury in connection to actions with certain skin care products used by an ethnic minority in NYC led to targeted local actions;
- reduction of blood PAH metabolites in the children of the former GDR after the German unification demonstrated the health and equity benefits of reduced air pollution in eastern Germany; and

- increasing levels of polybrominated diphenyl ether (PBDE) flame retardants in blood due to their mandated use in consumer products, particularly in California. The last exemplifies the complications in the management of competing risks, in this case the well known risks of fires and the poorly known risks of the increasing body burdens of PBDE.

Combining pharmacokinetic models with individual biosamples and other data allows the estimation of body burdens and daily intakes. These can then be attributed to sources, products, activities and lifestyles using statistical techniques. Further possibilities include exposure and body burden modelling, derivation of population intake fractions and risk modelling.
These advanced analyses do not require identification of each person (name, street address, etc.), but they do require that the concentration, physiological and questionnaire data are linked to [unidentifiable] individuals. This may be difficult to harmonise with the legal requirement for a "fully explicit and specific" consent from each sampled individual for each new data use.

The greatest societal benefits of European HBM Programme would be obtained in environmental health risk assessment and management, e.g., the implementation of REACH[2] by giving early warnings, pointing out vulnerable groups, providing guidance for policy targeting and helping in the assessment of policy accountability.

A triad of chemical risks would identify:
- **High concern** from increasing body burdens or high concentrations relative to biological guideline values in certain areas, population groups or users of certain products;
- **Low concern** from decreasing body burdens, absence of hot spots or high exposure groups, and low concentrations relative to biological guidelines;
- **Intermediate concern** in between.

Simultaneously with the progress towards a European programme, the feasibility study for a European Health Examination Survey (EHES, 2008)[3] proposed a protocol involving 4000 – 10 000 subjects in each participating country, minimum requirements for physiological, biological and questionnaire monitoring, nationally funded sampling, and – to ensure comparability across countries and time – common, EU funded coordination, protocols and database. A similar protocol could be feasible also for the European HBM Programme. Close collaboration between the two programmes would create a huge programme with many parties, interests and – obviously – conflicts. It would also provide considerable cost savings and create unforeseen research, risk/benefit and policy assessment opportunities.

The time window for coordinating the two projects is right now.

1/ http://www.invs.sante.fr/agenda/biosurveillance_2008/programme_en.htm
2/ http://ec.europa.eu/environment/chemicals/reach/reach_intro.htm
3/ http://www.ktl.fi/fehes

From human biomarkers to human biomonitoring in environmental health in Europe

Highlights of the Conference held in Paris on November 4-5, 2008

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Biomarkers

After exposure to an external agent, a continuum of biological events can occur that may result in clinical impairment of health. Biomarkers are used as indicators of these intervening events: from internal dose to biologically effective dose, early biological effect, altered structure or function, and finally to clinical disease. A biomarker is defined as *'any substance, structure or process that can be measured in the body or its products and may influence or predict the incidence or outcome of disease'* [1]. A distinction is made between biomarkers of exposure, of effect and of susceptibility (see box 1). In humans, blood, urine, breast milk and expelled air are most commonly measured, but hair, nails, fat, bone and other tissues may be sampled as well. Biomarkers are also frequently measured in plants, animals, or entire ecosystems, but this will not be further addressed in this special edition.

Human biomonitoring

Human biomonitoring (HBM) refers to monitoring activities using biomarkers that focus on environmental exposures, diseases and/or disorders and genetic susceptibility, and their potential relationships [3,4]. In principle, monitoring refers to a repeated or continued sampling and analysis; however, the term is also commonly used for "one time activities". Several examples are presented further down.
Human biomonitoring in environmental health should be differentiated from screening. Screening is essentially seeking to identify a disease or pre-disease condition in apparently healthy individuals. According to the UK National Screening Committee, screening is a public health service in which members of a defined population, who do not necessarily perceive that they are at risk of, or are already affected by, a disease or its complications, are asked a question or offered a test to identify

Box 1 **World Health Organisation (WHO) definition of biomarker types [2]**

Biomarker for exposure	An exogenous substance or first metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism
Biomarker for effect	A measurable biochemical, physiological, behavioural or other alteration within an organism, that depending upon the magnitude, can be recognized as associated with an established or possible health impairment or disease
Biomarker for susceptibility	An indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance

those individuals who are more likely to be helped than harmed by further tests or treatment to reduce the risk of disease or its complications [5]. In occupational health for instance, genetic screening may detect inherited characteristics, which can point to greater susceptibility to certain disorders in relation to certain occupational risks. In contrast, genetic biomonitoring finds changes in the hereditary material, which are the result of exposure to harmful substances. It should be noted, however, that hitherto pre-employment selection practices based on genetic screening test results are not considered part of a rational policy aiming at the protection of workers' health. Consequently, the use of those tests is generally not advocated and even prohibited in certain countries [6].

Clinical and occupational medicine offer historical and contemporary lessons on the value of analyzing human body fluids for indicators of exposure and adverse health risk and for assessing the efficiency of preventive measures. For more than a century, occupational physicians and industrial hygienists have used human biomarkers to monitor worker populations for exposure to a variety of hazardous substances, as part of a preventive approach, combined with workplace monitoring and hygienic measures. Biomarkers were and still are used extensively for metals such as lead, cadmium, mercury, nickel, chromium and arsenic, and for organic chemicals such as aniline, benzene, carbon disulfide, styrene, chlorobenzene and chlorinated aliphatic hydrocarbon solvents. Such repeated biological monitoring is seen as a component of medical surveillance that is the periodic examination of putatively exposed workers. Important messages are taken from their experience and expertise [7].

Added value of human biomonitoring in environment and health

In environmental health, human biomarkers are typically a tool:

- In research studies, to improve our knowledge on causal links between environmental factors and health by hypothesis generation and testing;
- In survey studies, where periodical measurements produce information on the prevalence of exposure to environmental agents and the related public health impact; and
- In raising awareness campaigns most often by NGO's.

Major examples of these can be found in:

- European research initiatives or projects [8];
- National programmes such as the US National Health and Nutrition Examination Survey [9]; the German Environmental Survey (GerES) [10]; the Flemish programme [11]; and
- Activities by various advocacy groups such as WWF and Health and Environment Alliance [12-14].

The power of human biomonitoring in environmental health is increasingly recognised at European level. Numerous debates are ongoing about its value in policy making as compared to other tools such as environmental monitoring and health surveillance, its usefulness for European policies such as REACH[1] [15,16], and the need for adequate resources [17]. In November 2008, the French Ministry of Health together with the French Institute for Public Health Surveillance (InVS) organised a conference under the French presidency of the EU with the aim of demonstrating the usefulness and added value of human biomonitoring as a tool for policy and for public health actions. The conference gathered various stakeholders and a wide range of topics were covered based on HBM programmes and activities implemented in different European countries and in North America [18].

Case examples, such as the study on mercury and pesticides exposure in New York City [19], illustrated how well-designed biomonitoring surveys can provide the evidence base to drive policy relevant recommendations and to what extent they have a strong potential in seeking attention for environmental health matters and changing beliefs and attitudes. The New York City study and several German studies among which GerES IV were also able to demonstrate, through the analysis of the relationship between exposure data and socio-economic factors, effects of environmental injustice. These studies influenced preventive measures, public health promotion and recommendations for policy. The potential sensitive nature of such results is shown for instance by the GerES data indicating a need for adaptation of the recommendations concerning breast feeding [20]. In Cyprus, the measurement of cotinine levels in saliva of children was used to evaluate the effectiveness of an aggressive anti-smoking campaign. The survey showed that important sources of exposure were still not controlled and that measures to prohibit tobacco smoke in public places were urgently needed [21].

Bearing in mind all these experiences, it is not a surprise that national programmes increasingly obtain a legal embedding, permitting repeated cycles of measurement. In Slovenia, the HBM programme is embedded in the Slovenian Chemical Act in Article 51 and linked to the Stockholm convention [22]. The aims of the biomononitoring programme are clearly defined and the different responsibilities distinguish the competent authorities from the scientists carrying out the programme. In Flanders (Belgium), environment and health has been put on the political agenda further to the dioxin crisis in 1998 and the first HBM Flemish study was carried out in 2001 [23]. As a result, HBM, the polluter pays principle and the precautionary principle have been embedded in the Flemish Decree of preventive health care in 2003.

Challenges

If biomarkers are to contribute to environmental health policy and interventions, they have to be relevant and accurate, provide information that cannot be obtained otherwise and bring about acceptable consequences for the study subjects (see box 2). This includes a strong need for external quality assessment schemes to ensure comparability of biomonitoring results. Between 1985 and 2008, for instance, the German External Quality assessment scheme for biological monitoring in occupational and environmental medicine developed 137 Standard Operating Procedures (SOPs) for hazardous substances in biological materials [24].

Much work is to be done in this respect as for most exposure biomarkers the relationship with health effects is currently unclear. This limits the interpretation of biomonitoring data in terms of health risk and the development of reference values and health-based values (see box 3) and therefore holds back the straightforward interpretation of data and their translation into policy actions [25].

[1]REACH is a the European Community Regulation on chemicals and their safe use (EC 1907/2006). It deals with the Registration, Evaluation, Authorisation of Chemical substances. REACH entered into force on 1 June 2007.

Box 2 Relevance, accuracy and necessity of human biomarkers [26]

Relevance	Suitability of the biomarker to provide useful information about the question under consideration.
Accuracy	Validity: degree to which a biomarker indicates what it claims to indicate. Function of specificity, sensitivity and predictive value of the marker. Measurement predictive value (in terms of analytical chemistry) to be distinguished from predictive value in terms of health effect. Reliability: the reproducibility of a result or the degree of similarity among results when the measurement is repeated under similar circumstances. Reliability depends on the variability of the manifestation that is measured and on the variability of the method of measurement and the skill with which it is done.
Necessity	Includes an evaluation of whether useful information cannot be obtained better by other approaches, such as questionnaires, environmental measurements or record reviews.

Box 3 Reference values, and health-based values

1. The German Commission on Human Biological Monitoring of the German Federal Environmental Agency develops [20,27,28]:
   Reference values: indicate the upper margin of the current background exposure of the general population to a given environmental toxin at a given time. They do not represent health-related criteria for the evaluation of human biological monitoring data.
   Human Biological Monitoring values: are derived from human toxicology and epidemiology studies and are intended to be used as a basis for a health-related evaluation of human biological monitoring data.
   - The HBM-I-value represents the concentration of a substance in human biological material below which - according to the knowledge and judgement of the Commission and with regard to the substance under consideration - there is no risk for adverse health effects and, consequently, no need for action. The HBM-I-value should be regarded as a verification or control value.
   - The HBM-II-value represents the concentration of a substance in a human biological material above which - according to the knowledge and judgement of the Commission and with regard to the substance under consideration - there is an increased risk for adverse health effects and, consequently, an acute need for exposure reduction measures and the provision of biomedical care (advice). The HBM-II-value should be regarded as an intervention or action level.
2. Within the work of the ESBIO [29] team, R. Smolders and G. Schoeters have given an overview of available benchmark or reference values [30].

Ethics and communication

As HBM involves taking samples in humans it raises important ethical and privacy issues. Communication is crucial both to obtain an authentic informed consent from the volunteered study subjects and to transmit results. According to the EU Privacy Directive [31], participants generally have the right to know their individual results, but also *not* to know them if they wish so. On an aggregated basis, results should also be transferred to policymakers and translated into concrete actions.

Improved communication strategies are thus needed, not only to allow ethically acceptable practices, but also to secure the real relevance/added value of human biomonitoring. Indeed, HBM can act as an important trigger for action at individual or at population/policy level, as already shown in occupational health. Knowing what you are being exposed to and in which manner can help to make informed decisions on health protection. The simple act of the measurement itself is an important message as such: environmental health is a matter of concern and authorities and individuals have to take their responsibility. HBM is a strong educational tool as the whole communication process allows study subjects to learn more about environmental health issues. The article on the Flemish programme [32] illustrates the importance of an open communication of biomonitoring results and the resulting policy answers as a means to broaden the social basis for environmental health policy and awareness raising. The innovative process to derive a phased action plan and the participatory approach that involves experts and a stakeholders jury could be inspiring even though its complexity should not be underestimated. In the same sense the New York City case study clearly shows the potential of HBM surveys as policy levers.

Research

HBM is not yet at its full potential. In order to guarantee scientific sound results in current and future surveys, specific research efforts are needed to allow e.g. (i) definition of harmonised procedures; (ii) definiton of the best (harmonised) ways to provide information to and obtain consent from study persons; (iii) correct interpretation of results; (iv) integration with other data (environmental and health); (v) translation into policy; and (vi) identification of new biomarkers for new/emerging chemicals, HPV chemicals, CMR chemicals, and for combined effects of mixtures of chemicals.

While surveys generally focus on exposure biomarkers, research projects more often include (early) effect biomarkers and genetic factors [8]. In this context, human biomarkers are increasingly used to study the impact of the environment on health, even in early life. The Pelagie cohort study in Brittany, France, for instance - a mother child cohort - assesses the impact of prenatal exposure to pesticides on intrauterine growth [33]. In Europe the number of mother child cohorts is increasing. They may provide essential knowledge for future prevention of adverse environmental exposure early in life and optimizations of health recommendations. However, large sample sizes are required and obtaining data and samples processed in the optimized way is a demanding effort [34].

The future: a European approach

The European Environment and Health Strategy (2003, European Commission) paid particular attention to the potential of HBM. The Environment and Health Action Plan 2004-2010 recognised the importance of the harmonization of activities across Europe [35-37]. However, although national programmes have to face common challenges and have their destinies united within one single political community, they are designed by and for individual Member States that are at the same time very different with respect to environmental exposures, health concerns, analytical capacities, political and health priorities, cultural background, and perception of ethics. Moreover, collaboration between several disciplines and fields is required for HBM programmes, which adds to the complexity. Harmonization efforts are therefore a challenge at the scientific, political, social, legal and ethical level.

The realization of platforms for networking and exchange of experiences has already been going on for some time within the research area; however, this could not create a functional EU wide harmonization in the field. A EU wide surveillance programme is considered the best way forward. It should lead to HBM data which are comparable between countries, to evaluate spatial and time trends, to identify and characterise vulnerable populations or highly exposed populations, to help policymakers understand what policies may need to be in place to reduce exposure if elimination is not possible, and to evaluate the impact of plans focusing on exposure reduction. In addition, the results would provide urgently needed background exposure data for numerous scientific studies. A pilot study, bringing together most European Member States, is expected to take a first and important concrete step in a process towards a fully operational, continuous, sustainable and scientifically sound EU HBM programme that can be exploited at the same level as for example its counterparts in the United States (NHANES).

Incorporation of Human Biomonitoring as a scientific and policy tool at a European level requires the availability of European-wide structures for gathering, storing and analyzing biomarker and other data. At the European HBM Conference held in Paris the following SWOT analysis related to HBM programmes in Europe was presented (see Box 4) to open the discussion on the implementation of HBM at EU level and national levels.

It is clear that there is a need to construct bridges between Member States efforts (at local, regional and national level) and European and International initiatives for the sake of harmonization and for the efficient use of best available knowledge as well as scientific and financial resources. Key elements for success relate to decision-making structures at EU level, strong scientific support, a transparent determination of EU HBM reference and health based values, funding for long-lasting programmes foreseen at a very early stage, legal instruments or policies that integrate capacities, competences skills, and infrastructures (labs, biobanks), and finally, a clear definition of the responsibilities and tasks at national and EU level.

Box 4 **SWOT analysis (adapted)**

STRENGTHS	WEAKNESSES
• Detection of time trends and exposure differences amongst sub-populations • Strong awareness raising and education tool (politicians and citizens) • Examples available of outputs that guided and evaluated Environmental Health policies • Cost efficiency already demonstrated in specified cases	• Heterogeneity of current actions • Lack of reference and health-based values to take actions • Lack of adequate capacities at national level • Limited understanding of the potential of HBM among stakeholders • Multiple research gaps
OPPORTUNITIES	**THREATS**
• Current development of HBM worldwide • Expected support for EU policies (REACH) • Development of Environment and Health strategies and plans at the level of WHO, EU, and MS* (NEHAPs)** • Limitation of resources calls for harmonization and mutualization of tools, avoiding duplication of efforts	• Lack of funding and competition for funding with other surveillance tools • Complexity and need for intersectoral and interdisciplinary work • Separate routes for Health Examination Surveys and HBM at EU or national levels

* MS: Member States
** NEHAP: National Environment and Health Action Plan

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UBA's Health related environmental monitoring in Germany

The BEH asked Marike Kolossa-Gehring (marike.kolossa@uba.de), **toxicologist and head of the Toxicology Section, Health-Related Environmental Monitoring of the German Federal Environment Agency (Umweltbundesamt - UBA), and Kerstin Becker, epidemiologist in the same Section, to give an overview in 10 points of the German biomonitoring programme (GerES) implemented since the mid 1980s.**

The German Environmental Survey - rationale and objectives

In the mid 1980s Germany, namely the Federal Environment Agency (UBA) established a complex Environmental Monitoring Programme which included humans as part of the environment. Since then, human biomonitoring (HBM) is used as the most appropriate tool to characterize and evaluate people's internal exposure to environmental pollutants.

In the 1980s HBM data came almost exclusively from occupational studies or small-scale studies on selected population groups and were therefore not sufficiently meaningful to evaluate the exposure of the general population. The only larger human biomonitoring programme was the campaign to measure the blood lead levels of the European population as a result of the EC council directive concerning lead [1].

UBA bundled the existing knowledge and implemented a nationwide population study to assess population exposure to environmental pollutants. This study called "Umwelt-Survey" ("German Environmental Survey", GerES, [2]) was carried out for the first time in 1985/1986 (West Germany; GerES I) with adults. In 1990/91, the survey was repeated in West Germany (GerES IIa). During the planning stage of GerES IIa Germany was re-unified. Therefore, GerES II was extended to East Germany (GerES IIb, [3]). GerES III (1998, reunified Germany) focussed on adults. GerES IV pilot and GerES IV (2003-2006) are the first GerES solely on children [4].

Experiences from GerESs and European and WHO initiatives changed the basic views and principles concerning HBM in Germany and promoted research on the interrelations between Environment and Health. In 2004 the European Commission adopted the Environment and Health (E&H) Action Plan 2004-2010 [5] and GerES IV became also a module of the German E&H Action Plan. In this framework the emphasis of GerES was no longer on monitoring chemical agents in the body alone. The objectives of GerES were "to observe the contamination of the population by substances, physical and biological parameters of the environment, and the factors that influence them". If possible, results should be linked with toxicological or health data.

Co-operation between health and environmental surveys

GerES was a cross-sectional study and had been conducted in close co-operation with the respective National Health Interview and Examination Surveys (NHIES) of the Robert Koch-Institute (RKI). Combining data from both surveys provides a sound basis for a representative nationwide environmental and health surveillance system.

Field work of the GerESs and of the NHIESs was always conducted in close co-operation. Senior scientists of UBA and RKI prepared the operation manual for the field procedures which was used in training of the field teams. The common field work saves money and effort. So it was possible, inter alia, to perform blood sampling for NHIES and GerES in one step.

A disadvantage of performing a common field work is the need to share investigation time. NHIES occupied the participants for about two hours time, GerES added another 90 minutes. From our experience this is the maximum one can expect from participants to spend especially if children are involved. Therefore, UBA had to renounce to include tests to measure cognitive and neuronal development of children in GerES IV.

The two surveys provide their data to each other and developments in environmental epidemiology resulted in an enhanced linking of the data to elucidate the health impact of environmental pollution. However, even if cross-sectional study design is not the best suited for this purpose GerES can at least give hints and insights. In the GerES IV we already developed in advance hypothesis for some topics like:

- Occurrence of mould spores in homes and allergic sensitization
- Contact allergies due to nickel, chromium or scents
- Impact of noise on hearing loss, stress and sleeping disturbances
- Irritation of the eyes and the respiratory system due to VOC in indoor air.

How is GerES linked to environmental monitoring?

In addition to evaluating internal exposure GerES investigated the contribution of different compartments (air, water, house dust, food) to the body burden and supported development of models for exposure assessment.

GerES included measurement of indoor air (VOC, mould fungi), household tap water (metals), house dust (biocides, mould, fungi) and noise in front of the sleeping rooms. Extensive questionnaires applied to the subjects or their parents supplied information on the closer living environment by investigating exposure conditions, i.e., food selection, housing conditions, quality of the residential environment and exposure relevant behaviour.

For the first time GerES IV data have been linked for testing purposes with data on outdoor air quality or soil contamination by using geographical information systems.

Major implication for public health interventions and environmental policies

HBM has been used and applied to Health Policy in Germany for several purposes. Examples are:

- Identification of the exposure pathways (phthalates, polycyclic aromatic hydrocarbons (PAH), metals).
- Development of strategies to prevent and reduce exposure (ban of PCP/ other persistent biocides in wood preservatives or lead in fuel).
- Basis for interpretation of exposure data in environmental medicine and to decide on treatment (statistically derived reference values, epidemiologically/toxicologically founded Human Biomonitoring Values).
- Reduction of the risk associated with amalgam fillings.
- Decline of heavy metal contamination via drinking water by revision of the German Drinking Water Ordinance.
- Control success of existing regulation and measures by following time trends. Most relevant results for children [6] the basis of results of GerES IV and GerES II (1990/92) are:

- PAH metabolite concentrations in urine decreased remarkably since 1990/92, especially for East-German children. The gap between the levels observed earlier in East-Germans and West-Germans has widely been closed. This finding is most likely related to the successful reduction of air pollution in East-Germany after the German reunification and is a proof of the adjustment of exposure levels in the re-unified Germany.
- The median level of Pentachlorophenol (PCP) in urine decreased from 4.5 µg/l to < 0.6 µg/l. This is due to the ban of production and application of PCP and PCP containing products in 1989.

- In both surveys around 50% of the children were living in households with at least one smoker and thus exposed to environmental tobacco smoke. The cotinine levels confirm the ongoing relevance of this exposure pathway, especially in families having a low socio-economic status.
- The concentration of lead in blood decreased continuously since the ban of leaded gasoline. The current lead level of 18 µg/l (32 µg/l in GerES II) is the lowest mean concentration reported from German studies on children so far.
- In 1990/92 the cadmium levels in blood and urine were close to the limit of detection (geometrical mean: 0.1 µg/l) and remained unchanged.
- In blood and urine mercury decreased due to a reduced exposure through fish consumption and amalgam fillings. In Germany amalgam fillings are no longer recommended for children.

Sensitive topics

Assessment of human biomonitoring data is a sensitive topic especially if threshold or limit values are not available. One approach is the calculation of daily intakes on the basis of measured metabolite concentrations which was used to assess organophosphates (GerES IV Pilot Study). Being fully aware of several limitations of this procedure, it gave hints that exposure to organophosphates of children in Germany might be too high [7].

Multivariate analysis showed that consumption of fruit juice was related to significantly higher level of organophosphate metabolites in children. Toxicity data for these metabolites are unfortunately not available. Since metabolites might come from pesticide residues or might have already been taken up with the juice, questions regarding the source of these metabolites have to be addressed. This is especially important for a decision on measures. Due to different responsibilities for environmental pollution and nutrition, UBA supplies the data and BfR (Federal Risk Assessment Agency) is responsible for consumer protection.

Another sensitive topic is breast feeding. GerES data demonstrated that older mothers giving birth to their first child transfer significantly more persistent pollutants to their child than younger mothers [8]. Even in children at the age of 12 years exposure was increased with an increasing duration of breast feeding. Therefore, we feel that the recommendations of the German Breastmilk Commission to breast feed 6 months should be reconsidered concerning older mothers.

Working on the upcoming issue of "environmental justice" results show that environmental pollution is not always a problem of disadvantaged children [9]. There are several pollutants which affect children from families with a high Socio-Economic Status (SES) index (parents' income, education, occupational status) more. Therefore, measures to reduce or prevent children's exposure have to be targeted to the specific SES groups.

The German HBM Commission

The mandate of the German Human Biomonitoring Commission, established in 1992, is to support the Federal Environment Agency in its work on human biomonitoring by providing expert advice [10]. The commission members are appointed every three years and represent scientific authorities at the level of Federal Government and German States (Länder), universities, public health institutes and clinical institutions. In addition the Commission invites permanent and advisory guests, i.e. experts who give advice on specific issues [11].

To achieve a harmonized assessment of internal exposure in environmental medicine, the Commission has developed criteria for the derivation of statistically derived reference and toxicologically/epidemiologically derived HBM values. Reference values permit to assess the exposure of individuals or population groups compared to the ubiquitous background exposure. Since environmental conditions are changing reference values have to be checked and updated continuously. Main source of information has been GerES. In analogy to a IUPAC (International Union of Pure and Applied Chemistry)-guideline, the Human Biomonitoring Commission uses the 95% confidence interval of the 95th percentile of a measured pollutant concentration level in the German population. The Commission has pointed out explicitly that the reference values are strictly statistically derived values, and give per se no information on health relevance.

The Commission draws up monographs which contain information on the following aspects of an environmental pollutant: occurrence, use and distribution in the environment; pathways of intake and toxicokinetics; possible exposure related factors; internal exposure; and health relevance. If information from toxicology and environmental epidemiology is regarded as sound the commission uses it to derive the HBM-values. Up to now, the Commission has derived HBM-values (HBM I and/or HBM II) for lead, cadmium, mercury, PCP and DEHP in body liquids (blood and urine).

Reference, HBM-I and HBM-II values: definition and use

The HBM-I-value indicates the threshold below which a risk of adverse health effects in the general population is not to be expected according to current knowledge. Adverse effects cannot be excluded with sufficient certainty for concentrations in the range between HBM-I- and HBM-II values. For results falling into this range, it is recommended to check the analytical results and explore whether the high level is due to specific sources which are then to be eliminated. If a result exceeds the HBM-II-value, an increased risk of health effects with the need for immediate action to reduce exposure is possible.

Reference values are extremely valuable to assess HBM results from an individual or of smaller or hot-spot studies. Numerous HBM-studies conducted in Germany have used the reference values of the Human Biomonitoring Commission to compare their study results with exposure of the general German population.

HBM-values can be used to assess health relevance of HBM data. According to GerES in 1990/92 (GerES II), for example, up to 2.4% of the adult population had elevated exposure levels, i.e., concentrations of lead, cadmium, mercury or PCP in blood or urine. In 1998 (GerES III) significantly less exceedances were observed. Only a few adults had concentrations of lead, mercury, or PCP in blood or urine above the HBM values.

In 1990/92 (GerES II), 2 to 11 children were exposed to lead in blood, cadmium in urine, mercury in urine, and PCP in urine in a concentration higher than HBM-I. In 2003/2006, only one child showed a concentration above the HBM-I for each lead or mercury in blood, and cadmium in urine. HBM values of PCP or Hg in urine were not exceeded.

The fact that cases exceeding the HBM values have become so rare is mainly due to preventive measures taken in the past [12].

The benefit from the German Specimen Bank

The German Environmental Specimen Bank (ESB) is a monitoring instrument of the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety, managed by the Federal Environment Agency [13], and operated by contracted research institutes and university groups with special competencies in the particular fields (*e.g.*, sampling of human, biological, and abiotic material, trace analysis of pollutants, cryobank operation).

Routine operation of the German ESB started in 1985. Human specimens are taken annually from students at four German universities and are archived as individual samples. Environmental specimens are also taken annually from representative marine, fresh water and terrestrial ecosystems. After pooling and homogenizing the samples are stored at temperatures below -150ºC.

After two decades of operation the ESB now provides a continuous historical record of the state of the environment in Germany in this period. It allows the retrospective monitoring of pollutants to identify temporal trends and spatial load differences. Target compounds may be those which had not yet been recognized as hazardous when the specimens were archived (emerging pollutants) or which could not be

analyzed with the desirable precision at that time. Thus the ESB makes it possible to analyze samples from the past using the analytical methods of the future.

To give an example: recently the focus in health related environmental monitoring has shifted to substances like polybrominated diphenyl ethers (PBDE)[1], perfluorooctanesulfonates (PFOS), and perfluorooctanoic acid (PFOA)[2] which are used as flame retardant.

Retrospective analyses have shown that corporal loads of dioxin as well as PFOS and PFOA in test persons have declined, whereas PBDE levels have increased. These German trends are corroborated by other national studies.

Future priorities and the German Health-Related Environmental Monitoring

GerES and the environmental specimen bank served as the basic elements of the German health-related environmental monitoring programme [14]. This program was implemented to track human exposure to hazardous substances which are then characterized and related to possible impacts on health.

German health-related environmental monitoring aims to:

- collect data about exposure to pollutants, noise, and about other environmental influences on the population,
- identify and quantify sources of exposure,
- illustrate temporal and spatial trends for purposes of forecasting exposure,
- identify "new" problematic substances (emerging substances) with high prevalence in the human body,
- investigate the socio demographically disproportionate distribution of environmental pollution,
- carry out a toxicological and health assessment,
- analyze the influence of environmental factors on public health and particularly susceptible subpopulations,
- draft and review the success of preventative and risk reduction measures within health and environmental policy programs,
- develop new analytical methods for human biomonitoring.

Main parts of these objectives can be achieved with ESP data, especially the monitoring of new and emerging substances in the frame of evaluating the success of REACH, for UBA a main task of future HBM. In this context European harmonisation and establishment of HBM has a high importance for UBA's work.

Additionally, a number of well centered research projects is under work which include, inter alia, human biomonitoring studies and especially cohort studies well suited to investigate the links between environmental exposures and health outcomes. Most recently UBA started to work on a concept for a birth cohort.

Critical steps and infrastructures needed when setting up a biomonitoring program at the national level

The most important requirement is political support which in most cases presumably might be the prerequisite to get financial funds. Thus it is essential to translate science to politics. Germany has the big advantage to have an established Human Biomonitoring Commission. This Commission is fulfilling this task and is very well accepted in the scientific community as well as by politicians.

A successful performance is safeguarded by a good central management, an operation manual, reliable laboratories and quality control of all steps. As mentioned in the beginning GerES was implemented in the 80s. In the beginning UBA measured only heavy metals in blood and urine and did this in own labs. During the following GerES the sampling, the instruments used and the pollutants analysed became more complex. However, UBA was able to learn about all critical points step by step which of course has been an advantage.

Last but not least participation might be the most critical issue because with a low response rate data cannot be regarded as representative on the national level. To achieve this again communication skills are needed. This means, on the one hand, that publicity is needed and an information campaign is necessary. On the other hand, ethics and data protection have to be safeguarded otherwise, at least in Germany, you would never convince people to take part in such a survey.

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[1] PBDE are flame retardants.

[2] PFOS and PFOA are typical exemples of perfluorinated chemicals (PFCs). PFCs are used to make materials oil- and water-resistent, among other applications.

Human biomonitoring in Flanders: some aspects related to study design, future, communication and ethics

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Abstract

Flanders is one of the few places in Europe with a legal basis to perform human biomonitoring (HBM). The HBM study is commissioned, steered and funded by the Flemish government and is carried out by the Center of Expertise for Environment and Health. This research consortium includes scientists from all Flemish universities and two Flemish research institutes.

The main purpose of the Flemish HBM program is to establish a surveillance network to make it possible to measure environmental pollution in the population and to investigate the relation between exposure and early health effects. In the first campaign (2001-2006) the question was whether living in different areas in Flanders resulted in a different exposure to environmental pollution. To make the translation of the HBM results into policy measures, the phased action plan was developed.

The second cycle of the Flemish HBM programme (2007-2011) is built on two pillars. First, reference values for the Flemish population will be obtained in a representative population sample for a broad series of pollutants. Second, targeted HBM will be performed in specific groups with a concern for environmental pollution pressure, the so-called hot spots. In both parts of the project, emphasis is placed on open and transparent communication and relevant interaction between scientists, policy makers, authorities, stakeholders and the public through a participative process.

HBM requires the collaboration of volunteers to donate blood, urine or other bodily tissues, and thus raises inevitable ethical questions. Some aspects showing that communication is at the heart of ethics are presented, as well as some difficulties from within the practices that arise in transnational research context.

Key words

Human biomonitoring, biomarkers of exposure, biomarkers of effect, surveillance

Human Biomonitoring in Flanders - first cycle (2001-2006)

A biomonitoring campaign in eight study areas and three age groups

In Flanders, a large human biomonitoring (HBM) campaign including more than 4,400 participants was carried out between 2001 and 2006. This project was implemented by the Flemish Centre of Expertise for Environment and Health, which was funded and steered by the Flemish government (Department of Economics, Science and Innovation; Flemish Agency for Care and Health; and Department of Environment, Nature and Energy). Within this Center, researchers from all Flemish universities and two research institutes provide different sorts of expertises: medical, environmental, statistical as well as social scientific.

The main purpose of the Flemish HBM program is to establish a surveillance network to make it possible to measure environmental pollution in the population and to investigate the relation of this exposure with early health effects. In the first campaign the question was whether living in different areas in Flanders resulted in a different exposure to environmental pollution. This project was carried out in eight study areas, covering 1/5th of both population and territory of Flanders. These areas were selected for their different environmental characteristics such as industrialised, rural, urban, near waste incinerators, near port areas and near fruit orchards. The focus was on three different age groups: newborns, adolescents and elderly people. The recruitment campaign for the three age groups was scheduled over three years (2002-2004). A Stratified Clustered Multi-Stage Design was used to select participants as a random sample of the population residing in the study areas. Sampling took place in three steps: first by study area, second by primary sampling unit (i.e. maternities (n=26) for newborns; schools (n=42) for adolescents, and communities (n=46) for adults), and third by random selection of the participants within the primary sampling units. The inclusion criteria were: 1) residing at least five years in the area; 2) giving written informed consent; 3) being able to fill in an extensive Dutch questionnaire. In the newborn study, twin pairs (n=10) were allowed; in the adult study, spouses (n=401) could also participate in the trial. In the newborn study, 97% of the eligible mothers agreed to deliver cord blood and answer the questionnaire. In the adolescent and adult study, invitation letters were sent via the schools and by regular post. 71.6% of the adolescents and 47.5% of the adults replied to the invitation, and respectively 85.7% and 75.3% of those who answered agreed to participate.

In each of these age groups selected pollutants and health effects were measured (table 1). The biomarkers of exposure were chosen because validated analytical methods were available and because they had known human health effects. Sample size calculations were based on data from a pilot project on HBM which was conducted in 1999 [1]. The age groups were chosen for various reasons. Newborns represent the most vulnerable age group and are interesting to follow-up in a birth cohort. The results of the neurological follow-up study and the follow-up study on asthma and allergy will become available in the next years. Adolescents represent the local environment since they mostly have not moved a lot. Young people show recent exposure, even for cumulative toxins. Elderly people are the most interesting target group to study cumulative exposure and chronic health effects such as diabetes or cancer.

In most cases, biomarkers of exposure did not exceed the actual norms or guidance values, as far as those are existing. For each of the study areas, anomalies in specific results were detected in comparison with the Flemish reference mean and reference 90th percentile (P90). Living in different study areas had a measurable impact on the results of cadmium, lead and chlorinated compounds [2,3]. Figure 1 shows an overview of the group results of the biomarkers of exposure for each age group in the different study areas.

In the environment, people are exposed to a mixture of pollutants. For this reason and also in order to look for relations with the measured

Table 1 **Overview of biomarkers of exposure and biomarkers of effect in the Flemish biomonitoring study 2001-2006 (Flanders, Belgium)**

	Newborns N=1200	Adolescents (14-15 y) N=1600	Adults (50-65 y) N=1600
Markers of exposure	**Cord blood:** cadmium, lead **Cord blood serum:** marker PCBs, pesticides, dioxin-activity	**Blood:** cadmium, lead **Serum:** marker PCBs, pesticides **Urine:** 1-OH pyrene, t,t'-muconic acid, cadmium	**Serum:** marker PCBs, pesticides, dioxin-activity **Urine:** 1-OH pyrene, t,t'-muconic acid
Markers of effect	Biometry, TSH (heel prick), Apgar score, time to pregnancy **Questionnaire:** Asthma & allergy **Follow up** of part of children	**Blood:** Comet test **Serum:** Hormone balance Biometry, sexual development **Questionnaire:** Asthma & allergy	**Blood:** Comet test **Serum:** Tumour markers **Urine:** 8-OH dG **Questionnaire:** Asthma & allergy
Co-variables	**Questionnaire:** General + food **Biochemical analyses:** Cholesterol, iron status cord blood	**Questionnaire:** General + food **Biochemical analyses:** Cholesterol, iron status blood, urinary creatinine	**Questionnaire:** General + food **Biochemical analyses:** Cholesterol, iron status blood, urinary creatinine

pollutants, biomarkers of effect were investigated. Small but significant differences in health effects were detected between the eight study areas, e.g. in the prevalence of asthma or pubertal development. Also, statistically significant dose-effect relationships were observed.

As communication and transparency are considered very important through the HBM campaign, a communication plan was developed [4]. In order to be transparent about the study, the research centre and the authorities organized information meetings before the actual start of the study to introduce the aims and means of the project in different regions in Flanders. Further, the dissemination of information was improved through a website.

In order to be transparent about the HBM results, study reports were made public. The ministers of Public Health and Environment together with the authorities and the research centre, organized press conferences and information meetings. Part of the complexity of communication was timing, leading to discussions about the best possible combination between a press conference with the ministers, an information meeting, informing local authorities and the principle of 'participants first'. While individual participants should by moral rights receive the reports (non-technical versions) before the press, at the same time the risk of participants leaking information to the press before a ministerial press conference should be taken into account. Very precise timing and tight schedules were therefore essential.

With regard to the individual results participants were given the opportunity to choose between several options:

- to receive the individual results at home;
- to have the individual results sent to their general practitioner;
- to receive no individual results at all.

Communication of individual results is complicated by the fact that interpretation on an individual level is often difficult. In order to give scientific interpretation meaningful for individuals, the quality of the biomarkers used in the HBM research is essential, and not all markers are good predictors of health risk on an individual level. Another essential factor is the availability of reference values or norms in humans with regard to health risk. International norms are available for lead only. This means that individual results have to be treated very carefully. The research centre provided the available international scientific information, together with information about uncertainties or unknowns. Information was also given on possible risks of certain substances and available preventive measures to lower the risks. The network of local environmental health professionals was informed, as well as general practitioners (if requested by participants).

A digital newsletter 'De Biomonitor' was developed, newsletter of the 'Flemish Environment and Health network', including the ministries of Public Health and Environment, the network of local environmental health professionals and the Center. Part of the aim of the newsletter is to publish the results from the HBM program and news about environmental health. 'Outsiders' are also invited to give their opinion about the study. Furthermore, other actors within the field can submit an article or a comment.

Interpretation of the study results: a phased action plan

The overview in Figure 1 shows a big amount and a complex mixture of results. Ordered by the authorities, the Centre of Expertise for Environment and Health together with scientists and the authorities developed an action plan to set policy priorities with regard to the HBM results [5,6]. This action plan included four successive phases, each focussing on different aspects:

- Pre-phase: difference with regard to reference value, pre-selection of anomalies/cases;
- Phase 1: assessment of priorities for policy making;
- Phase 2: identification of the cause and source;
- Phase 3: policy actions and evaluation.

Discussions in the working group mainly focussed on environmental and medical scientific interpretation of HBM data. It was thought of as a merely scientific quest: with the right group of experts the interpretation with regard to policy priorities would follow automatically. While trying to build bridges towards policy interpretation, though, the limitations of an exclusively scientific endeavour clearly showed: no scientist or group of scientists could claim they possessed the necessary and overarching knowledge to assess the policy priorities since also other than medical and environmental (scientific) factors had to be taken into account (economics, social preferences, feasibility of policy measures, issues introduced by the social scientists).

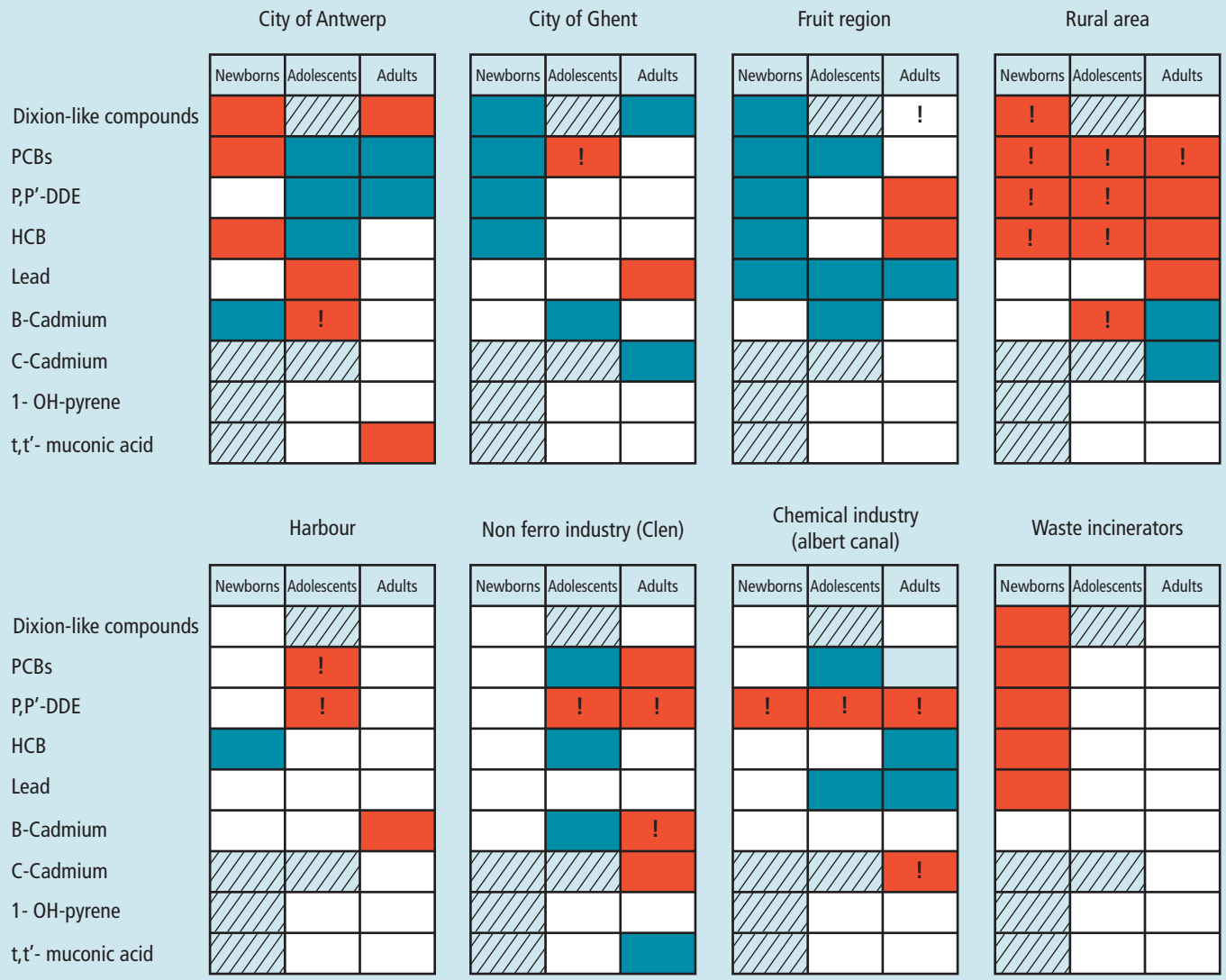
The social scientists proposed the formation of a stakeholder jury that would judge relevant data and knowledge in order to give advice to the government. Furthermore the social scientists developed a practice cycle with the different procedural steps, actors and roles for each phase of the action plan:

1. deciding how to operate and which actors to involve during the process
2. desk research on the study results
3. expert consultation
4. bringing a synthesis of the desk research and expert consultation before a jury of stakeholders
5. a synthesis of desk research, expert consultation and jury advice is presented to the decision makers
6. the decision makers decide on next steps.

During all of these steps, external communication about the process is important and is therefore included. The range of addressees for this communication depends on the decision making context.

This approach can be characterized as an example of the analytical-deliberative approach [7,8] or of the extended peer review [9,10]: next to experts stakeholders are also consulted. The action plan has rather successfully been tested in practice (for further reading see: [11, 12]).

Figure 1 **Overview of group results for biomarkers of exposure in three age groups in the different study areas (Flanders, Belgium)**



Red: significantly higher than reference mean
Blue: significantly lower than reference mean
! : significantly more than 10% of values above P90

Human Biomonitoring in Flanders - second cycle (2007-2011)

At the end of 2006, the Flemish government decided to continue the HBM program and start a new five-year cycle of surveillance. This current campaign runs from 2007 until 2011 and will consist of two main pillars. First, reference values for the Flemish population will be obtained in a representative population sample for a broad series of pollutants. Second, targeted HBM will be performed in specific areas with a concern for environmental pollution pressure, the so-called hot spots. In both parts of the project, emphasis is placed on open and transparent communication and maximum interaction between scientists, policy makers, authorities, stakeholders and the public through a participative process.

Reference values for Flanders

The second part of the HBM program builds further on the previous campaign (2001-2006) and aims to follow trends of internal pollutant levels in the Flemish population.

Three age groups are selected for biomonitoring. A newborn cohort (n=250) allows to assess exposure in the most vulnerable age group, i.e. the newborn baby. Moreover, follow-up of the children will allow to associate prenatal exposure with development later in life. 14-15 year old adolescents (n=200) represent an interesting group, since biomarkers in young people show recent exposure, even for cumulative toxins. The HBM data in adolescents can be linked with health information that is routinely collected through clinical examination by the school doctor. A third group are adults between 20 and 40 years (n=200) which are recruited to study associations between exposure and health outcome parameters such as fertility, asthma and allergy and cardiovascular effects. Based on the data of the first Flemish HBM study, sample size calculations were performed in order to select the number of participants in each age group.

Stakeholders could suggest pollutants of interest to be included in the Flemish HBM campaign. For all potentially interesting chemicals, fact sheets were composed with extensive information on the pollutant characteristics (e.g. production volumes, guidelines for internal or external exposure, margins of safety, health relevance, policy relevance etc.) and on the technical aspects of the corresponding biomarker (e.g. available measurement techniques, sample volume, price, available data in the international literature, expected levels in the Flemish population, etc.). Based on these fact sheets and on the available budget, an expert committee within the research consortium selected a panel of exposure markers to be measured in each age group. The set of biomarkers includes both historical pollutants and new emerging chemicals (table 2). The 'classical very toxic very persistent' pollutants like heavy metals, PCBs, dioxins, etc. are to date still measurable in the Flemish environment. By comparing the data of the first and second HBM campaign, temporal trends can be studied. This information will be

Table 2 **Overview of biomarkers of exposure and biomarkers of effect in the Flemish biomonitoring study 2007-2011 (Flanders, Belgium)**

Newborns & mothers N = 250	Adolescents (14-15 y) N = 200	Adults (20-40 y) N = 200
	Biomarkers of exposure	
Cord blood: heavy metals; PCBs; p,p'-DDE; hexachlorobenzene; Calux® assay; brominated compounds (PBDEs, HBCD); bisphenol A; TBBPA; perfluoro compounds (PFOS, PFOA) **Maternal blood:** heavy metals **Maternal hair:** (Me)Hg	**Blood:** heavy metals; PCBs; p,p'-DDE; hexachlorobenzene; Calux® assay; brominated compounds (PBDE, HBCD); TBBPA; nitro- and polycyclic musks **Hair:** (Me)Hg **Urine:** total As and toxic relevant As; 1-OH-pyrene; t,t'-muconic acid; phthalates; dialkyl phosphate metabolites; 2,5-DCP; p-OH-benzoïc acid; bisphenol A; cotinine	**Blood:** perfluoro compounds (PFOS, PFOA) **Urine:** total As and toxic relevant As; 1-OH-pyrene; t,t'-muconic acid; phthalates; dialkyl phosphate metabolites; 2,5-DCP; p-OH-benzoïc acid; cotinine
	Biomarkers of effect	
Cord blood: thyroid hormones; sex hormones; insulin, leptin **Birth registry:** length; weight; head circumference; gestational age **Questionnaire:** asthma & allergy of the mother; fertility and miscarriages of the mother **Follow-up study:** neurological development	**Blood:** thyroid hormones, sex hormones (in boys only), comet assay, gene expression **Medical files of school doctor:** growth, pubertal development, gynaecomastia **Fieldwork:** Neurobehavioral Evaluation System (NES test) **Questionnaire:** asthma & allergy, age at menarche in girls, ADHD questions	**Blood:** cardiovascular markers, sex hormones (in men) **Questionnaire:** asthma & allergy, fertility, miscarriages (in women)

PCBs: polychlorinated biphenyls; p,p'-DDE = p,p'-dichloor diphenyl dichloro-ethane (metabolite of DDT); PBDE = polybrominated diphenyl ethers; HBCD = hexabromocyclododecane; TBBPA: tetrabromobisphenol A; (Me)Hg = (methyl) mercury; PFOS = perfluoro-octane sulfonate; PFOA = perfluoro-octanoic acid; As = arsenic.

complemented with exposure data on 'new' and emerging pollutants. The current surveillance campaign will allow to assess for the first time exposure to pollutants such as polybrominated flame retardants, phthalates, perfluoro compounds, etc. in the general Flemish population. Biomarkers of effect have a high added value. Measurements of biomarkers of exposure and biomarkers of effect (table 2) in the same individuals allow to evaluate dose-effect relationships which may provide insight in the associations between exposure to pollutants and biological effects. Moreover, biomarkers of effect may provide a measure for integrated exposure since multiple pollutants may affect biological function through a similar mechanism, e.g. several pollutants have immune-disrupting properties and thus may contribute to the development of asthma. In adolescent and adults, cross-sectional data are available; in the newborns, prospective data will be collected in a follow-up study.

Biomonitoring in hot spots

Targeted HBM will be performed in hot spots, i.e. geographical areas or population groups with a concern for environmental pollution pressure.

For the selection of the hot spots, a one-year participative process was developed which includes participation of a heterogeneous group of stakeholders such as researchers, local environmental health professionals, health workers, governmental organizations (e.g. health registries, health and environmental administrations and agencies, etc.), local communities, industry, union representatives, consumer groups, local action groups, etc. The selection process involved a broad, open call for suggestions of hot spots, scientific documentation by researchers of the consortium, expert evaluations from external scientists, social scientists and policy makers and an advisory role from local experts and societal action groups. The aim of this process was not only to select relevant and interesting hot spots, but also to create a platform, so that the performance of the HBM, communication of the results and possible policy interpretations or proposals for action will have a large base and reach a broad public. Intensive interactions between different stakeholders in all stages of the research process will increase the rate of success of an efficient "environment and health" policy.

Ethics and human biomonitoring

Since HBM requires the collaboration of volunteers to donate blood, urine or other bodily tissues, it raises inevitable ethical questions. Surveillance or studies using biomarkers can only take place under strict conditions that guarantee full respect for human dignity. These are regulated in the recommendations from the Convention for Human Rights and Biomedical Science [13], the European Privacy Directive [14], and in many other (inter)national and European declarations, conventions and regulations, implemented by national law.

Participating in environmental health studies does generally not offer any immediate advantage to the volunteer, but renders an important service to the society by contributing to scientific knowledge and better policy. In contrast with the clinical context, the notion of public interest moves more to the forefront.

Some aspects on communication related to study design are described above. In addition, communication is crucial at recruitment and when disseminating results, on individual as well as on collective level.

It is firstly paramount that participants consent in participating, based on good information about the goals, risks, benefits and expectations of the study. It is the duty of each researcher to strive for an informed consent, as authentic as possible. The value in terms of authenticity of informed consent is primarily related to the communication between persons. Communication is context-based and depends upon the implicit interests and competences of participants [15]. Each person has his own stakes and believes. Because communication is always considered to be incomplete or partial, rooted in background knowledge and opaque, the ideal of "fully explicit and specific" consent may not be feasible. Therefore there is reason to be suspicious of the (legal) prerequisite that informed consent needs to be explicit and specific. Communication is furthermore influenced by the complex interaction between the personalities of the persons involved.

To optimally utilize the collected biological material, a later and unforeseen use of samples or data may be recommended, for which no explicit consent was asked. In principle a new consent should then be asked. This may cause not only a lot of extra work, it is also uncertain that each participant will send a new written consent, often out of practical reasons, causing in turn a possible bias in research results due to incomplete data.

Suitable communication strategies are thus to be very carefully prepared for all phases of the study, including at the presentation of the results. In Flanders, volunteers participating in HBM studies have a right to know the results, as well as the right not to know. This may create difficulties as HBM results are often difficult to interpret, especially in terms of future health at the individual level.

On the larger scale, transnational research is key for further investigation of the health impact of environmental stressors. Transfer of sensitive personal data or samples from one Member State to another is often required. Study participants should be protected equally throughout the whole EU, but differences in national legislation and in interpretation of EU or international guidelines exists.

The foremost question to be asked is if the current ethical and legal context within the field of environmental and public health surveillance and research and its related practices truly protect the autonomy and ultimately the dignity of the study participants ? It is obvious that understandable information and good communication practices go beyond a fully standardized juridical informed consent procedure.

Ethically correct conducted surveillance and studies, including appropriate communication strategies, avoiding unnecessary bureaucratic paperwork, will in the long term create openness, transparency and participation, pillars of democratic society and will benefit the research community as well as the general public. Societal acceptance of practices in general will depend on good communication at all levels.

At the long term, the scientific community will benefit from the trust that is built up by fully respecting the dignity and integrity of each study participant and improving solidarity, which is crucial for guaranteeing the disposition of the necessary samples for important research.

The legal basis and future of HBM in Flanders

Flanders is one of the few places in Europe with a legal basis to perform HBM.

In the aftermath of a series of environmental health problems in the nineties, societal anxiety and pressure resulted in the legal recognition of environmental health as an element of preventive public health policy. In 2003, the Flemish government voted the Decree on preventive health care. Hereby different policy initiatives are made possible.

Elements of the Decree are, among other, the use of the precautionary and the polluter pays principles, setting up structures for networking, preventive action and informing the public. The Decree also makes it mandatory for the Flemish government to perform a HBM program. This is as such an important policy signal.

The Flemish government can establish limit values in human beings and take measures to reduce exposure and to protect public health if the limit values are exceeded, whether there is scientific proof or probability of harmful impact on health. It can establish a network of surveillance of the exposure measured in human beings and/or effects of physical and chemical factors on the population, with the aim to be able to take measures to protect the public health.

HBM is a relatively new tool for environmental health policy. There are a lot of unanswered scientific questions and the interpretation of the results in public health terms is very complex and still in development. This makes that in Flanders, HBM programme for the moment is still outsourced to the scientific community.

From the policy perspective it is important to assure the long term continuity of the HBM programme. Notwithstanding that support from the scientific community stays important in the long run, academic groups possibly are not the best organisations to do routine surveillance. Therefore, once HBM is, from a scientific public health point of view, more stable and established, it would be preferable to embed it in a governmental institution rather than to outsource it. As such, it would follow the example of the water, air and soil monitoring. Translation of the HBM results into policy measures and setting priorities remains very important.

Another question the authorities are exploring is how fundamental research projects can be connected to the logistical framework of the surveillance programme. A large part of the cost of surveillance is explained by the requirements of field work with recruitment and collecting samples. Connecting both, surveillance and research projects, can result in reducing the overall cost and enhance the research needed to meet the upcoming (emerging) issues in environmental health.

For comparison of the results with other European countries, harmonisation is needed. Finding a balance between a European harmonisation and the possibility for each country to meet the local problems and needs is important.

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Biomonitoring as a policy lever: a case study of mercury and pesticide surveillance in New York City

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Abstract

Introduction - In 2004, the New York City (NYC) conducted a population based environmental biomonitoring study to characterize exposures to selected biomarkers and to inform the choice and conduct of public health and policy actions to reduce exposures.

Biomonitoring methods - The survey collected and analyzed urine and blood to evaluate inorganic and organic mercury and urinary metabolites of organophosphorus and pyrethroid pesticides (n=1,811).

Biomonitoring results - 95th percentile levels of inorganic mercury among those born in the Dominican Republic were higher than others, largely attributable to the use of illegally imported mercury containing skin lightening creams. Total mercury was three times higher in NYC than the United States (US), with population differences within NYC largely explained by varying frequencies of fish consumption. Pesticide exposures were 4 to 14 times higher in NYC than in the US.

Policy actions and discussion - Biomonitoring led NYC to actions that included the embargo of products, expanded inter-governmental oversight of mercury in fish, public and healthcare provider education campaigns, and local efforts to restrict the use and availability of pesticides. The article presents and discusses a policy framework to explain why environmental biomonitoring results appear to readily influence public policy.

Key words

Human biomonitoring, pesticides, mercury, New York City, policy analysis, NYCHANES

Introduction

Over the past several years, the New York City (NYC) Department of Health has enhanced its environmental health surveillance activities to encompass a variety of new content areas and new sources of data that describe the city and its population[1]. In 2004, New York City conducted the first community analog of the United States' National Health and Nutrition Examination Survey (NHANES). The NYC version, NYCHANES examined 1,999 people representing the adult non-institutionalized population of NYC. The primary aim of NYCHANES was to provide objectively measured prevalence estimates of and information about the awareness, treatment and control of chronic conditions [2]. NYC conducted a biomonitoring study that took advantage of the extensive infrastructure and specimen gathering of NYCHANE. Table lists seven key objectives of NYC's environmental biomonitoring efforts. The final objective - to inform the choice and conduct of public health and policy actions to reduce exposures - may be crucial to draw attention and resources to future biomonitoring efforts. (table)

Biomonitoring methods

NYCHANES was a population-based cross-sectional study of New York City adults aged 20 and older conducted between June and October, 2004. Details about NYCHANES' study design, sampling and collection methods were previously described [3]. The biomonitoring component of the survey selected environmental biomarkers, including inorganic and organic mercury and urinary metabolites of organophosphorus and pyrethroid pesticides. Urine and blood were collected on 1,811 subjects. Metals were analyzed by the New York State Wadsworth Laboratories, and pesticides by the United States Centers for Disease Control and Prevention (US CDC) National Center for Environmental Health Laboratories.

Biomonitoring results

Inorganic mercury

On average, levels of inorganic mercury were low in NYC. However, the upper 95th percentile of Hispanic New Yorkers born in the Dominican Republic had exposures exceeding 21.2 µg/l, more than six times that of the 95th percentile of other New Yorkers. We conducted follow up interviews and home visits to many of these participants, and determined that each of the cases we were able to interview were attributable to the use of mercury containing skin lightening creams manufactured principally in the Dominican Republic and illegally imported to the US. While the number of participants with significantly elevated inorganic mercury from the use of these products was small, they represented as many as 25,000 New Yorkers.

Organic mercury

We also measured total mercury in blood. We found that New Yorkers, on average, had mercury exposure three times that of the United States overall [4]. Levels were particularly high among Asian New Yorkers, and highest among the foreign born Chinese (Figure 1). Fish consumption was the greatest driver of mercury levels, with a strong linear relationship between frequency of seafood consumption and measured mercury levels. Mercury levels were three times higher in NYC even among those who did not consume fish.

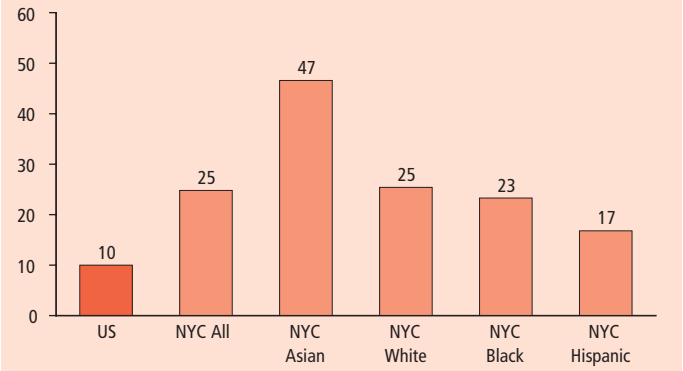
Pesticides

NYCHANES measured urinary metabolites of organophosphorus (OP) and pyrethroid pesticides using methods described previously and employed in NHANES [5,6]. We completed analysis of a simple random sample of 380 subjects, and expect to report findings on the full 1,811

Table **Objectives of the NYC Environmental Biomonitoring Program (2004)**

The objectives of the NYC biomonitoring program were to:	
1.	Estimate the population distribution of exposure to heavy metals, pesticides and cotinine.
2.	Compare NYC's exposure to that of referent populations, in particular, the United States as a whole.
3.	Identify sub-populations at greater or lesser risks of exposure.
4.	Analyze risk factors for environmental exposures.
5.	Determine baseline exposures against which future findings may be compared.
6.	Establish a population-based serology repository for use in exploring emerging environmental health issues.
7.	Inform the choice and conduct of public health and policy actions to reduce exposures.

Figure 1 **Percent of Adults Above Reportable Mercury Levels of 5 µg/l (New York City)**



samples in 2010. Metabolite concentrations of each class of pesticides in NYC were 4 to 14 times higher than in the US [7]. Figure 2 compares 95th percentile levels of the total OP and a representative pyrethroid metabolite, phenoxybenzoic acid (PBA), indicative of a broad group of pesticides. We observed significant disparities in exposure by gender and by whether the participant's home had recently been visited by a pest control professional (PCP). PCPs are licensed and regulated by the State of New York. Concentrations of pesticide metabolites were two to four times higher among women than men. This is significant because in-utero exposures to pesticides have been associated with lower birthweight and cognitive delays in children [8].

These findings demonstrate three important contributions made by the biomonitoring program. First, it documented disparate exposures within the NYC population; second, it found that exposures are substantially higher in NYC than the US; third that women may be exposed more to agents associated with reproductive health outcomes; and finally, that activities regulated by government influence exposure.

Policy actions undertaken in response to biomonitoring findings

Policies are actions that guide decisions and other actions undertaken by governmental and non-governmental and private sectors. In keeping with the intent of the NYC biomonitoring program, findings were rapidly assessed for their public health significance and to inform opportunities to reduce exposures.

Inorganic mercury

Following the discovery of the skin lightening creams, the NYC Health Department sent inspectors to more than 400 stores in neighborhoods with the greatest number of people from the Dominican Republic, based on the 2000 US Census. Inspections found 13 brands of mercury containing skin lightening creams that were embargoed by the Department at more than 100 stores. To help inform consumers, orders were issued requiring stores with embargoed products to post warning signs for six months (Figure 3). Given the increasing frequency with which contaminated imported products are discovered, the Department created a unit within its lead poisoning prevention program to continue store inspections and public outreach. With the cooperation of the Pan American Health Association, we reached out to the Dominican Republic's Ministry of Health, which in turn conducted inspections of the laboratories and ordered cessation of production in at least one. Extensive significant print and television media of the findings contributed to greater public awareness of the existence of these products.

Organic mercury

Our biomonitoring findings made the case that federal and state authorities' prior emphasis on avoidance of mercury exposure from freshwater sport fish was inadequate to address the population sources of exposure to mercury, namely commercially sold fish. Biomonitoring led to close collaboration among the Health Department, the US EPA and the State Department of Agriculture and Markets. All three agencies collaborated on sampling of commercial fish sold in NYC, findings from which will be made public in 2009. Using its Health Alert Network, NYC issued clinical guidance to most physicians and nurses within NYC on organic mercury exposure and health risks, encouraging patient education and discouraging routine testing. The Health Department also created its first fish-related educational campaign that has since circulated 200,000 fish consumption guides in three languages to "Eat Fish, Choose Wisely" (Figure 4).

Pesticides

In 1999, an environmental advocacy group published results of its analysis of a unique New York State database of pesticide applications, showing that four of the five counties that comprise NYC ranked in the top ten for pesticide use statewide, outranking many highly productive agricultural counties and suburban counties with significant acreage of tended lawns [9]. NYC's biomonitoring results confirmed these advocates' claims that urban pesticide exposures may be greater than previously acknowledged. Since the study, NYC became the largest city

Figure 2 **95th percentile pesticide metabolite exposures in NYCHANES by gender and recent presence of pest control professional (New York City)**

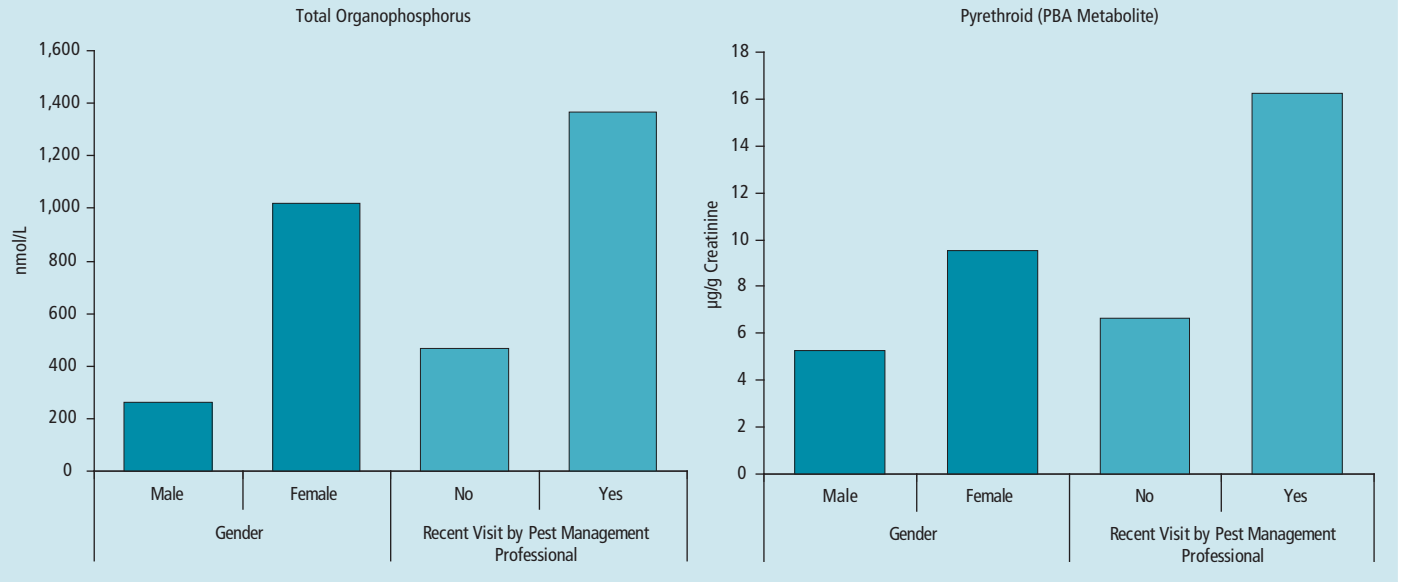


Figure 3 **NYC-mandated store window posting warning of mercury in skin lightening creams (English and Spanish).**

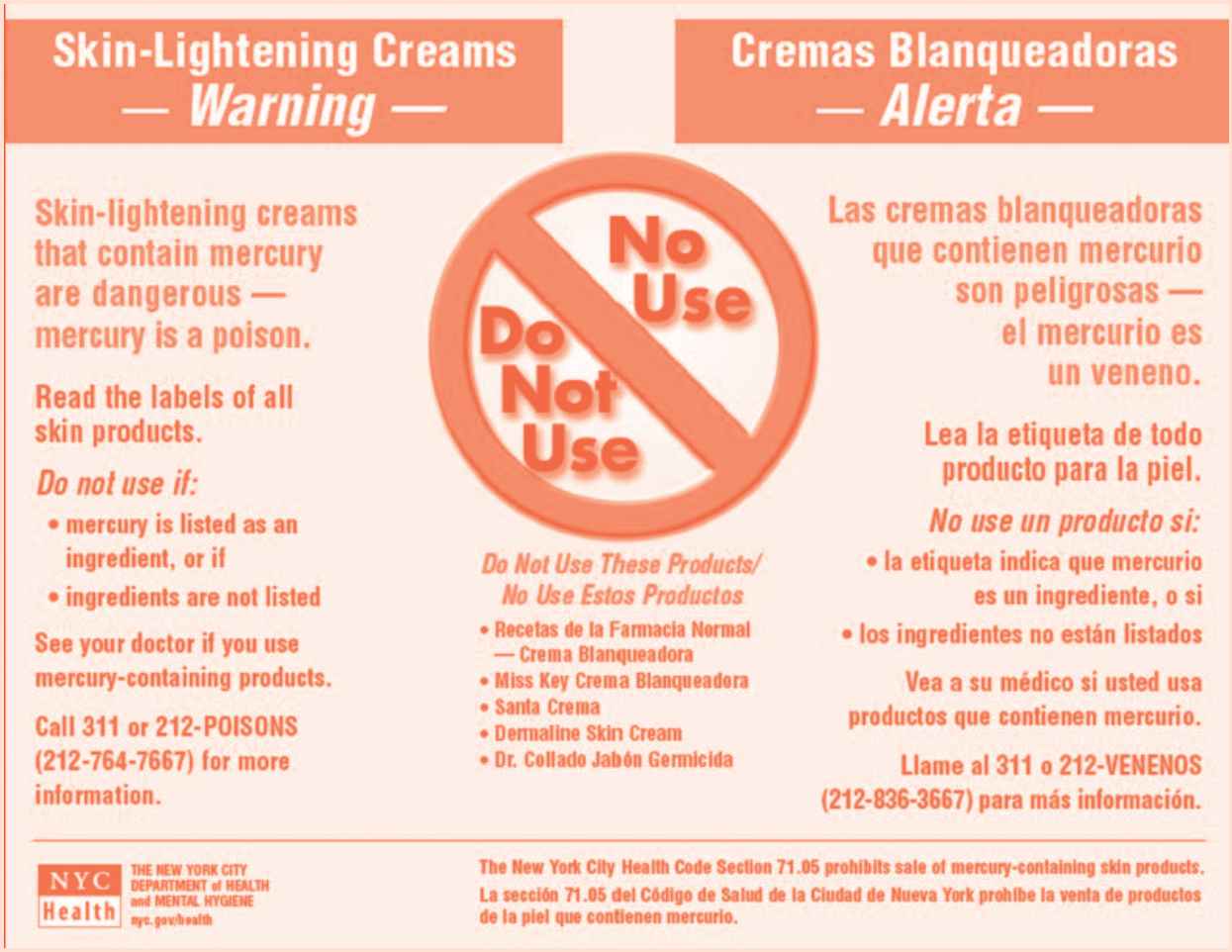
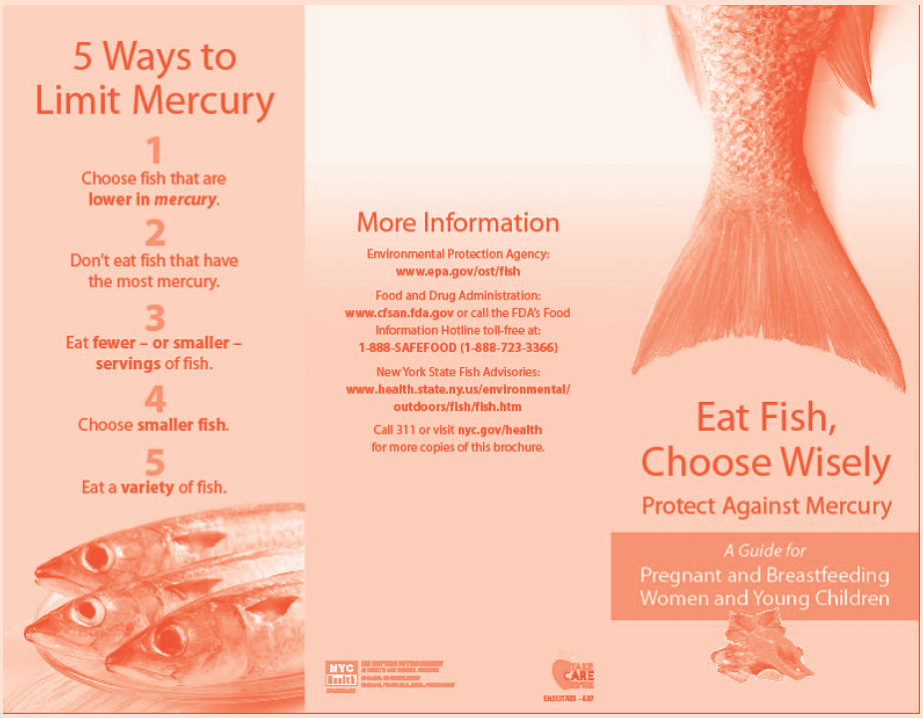


Figure 4 **Mercury in fish guidance cover sheet (English) (New York City)**



in the US to restrict local governmental use of pesticides, and now requires reporting and public disclosure of pesticide use and trends, in part to drive demand for safer pest control. Staff at federal and state agencies to whom these results have been presented report that these data have led to a greater recognition that urban exposures are significant. NYC is now represented on an EPA's public advisory committee on pesticides, and will soon join the EPA and state agencies in hosting a symposium on the problem of urban pesticide use. Recently, NYC helped to obtain the commitment of New York State's pesticide regulators to prohibit the use of structural pesticide products formulated as total release foggers [10].

Discussion

In the relatively brief time since NYCHANES' reporting of findings, NYC has been able to take and move other governmental agencies to take a variety of policy actions that collectively have significant potential to reduce future exposures to mercury and pesticides.

A framework for understanding biomonitoring's potential to affect policy

In the early 1990s, two North American social scientists, Paul Sabatier and Hank Jenkins-Smith, described a framework for considering how policy decisions are made amidst a system of policy information, beliefs and resources [11]. Policy actors may include local, state and federal governmental agencies, elected officials, health organizations, advocates, industry and others, depending on the policy problem. Through negotiation, conflict or consensus, these actors determine policy decisions, which in turn yield results that cycle back to ongoing policy reanalysis and reformation. External to this policy system are less tractable but important factors that include how a problem is defined, the information available, and the rules of engagement. Important for this discussion is that policy systems tend to maintain the status quo until a significant change in any element introduces sufficient disequilibrium.

Why biomonitoring has the potential to readily influence policy

In considering the variety of policy impacts the biomonitoring program in NYC has been able to achieve, it is useful to consider which elements in this policy framework were influenced most by biomonitoring, and how.

- Biomonitoring can have profound impacts on a variety of beliefs. In the case of pesticides, for example, regulators commonly believe that the registration and approval process for products is sufficient to ensure that exposures will be minimal, and that adherence to use instructions ensures a products' safety. NYC's findings challenged those beliefs.
- Biomonitoring can redefine the scope of problem. NYC's findings redefined the problem of "mercury in fish" to "mercury in humans due to fish." When the problem of pesticides was redefined with the inclusion of exposure and not just use, governmental agencies could consider what role they may play in preventing exposure. By virtue of uniformly higher exposures in NYC, defining pesticides and mercury as a problem of urban life prompted state and federal agencies to be more sensitive to urban concerns.
- Biomonitoring can influence the relative priority of different values. In the US, agencies charged with regulating consumer products and food may have dual roles; protection of the public and promotion of commerce. Human exposure data helps tip the scale to public protection.
- Biomonitoring may also influence people's beliefs that a problem is susceptible to change. By quantifying a problem and demonstrating disparate impact on subpopulations, biomonitoring demonstrates the potential to influence exposure, paving the way for policy actors to consider ways in which their own actions can influence the potential for, or against, exposure.
- Finally, biomonitoring results influence belief in the efficacy of governmental action. Whether comparing baseline levels to a standard, or tracking levels over time, exposure data can be used to evaluate the effect of governmental actions intended to influence exposure in a shorter timeframe than health outcome data, especially for exposures associated with chronic illnesses. And, the ability to evaluate and demonstrate efficacy may influence the willingness to consider policy changes themselves.

Limitations

The generalizability of these outcomes from NYC's biomonitoring efforts may be limited by a number of factors. NYC's study was conducted as part of a broader population-based health and nutrition survey. It is likely that its findings were compelling enough to influence policy in part because the findings were not profoundly limited by response bias. In addition, NYC chose the subjects of its biomonitoring - mercury and pesticides - mindful of its potential to interpret and act on findings. Not all biomarkers in human biomonitoring avail themselves so readily to interpretation given the absence of a standard or reference dose, nor to action given uncertainties over sources of exposure.

Conclusion

NYC's population-based biomonitoring program supplemented more commonly available health data, identified populations at greater risk of exposure and echoed public concerns by implicitly emphasizing primary prevention of exposure rather than hazard mitigation or health care. Biomonitoring is but one of many environmental public health surveillance instruments. However, its unique capacity to verify human exposure enhances its ability to influence policy by modifying assumptions, creating disequilibrium between competing assumptions regarding risk, engaging new policy stakeholders, and modifying the beliefs of key policy actors.

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Urinary biomarkers for pesticide exposure in pregnant women of the Pelagie cohort study conducted in Brittany, France (2002-2006)*

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Abstract

Although pesticides have many applications, they are primarily used for agricultural purposes. Pesticide exposure sources in humans are numerous. Overall pesticide impregnation levels in France and the majority of European countries have yet to be clearly established. One benefit of using biomarkers to assess exposure is that it covers all possible exposure routes. Given the high sensitivity of faetuses to toxic compounds measuring the exposure of pregnant women has become a critical public health issue.

The Pelagie cohort has included nearly 3,500 pregnant women from Brittany (France) over the 2002-2006 period. Urine samples were taken in the early stages of pregnancy, 546 of which underwent testing for chemical pesticides. The objective was to assess pesticide impregnation levels and scope among pregnant women, more specifically in regards to herbicides of the triazine family, prohibited in France since late 2003 but still present in the environment, and to organophosphorous insecticides, used both for agricultural and non-agricultural purposes.

Findings confirm the presence of pesticide residues in a majority of urine samples of pregnant women, some molecules being breakdown products remaining in the environment. These pesticide residues are usually numerous and their impact, either individually or jointly, on the fœtus and its development has yet to be clearly established in epidemiological literature. They are soon to be assessed in the Pelagie cohort.

Key words

Biomarkers of exposures, pesticides, pregnancy, Britanny

Introduction

Most pesticides are used in an agricultural context for treating crops. But pesticides are also used for numerous other purposes such as: maintenance of private gardens, alleyways and vegetable gardens, domestic mosquito and ant control, veterinary use or antiparasitic agent, including for domestic animals, industrial use, and community use for cleaning public gardens, roads and railways. Such varied uses lead to soil, water and air contamination by pesticide molecules which in turn contaminate plants, animals and humans [1].

Many surveys indicate environmental contamination through pesticides. In 2006, the IFEN (French Environment Institute) identified pesticide residues in French waterways in 90% of the thousand stations spread over the territory, establishing average to poor water quality in over one-third of sites tested for pesticides [2]. Pesticide traces were detected in half of underground water testing stations. A small portion of the population (1.5% for Brittany, 5% for France) was thus exposed to tap water that exceeded the 0.1µg/l regulatory limit for pesticides [3]. Rainwaters are locally and punctually very contaminated by pesticides [4]. Air quality monitoring agencies of various French regions regularly detect the presence of pesticides in the atmosphere [5,6]. Various surveys have also detected pesticide residues in French households [7]. A recent European survey has confirmed the presence of pesticide residues in nearly half of all fruits, vegetables and cereals available throughout the European Union [8]. Finally, our daily activities, including feeding and domestic occupations, expose us to pesticide residues through ingestion, inhalation or skin contact. Such exposure is generally deemed of a low level, but of a continuous or repeated nature. The fact remains that current pesticide exposure levels are essentially unknown in France and in the majority of European countries.

Exposure to pesticides is a critical issue for the population in Brittany, given intensive regional agricultural activities. The Pelagie cohort was conducted in order to assess the impact of environmental contaminants in Brittany on intrauterine and infant development. The Pelagie cohort relies on the use of pesticide exposure biomarkers that reflect the internal dose or biological impregnation levels and cover all potential exposure routes, through the collection of urine samples from women at the beginning of their pregnancy, between 2002 and 2006. We focused primarily on herbicides of the triazine family and on organophosphorous insecticides, which are suspected to be toxic for reproduction and neurodevelopment. Atrazine and simazine, both of the triazine class, are herbicides used in corn crops. Their use has been prohibited in France since late 2003. Their breakdown products are easily disseminated through waterways and are still very much present in the environment, primarily in dealkylated (water) and hydroxylated forms (soil and plants), either month or years after being used. Worldwide, atrazine remains of the most used herbicides. Embryotoxic and embryolethal effects, as well as dysfunctions of endocrine and immune systems, have been reported in animals following prenatal exposure to atrazine [9]. Organophosphorous insecticides have essentially replaced organochlorine pesticides in many crops and are present in non-agricultural products, such as those used at home and on animals. Few animal studies have revealed teratological effects following prenatal exposure to organophosphorous insecticides. However, the observation of neurotoxic effects following moderate and even low exposure levels remains a great source of concern [10,11].

This article intends to describe exposure levels to various pesticides, based on urine samples of women from the Pelagie cohort. Findings were presented at the European Conference on Human Biomonitoring, held in Paris in November 2008[1].

Methodology

The Pelagie Study

This epidemiological study is based on the Pelagie cohort including nearly 3,500 pregnant women in the early stages of pregnancy and planning the follow-up of infants over a period of several years.

1 http://www.invs.sante.fr/agenda/biosurveillance_2008/programme_en.htm

Pregnant women were recruited during medical consultations at the beginning of pregnancy (prior to 19 weeks of amenorrhea) from the Brittany region (Ille-et-Vilaine, Finistère and Côtes-d'Armor) over a period of more than three years (2002 - February 2006). Each participating doctor informed the woman of the objectives and methodology of the study and asked for their consent to participate. A questionnaire was then handed to the pregnant woman during her initial visit and she was asked to fill it up at home and to return it directly to INSERM U625. She also had to return a morning urine sample (sample the most susceptible to contain recent traces of exposure to the non persistent pesticides targeted by the study [12] via the provided stamped packaging. Early pregnancy urine samples were thus collected from nearly 95% of women participating in the Pelagie study.

Various samples were taken at birth: an umbilical cord blood sample, a hair strand from the mother, and a placenta sample. Midwives also collected information on pregnancy and birth conditions. The infant's health status was assessed by the pediatric perinatalogist. Biological samples were stored and frozen, specifically at -20°C for urine samples.

Urine chemical determination

Chemical pesticide determination of urine samples was based on a case-cohort design, including a group of 569-children (18% of the liveborn singleton cohort with no maternal hypertension) selected at random amongst the cohort and referred to as the sub-cohort [13]. A total of 52 molecules was analyzed in urine, including 12 from the triazine class, 32 from the organophosphorous class, 6 from the amide class and 2 from the carbamate class.

Chemical analyses were carried out by the IDHESA Institute (Plouzané, Finistère) on a maximum 10 ml urine sample, using both liquid chromatography and triple quadrupole mass spectrometry (LC/MSMS) following solid phase extraction. Pesticides in urine samples were extracted using an online solid phase extraction system of the Symbiosis Prospekt II type and a Hysphere C18 HD cartridge, and eluted during the chromatography mobile phase. Separation was achieved by liquid-phase chromatography (Alliance Waters, Separations Module 2690), using a Synergi Fusion RP C18 column, (250 X 2 mm, 4 µm) and an elution gradient: acetonitrile/formic acid 0.01% and 5mm ammonium formate/formic acid 0.01%. Detection relied on LC/MSMS (Quattro Ultima, Micromass/Waters). Reference standards were provided by Riedel-de-Haën Fine Chemicals and Dr. Ehrenstorfer GmBH. Three internal standards were used for extraction and detection controls: deuterated simazine 10, deuterated diuron 6 and DMButylphosphate. Given the number of molecules analyzed simultaneously, this methodology is quite innovative and allows for greater detection limits by using LC/MSMS (minimum of 2 transitions) and 3 internal standards. Detection and quantification limits (QL) for each molecule targeted by the laboratory are stated in Table 1. QLs range from 0.001 to 1.7µg/l. Concentration range linearity was observed from 0.010 to 10 µg/l for pesticides of the lowest detection limits and from the detection limit for other pesticides. Average recoveries were 100% +/- 20%, with variation coefficients ranging from 0.1 to 13.9%.

Statistical analysis

Chemical determination values are expressed in µg/L. For each compound, non-quantified values have been replaced by the detection limit divided by the square root of two. Molar concentrations, expressed in nmol/L, were measured by dividing chemical determination value by the molar mass. Molar concentrations were then summed up to compute exposure biomarkers for several molecules. Adjusted concentrations based on urinary creatinine, a urine dilution index, were calculated. Geometric means were calculated in order to limit the influence of extreme values. The SAS® software was used for all calculations (SAS/STAT version 9.1, SAS Institute, Inc., Cary, NC, USA).

Findings

Among the 569 women from the Pelagie subcohort, 23 women failed to provide a urine sample or did so too late regarding the inclusion criteria. Consequently, results of urine pesticide determination are available for 546 women (96%) of the sub-cohort (Table 2). Most of these women came from the Ille-et-Vilaine department. Nearly three quarters of these women belonged to the 24 to 35 age group and 45% were primiparous. More than 60% of participants reported two years of post-baccalaureate schooling. Roughly 27% of women reported having smoked at the beginning of pregnancy. A total of 15% of women reported having consumed one glass of alcohol a day in the early stages of pregnancy while only 1% reported having consumed more than one glass a day. Urine samples were collected after 11 weeks of pregnancy from half of the women. Frequency of urine collection varied slightly according to seasons (28% in Autumn, 26% in Spring) for which

Table 1 **List of triazine compounds and organophosphorous insecticides (OP) in decreasing order of quantified values in µg/L amongst the 546 urine samples collected from pregnant women of the Pelagie study cohort (Brittany, France, 2002-2006)**

Family	Metabolite	Detection Limit (µg/L)	Quantification Limit (QL ; µg/l)	n (>QL)	%(>QL)
OP	DMP	0.058	0.2	458	83.88
OP	**Diazinon**	0.0002	0.001	206	37.73
OP	DEDTP	0.005	0.02	195	35.71
OP	**Ethion**	0.003	0.009	168	30.77
OP	DMTP	0.32	1	154	28.21
OP	chlorpyrifos-oxon	0.000	0.002	139	25.46
OP	**Phoxim**	0.026	0.09	137	25.09
OP	DMDTP	0.127	0.45	110	20.15
OP	Paraoxon-methyl	0.0225	0.75	106	19.41
OP	DEP	0.366	1.25	96	17.58
Triazine	Atrazine desipropopyl-2-hydroxy	0.043	0.15	88	16.12
Triazine	Atrazine desethyl desisopropyl 2-hydroxy	0.067	0.25	77	14.10
OP	4-nitrophenol	0.062	0.21	75	13.74
OP	**Phorate**	0.003	0.01	68	12.45
OP	**Chlorpyrifos**	0.01	0.035	66	12.09
OP	**Chlormephos**	0.017	0.06	62	11.36
Triazine	Atrazine desethyl	0.001	0.003	58	10.62
OP	4-nitrophenyl (sulfate) potassium salt	0.022	0.08	58	10.62
Triazine	Atrazine-2-Hydroxy	0.005	0.02	56	10.26
OP	Paraoxon-ethyl	0.015	0.05	56	10.26
OP	TCPY :3.5.6-trichloro-2-pyridinol	0.042	0.15	52	9.52
OP	DETP	0.508	1.7	47	8.61
Triazine	Simazine 2-hydroxy	0.006	0.02	46	8.42
OP	Terbufos-sulfoxyde	0.002	0.008	43	7.88
Triazine	Simazine mercapturate	0.014	0.06	42	7.69
Triazine	Atrazine desethyl desisopropyl	0.135	0.5	39	7.14
OP	Terbufos- oxon-sulfone	0.014	0.045	36	6.59
OP	**Parathion-ethyl**	0.362	1.5	36	6.59
OP	**Parathion-methyl**	0.07	0.25	34	6.23
OP	**Malathion**	0.002	0.006	33	6.04
OP	chlorpyrifos methyl-oxon	0.015	0.05	31	5.68
OP	**Fenthion**	0.014	0.05	30	5.49
Triazine	Atrazine desethyl 2-hydroxy	0.094	0.315	27	4.95
OP	Terbufos-oxon	0.003	0.009	27	4.95
OP	Terbufos-sulfone	0.003	0.01	27	4.95
Triazine	Atrazine desipropopyl	0.262	0.9	24	4.40
Triazine	Atrazine mercapturate	0.005	0.02	22	4.03
OP	Chlorpyrifos-methyl	0.043	0.15	17	3.11
OP	Terbufos-oxon- sulfoxyde	0.008	0.03	17	3.11
OP	**Dichlorvos**	0.268	0.9	14	2.56
OP	**Terbufos**	0.018	0.06	14	2.56
Triazine	**Atrazine**	0.01	0.05	9	1.65
OP	**Omethoate**	0.033	0.15	9	1.65
Triazine	**Simazine**	0.054	0.2	5	0.92

In bold : Parent compound
"n(>QL) : Number of quantified values ; %(>QL) : Percentage of quantified values"

Table 2 **Description of the 546 pregnant women* of the Pelagie sub-cohort (Brittany, France, 2002-2006)**

Inclusion year		
2002	n=77	14.1 %
2003	n=197	36.1 %
2004	n=198	36.3 %
2005-2006	n=74	13.6 %
Department of residence		
Ille-et-Vilaine	n=342	62.6 %
Côtes-d'Armor	n=147	26.9 %
Finistère	n=33	6.0 %
Other departments or unspecified	n=24	4.4 %
Housing zone		
Municipality with <20000 inhabitants	n=443	81.1 %
Municipality with >20000 inhabitants	n=103	18.9 %
Pregnant woman's age at inclusion		
< 25 years	n=67	12.3 %
25 - 29 years	n=212	38.9 %
30 - 34 years	n=191	35.1 %
35 years	n=75	13.8 %
Median age (min-max)	29.9 y	(19.5-44) y
Median BMI (min-max)	21.3 kg/m^2	(15-44) kg/m^2
Parity		
1	n=245	45.0 %
2	n=197	36.1 %
	n=103	18.9 %
Mother's level of education		
Pre-baccalaureate	n=102	18.7 %
Baccalaureate	n=105	19.3 %
Post-baccalaureate	n=338	62.0 %
Mother's smoker status		
Non smoker	n=390	72.4 %
Ex-smoker in early pregnancy prior to inclusion	n=62	11.5 %
Smoker upon inclusion	n=87	16.1 %
Mother's alcohol consumption in early pregnancy		
Never or rarely	n=459	85.3 %
once a day	n=73	13.6 %
> once a day	n=6	1.1 %
Season of urine sampling		
Spring	n=144	26.4 %
Summer	n=115	21.1 %
Autumn	n=155	28.4 %
Winter	n=131	24.0 %
Median gestational age (min-max) at time of urine sampling	10.3 weeks	(2-18) weeks
Median creatinine level (min-max)	1 g/l	(0.19-3.51) g/l

"*Sub-cohort women from whom a urine sample was collected and with a urinary creatinine concentration higher than 100mg/l (n=546 of 569 ; 96 %)"
"n : total number ; % : percentage ; BMI : Body Mass Index"

agricultural applications may vary. Creatinine concentrations ranged from 185 to 3511 mg/L (median=1004 mg/L).

The 44 targeted molecules (triazines and organophosphorous) were quantified in 1 to 84% of the 546 urine samples of the sub-cohort (Table 1). The 10 most prevailing molecules belonged to the organophosphorous insecticide class, their quantification limits not necessarily being the lowest ones. Only 1.6% of the sub-cohort urine samples showed no trace of the 52 determined molecules (i.e. no quantified molecule). A majority of urine samples (54%) contained at least 8 quantified molecules, 10% of samples contained at least 13 molecules with one sample containing up to 28 quantified molecules (Figure 1).

Urine concentrations of triazine and organophosphorous insecticide residues for the 546 pregnant women of the Pelagie sub-cohort are provided in Tables 3 and 4, with and without adjustment on creatinine level. Traces of exposure to atrazine and to simazine, assessed for each with the mother substance and its mercapturic metabolite, were observed in a minority of women (respectively 5 and 8%; Table 3). However, their dealkylated and hydroxylated metabolites were observed respectively in 20% and 40% of urine samples. Average urine concentrations are the lowest for atrazine and its mercapturic metabolite, followed by simazine and its mercapturic metabolite for which levels reach at least 0.1 µg/l in 5% of the population under study. Average urine concentrations of dealkylated and hydroxylated metabolites of triazine compounds are higher and comparable (respectively 2.2 nmol/l and 2.0 nmol/l).

Organophosphorous insecticide compounds for which a urinary level of 0.1 µg/l has been reached in at least 5% of urine samples are listed in Table 4. These compounds are: chlormephos (corn crops), chlorpyrifos (various crops, domestic ant, cockroach and other insect control products), TCPy, a chloryrifos metabolite, ethion (cereal and vegetable crops), parathion-ethyl (various crops, prohibited since late 2002), parathion-methyl (various crops, prohibited since late 2003), metabolites of parathions (oxon and others: paraoxon-ethyl, paraoxon-methyl, 4-nitrophenol, 4-nitrophenyl potassium salt), phorate (corn crops, prohibited since late 2003) and phoxim (various crops, ant control products). The table 4 shows that dialkylphosphate metabolites (DAP), common tomany organophosphorous insecticides, were found in nearly 90% of urine samples. The majority of these samples contained metabolites of both classes, the ethyl class (DE) and the methyl class (DM). Among the six DAP metabolites, DETP showed the lowest quantification frequency at 9%. The highest average urinary concentrations among the six DAP metabolites were observed for the DMP (12 nmol/l; common and specific metabolite to organophosphorous insecticides) and DMTP (3.1 nmol/l; common but non specific metabolite to organophosphorous insecticides). Finally, residues of triazine compounds in urine samples were less frequent and of lower concentrations than those of organophosphorous insecticides and DAP metabolites.

Tables 3 and 4 show similar median and average urine concentrations, with and without adjustment on creatinine level.

Table 3 **Urinary levels of triazine compounds (herbicides) for the 546 pregnant women of the Pelagie study sub-cohort (Brittany, France, 2002-2006)**

		Urine Concentration							Creatinine-Adjusted Urine Concentration						
	% (>QL)	Median	p75	p90	p95	p99	Max	Geo. Mean	Median	p75	p90	p95	p99	Max	Geo. Mean
		µg/L							µg/g creat.						
Atrazine	2 %	**<QL**	<QL	<QL	<QL	0.08	0.52	**0.007**	**<QL**	<QL	<QL	<QL	0.07	1.29	**0.007**
Atrazine mercapturate	4 %	**<QL**	<QL	<QL	<QL	0.08	0.68	**0.004**	**<QL**	<QL	<QL	<QL	0.09	1.67	**0.004**
Simazine	1 %	**<QL**	<QL	<QL	<QL	0.04	1.83	**0.04**	**<QL**	<QL	<QL	<QL	0.21	2.50	**0.04**
Simazine mercapturate	8 %	**<QL**	<QL	<QL	0.3	1.6	4.6	**0.01**	**<QL**	<QL	<QL	0.2	2.6	8.3	**0.01**
		nmol/L							nmol/g creat.						
Atrazine or atrazine mercapturate	5 %	**<QL**	<QL	<QL	0.1	0.7	4.4	**0.05**	**<QL**	<QL	<QL	0.1	1.0	10.9	**0.05**
Simazine or simazine mercapturate	8 %	**<QL**	<QL	<QL	1.3	7.8	14.2	**0.26**	**<QL**	<QL	<QL	1.2	9.8	25.6	**0.27**
Triazine dealkylated metabolites	20 %	**<QL**	<QL	5.9	11.8	33.8	76.5	**2.2**	**<QL**	<QL	5.7	12.6	38.6	98.7	**2.3**
Triazine hydroxylated metabolites	40 %	**<QL**	3.8	10.7	19.5	46.8	66.9	**2.0**	**<QL**	3.5	12.4	21.7	67.9	138.2	**2.0**

In bold : parent compound
"QL : Quantification Limit ; %(>QL) : Percentage of quantified values ; <QL : Non-quantified value ; pXX : XXth percentile ; Max : Maximum ; Geo. Mean : Geometric Mean "

Figure 1 **Number of quantified molecules in urine samples out of 52 molecules (Britany, France, 2002-2006)**

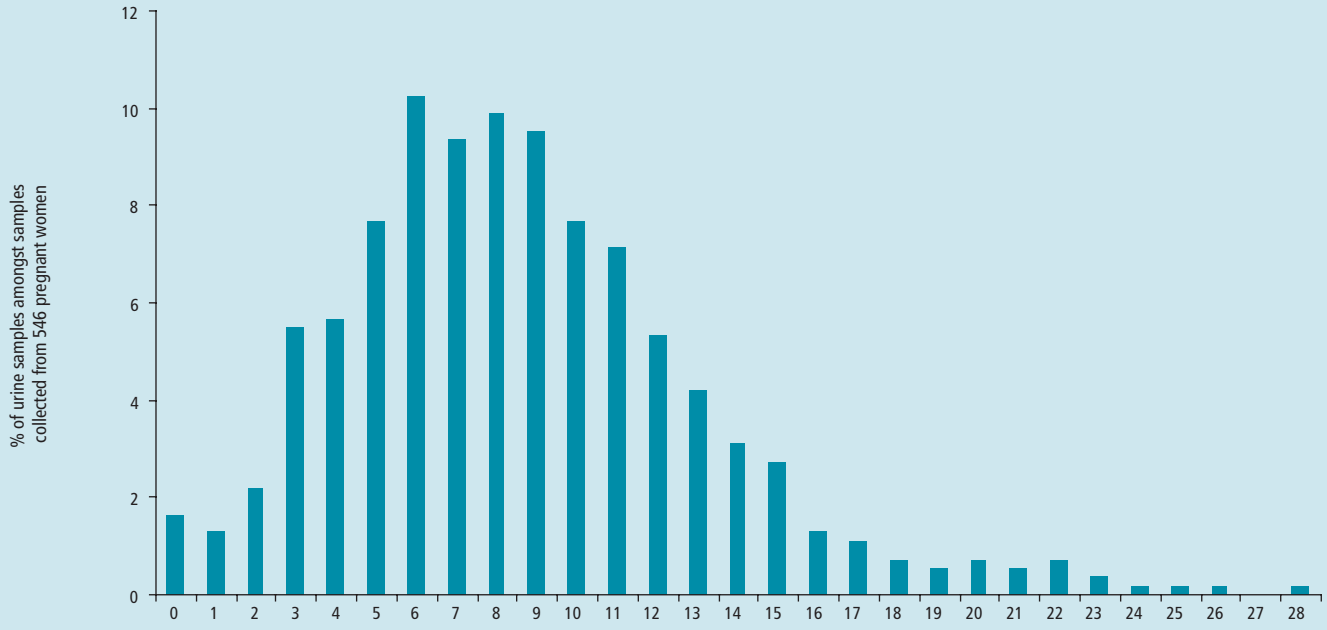

Table 4 **Urinary levels of organophosphorous insecticides for the 546 pregnant women of the Pelagie study sub-cohort (Brittany, France, 2002-2006) (for molecules with a 95thpercentile over 0.1µg/l and for dialkylphosphate metabolites, DAP)**

		Urine Concentration							Creatinine-Adjusted Urine Concentration						
	% (>QL)	Median	p75	p90	p95	p99	Max	Geo. Mean	Median	p75	p90	p95	p99	Max	Geo. Mean
		µg/l							µg/g creat.						
Chlormephos	11 %	**<QL**	<QL	0.09	0.5	2.1	7.1	**0.02**	**<QL**	<QL	0.09	0.4	2.3	16.7	0.02
Chlorpyrifos	12 %	**<QL**	<QL	0.04	0.1	0.7	10.0	**0.010**	**<QL**	<QL	0.04	0.2	0.9	8.4	0.010
TCPY : 3.5,6-trichloro-2-pyridinol	10 %	**<QL**	<QL	0.03	0.4	2.8	5.4	**0.04**	**<QL**	<QL	0.10	0.4	2.9	7.7	0.04
Ethion	31 %	**<QL**	0.02	0.08	0.15	1.1	3.1	**0.006**	**<QL**	0.02	0.08	0.2	0.9	3.2	0.006
Parathion-ethyl	7 %	**<QL**	<QL	<QL	2.0	8.7	14.1	**0.3**	**<QL**	<QL	<QL	2.3	9.4	28.0	0.3
Paraoxon-ethyl	10 %	**<QL**	<QL	0.05	0.16	1.0	2.6	**0.01**	**<QL**	<QL	0.05	0.14	0.99	2.28	0.01
Parathion-methyl	6 %	**<QL**	<QL	<QL	0.4	3.0	14.4	**0.06**	**<QL**	<QL	<QL	0.30	3.6	19.0	0.06
Paraoxon-methyl	19 %	**<QL**	<QL	1.3	1.9	5.8	27.0	**0.25**	**<QL**	0.3	1.2	2.5	7.5	65.9	0.25
Common metabolites of parathions :															
4-nitrophenol	14 %	**<QL**	<QL	0.3	0.6	2.2	3.9	**0.06**	**<QL**	<QL	0.3	0.8	2.4	11.3	0.06
4-nitrophenyl potassium salt	11 %	**<QL**	<QL	0.1	1.6	6.7	34.1	**0.02**	**<QL**	<QL	0.1	1.2	6.9	35.5	0.02
Phorate	12 %	**<QL**	<QL	0.02	0.17	1.9	3.8	**0.004**	**<QL**	<QL	0.02	0.17	2.6	5.2	0.004
Phoxim	25 %	**<QL**	0.09	1.6	2.4	5.9	11.0	**0.05**	**<QL**	0.10	1.7	3.0	8.0	16.0	0.05
Dialkylphosphate metabolites :															
DEP	18 %	**<QL**	<QL	2.7	4.4	11.2	69.1	**0.4**	**<QL**	<QL	2.4	5.7	11.5	82.4	0.4
DETP	9 %	**<QL**	<QL	<QL	2.9	11.5	34.4	**0.4**	**<QL**	<QL	<QL	3.1	13.8	33.2	0.5
DEDTP	36 %	**<QL**	0.1	1.6	3.5	13.5	37.2	**0.02**	**<QL**	0.1	1.6	3.8	18.4	35.1	0.02
DMP	84 %	**2.3**	6.3	11.1	15.8	32.0	112.9	**1.5**	**2.3**	6.3	11.8	16.5	45.7	134.4	1.5
DMTP	28 %	**<QL**	1.3	5.3	9.4	37.6	192.2	**0.5**	**<QL**	1.3	5.5	12.0	30.0	115.4	0.5
DMDTP	20 %	**<QL**	<QL	1.7	4.0	14.0	71.4	**0.2**	**<QL**	<QL	1.6	4.9	14.7	217.0	0.2
		nmol/l							nmol/g creat.						
Dialkylphosphate metabolites :															
DEP	18 %	**<QL**	<QL	17.6	28.9	73.0	451.4	**2.6**	**<QL**	3.2	15.9	36.9	75.3	538.0	2.7
DETP	9 %	**<QL**	<QL	<QL	13.7	55.0	164.6	**2.1**	**<QL**	<QL	<QL	14.6	65.7	158.7	2.2
DEDTP	36 %	**<QL**	0.5	6.9	15.7	60.2	166.0	**0.09**	**<QL**	0.5	7.3	16.8	82.0	156.3	0.09
Total DE (DEP+DETP+DEDTP)	50 %	**3.5**	13.4	35.2	60.1	132.4	453.9	**6.9**	**4.9**	13.5	39.1	56.5	159.5	541.0	7.0
DMP	84 %	**18.5**	49.9	88.0	125.1	253.9	895.6	**12.0**	**18.0**	49.9	93.8	131.0	362.7	1065.9	3.6
DMTP	28 %	**<QL**	8.1	32.6	57.2	229.4	1171.2	**3.1**	**<QL**	7.7	33.3	73.1	183.0	703.4	3.2
DMDTP	20 %	**<QL**	<QL	10.7	25.0	88.5	451.4	**1.1**	**<QL**	<QL	9.9	31.1	92.9	1372.0	1.1
Total DM (DMP+DMTP+DMDTP)	89 %	**31.0**	70.8	128.3	183.2	539.6	1482.8	**27.0**	**31.0**	73.4	141.4	201.4	697.9	1456.2	27.3
Total DAP (DE+DM)	91 %	**42.8**	88.6	160.9	221.2	568.3	1487.2	**40.2**	**41.9**	94.8	173.0	240.2	700.9	1583.7	40.7

In bold : parent compound
"QL : Quantification Limit ; %(>QL) : Percentage of quantified values ; <QL : Non-quantified value ; pXX : XXth percentile ; Max : maximum ; Geo. Mean : Geometric mean"
"DEP : diethylphosphate ; DETP : diethylthiophosphate ; DEDTP : diethyldithiophosphate ; DMP : dimethylphosphate ; DMTPœ dimehylthiophosphate ; DMDTP : dimethyldithiophosphate"

Discussion

Our findings show that pesticide residues are observed in the majority of urine samples of pregnant women of the Pelagie sub-cohort. A French study conducted on twenty adults living on the Ile-de-France region shows higher median concentrations of DAP metabolites in urine than those observed among the Pelagie cohort (7 nmol/g creatinine (DE) and 221 nmol/g creatinine (DM) versus 5 nmol/g creatinine (DE) and 31 nmol/g creatinine (DM) for the Pelagie study) [15,7].

The Pelagie cohort is the first French study assessing levels of multiple pesticide impregnation among pregnant women of the general population. Compared to a study of 100 pregnant Dutch women, only 5% of the Pelagie cohort show urinary levels of DAP and TCPy metabolites higher than the median levels observed in the Dutch study [14]. Median DAP concentrations in urine of pregnant women reported by a nation-wide American study and by studies in New York and in California's agricultural region, are also higher than those observed for the Pelagie sub-cohort (up to +270% compared to California) [15-19]. To our knowledge, no study has measured atrazine concentrations and that of its metabolites in the urine of pregnant women. An American study did report atrazine mercapturate concentrations of respectively 0.013 to 2.8 µg/l and 0.003 to 2.2 µg/l in the urine of 24 mothers and 51 children of non-farming families in Iowa [20]. Findings of the Pelagie cohort indicate that triazine metabolites are more frequent in urine and in higher concentrations than the parent molecules which probably confirms the persistence of such breakdown products in the environment.

Caution must however be used in interpreting these results. The uniqueness of urine sampling from the Pelagie cohort as well as inter-laboratory analytical variability may in part explain the different urine concentrations observed amongst studies. Furthermore, the pregnancy stage at which the urine sample was collected is varying and could have an impact on both pesticide metabolite concentrations in urine and creatinine levels. Finally, it is important to note that the Pelagie study is not representative of the pregnant women population of Brittany. The Pelagie cohort distinguishes itself by a high academic attainment (60% of educational level higher than the baccalaureate compared to 44% for the 2003 national perinatal study [21]).

In conclusion, findings of the Pelagie study provide an overview of urine exposure levels to herbicides of the triazine family, now prohibited in France, and to agricultural and non-agricultural organophosphorous insecticides in French pregnant women.

Various hypotheses have been made in recent years as to the potential impact of exposure to pesticides on intra-uterine development. Some epidemiological studies suggest an increased risk of miscarriage, congenital defects and stillbirths for parents who make professional use of pesticides, which usually implies greater exposure levels than environmental exposure [22]. Studies on the impact of environmental exposure to pesticides are more recent and their findings vary [23]. Three recent American cohort studies, relying on biomarkers of exposure to organophosphorous insecticides, offer similar findings when taking into account genetic susceptibility and suggest a negative impact of environmental exposure to organophosphorous insecticides on intra-uterine development [16,17,19,24,25]. These relations between biomarker levels for exposure to pesticides and intra-uterine development parameters are currently being analyzed within the Pelagie study to either confirm or infirm these findings.

Acknowledgements

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Human biomonitoring in Cyprus : Cotinine in children – the impact of smoking, 2004-2008

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Abstract

Objectives – This study describes a comprehensive anti-smoking campaign carried out in Cyprus over 2004-2008 with the aim to reduce children exposure to passive smoking and prevent the related health impacts.

Methods – Questionnaires were used in 2005 to evaluate parents' knowledge, attitudes and practices. Based on the results, an intervention program was developed and implemented up to 2008. The intervention was evaluated in a pilot study in which nicotine was assessed in indoor air and the total exposure of the children to environmental tobacco smoke (ETS) was evaluated from the cotinine level in their saliva.

Results – In 2005 at least one parent was an active smoker in 42% of the households and 72% of smokers smoked in their homes. Parents were not fully aware of the effects of ETS on children and had erroneous perceptions on how to protect them. Nicotine measurements in the air after the intervention (2008) showed a definite improvement in smokers' practices since only 41% of the smokers smoked in their homes. Associated cotinine measurements in children's saliva showed that 97% of children were exposed to ETS, regardless whether their parents smoked or not. No significant difference in the levels of cotinine was found between the two groups (smokers' vs. non smockers' children) and there was no correlation between cotinine levels and the levels of nicotine found in the family home.

Conclusion – The intervention led to an improvement in smoking parents' practices at home, however children are still substantially exposed to ETS outside the family home.

Key words

Children, passive smoking, parents smoking behaviour, salivary cotinine, air nicotine, measures of precaution

Introduction

Environmental tobacco smoke (ETS) consists of more than 4,000 chemical compounds, many of which are endocrine disruptors and more than 40 are known carcinogens [1]. The International Agency for Research on Cancer and the US Environmental Protection Agency classify ETS as a "group A carcinogen", which is defined as a substance known to cause cancer in humans, with no safe level of exposure [2,3].

Exposure to ETS, also known as passive smoking, is associated with adverse health effects in children and non-smoking adults, such as respiratory disorders, middle ear disease, sudden infant death syndrome (SIDS), compromised cognitive abilities, heart disease and cancer [3,4,5]. Children are especially at high risk of toxicity because of differences in their pulmonary physiology and their higher respiratory ventilation rate per minute [6,7], their body is under rapid development and their biochemical defence mechanisms are still immature. Foetuses are at even greater risk and therefore pregnant women must eliminate their exposure to ETS.

It is estimated that almost half of the children in the world are exposed to ETS [8] and that in the USA ETS causes the death of about 6,000 children younger than 5 each year [9]. Teenagers whose parents are active smokers are more likely to begin smoking [10]. Children's ETS exposure occurs primarily in the home, where legislative measures against it can not be applied and it is completely up to the parents to minimise it.

This study describes a comprehensive anti-smoking campaign carried out in Cyprus over 2004-2008 with the aim to reduce children exposure to passive smoking and prevent the related health impacts. The specific targets were:

- evaluation of knowledge and attitudes of Cypriot parents in relation to passive smoking and the practices followed in every day life,
- design and implementation of an antismoking campaign optimised according to the results of the initial evaluation,
- evaluation of the effectiveness of the intervention with experimental determination of the exposure of children to ETS by measuring nicotine in the air of children's homes and cotinine in their saliva.

Methods

Parents knowledge/attitudes/practices

Questionnaires were developed in 2004 that included:

- demographic information,
- the practices followed by parents with regards to active and passive smoking,
- the parents' knowledge of the effects of ETS exposure on health,
- the parents' knowledge and views regarding existing smoking prohibitions and the need for further restrictions,
- smoking practices in the family home,
- parents' receptiveness to receiving more information and above all to changing their manners when needed.

The questionnaires were distributed to parents of preschool children in Nicosia and Larnaca from 11/2004 to 3/2005. The questionnaires were evaluated with the Epi-Info® epidemiological software [11].

Intervention

An intervention programme was developed based on the evaluation of the questionnaires and run in 2005-2008. Informative leaflets were prepared and distributed to 50,000 elementary school children. The pupils discussed in the classroom the information provided in the leaflets and the following were stressed:

- why they should not begin to smoke,
- how children can protect themselves from ETS exposure,
- every child has and should demand the right to clean air, free of ETS pollution.

The pupils were asked to pass the informative leaflets and knowledge to their families. Furthermore, a short video on passive smoking and children was produced and distributed to schools and health visitors, related seminars were widely offered to concerned groups and the

importance of limiting and even eliminating children's exposure to ETS was stressed extensively in numerous TV and radio programmes.

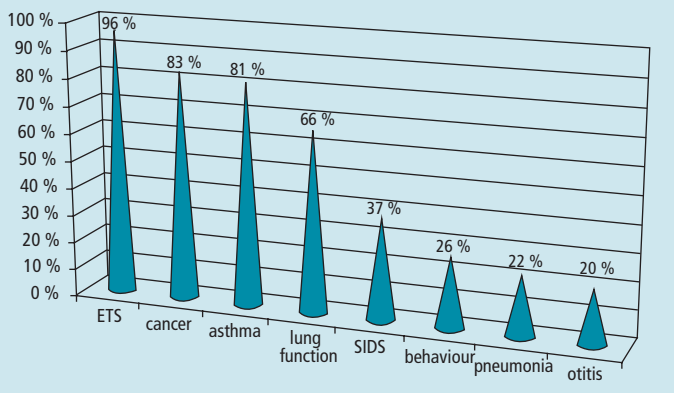
Experimental measurements

During 2007-2008 the level of ETS in homes of young children was determined by measuring nicotine in the indoor air and the total exposure of the children to ETS was assessed by determining the levels of cotinine in their saliva. Participants were recruited through their school and a written consent of at least one parent was obtained. An appropriately trained health visitor and a chemist scientist visited participants at home, explained the goal of the study and how the participants would be involved. The child was asked to provide the saliva sample in a sterile container and received a small gift for her/his participation. At that time, an air pump with an XAD-4 40/80 mg adsorbing tube was placed in the home and collected air with a flow rate of 1,000 mL/min for 3 days. This method is based on the standard method number 2551 of the National Institute of Occupational Safety and Health (NIOSH - http://www.cdc.gov/niosh/nmam/). When the pump was retrieved on the fourth day, a second saliva sample was obtained from the child. If the child did not wish to participate at any stage of the study, then his/her wish was honored. Individual results were not communicated to participants, unless they indicated an interest in receiving the information.

Nicotine in the indoor air was determined according to the method described by Michael CM *et al* [12].The samples were stored at 4ºC and analyzed within 7 days. The adsorbed air sample was extracted with ethyl acetate and 0.01% triethylamine, using quinoline as an internal standard. Nicotine levels were determined by GC-FID and GC-MS (column: EC-1, 30m wide bore). Blank tubes, duplicate standards and spiked samples were analyzed. Quinoline was used as an internal standard in the analysis of samples. The detection limit of the instrument was determined to be 0.1 μg/ml. Sample recovery was in the range of 99.5-100.5%.

Saliva samples were stored at -20ºC until analyzed and were proven to be stable for up to 9 months. The whole volume of each sample was centrifuged at 6,000 rpm for 15 minutes. 0.5 ml of the resulting supernatant was transferred to new tube and 20-30 mg NaCl were added. The sample was vortexed and then microextracted with 200 μl dichloromethane. The extract was placed in an ultrasonic bath for 15 minutes and subsequently centrifuged at 5,000 rpm for 15 minutes. The organic layer was then transferred in hexane using 50 ppb of D3-cotinine as an internal standard [13]. The samples were analyzed by GC-MS (Agilent 6890/5973N, column HP-SMS, 30m ID= 0.25 mm, film 0.25 μm). Standard solutions of cotinine were used in the range 10-100 ppb and contained 50 ppb D3-cotinine as an internal standard. The standard curve was the D3-cotinine / cotinine peaks as a function of the reverse of the concentration of cotinine in the standard solutions. The linearity of the standard curve was R^2=0.9996. Spikes in the range 2-50 ppb were analyzed and the average recovery was found to be 63%. The uncertainty (2 σ) was determined to be at different cotinine levels (in ppb) as follows: 4±1, 10±1, 50±10.

Figure 1 **Cypriot parents' degree of knowledge regarding ETS and its association with adverse health effects (Cyprus, 2004-2008)**



A total of 524 parents responded to questionnaires in the period 11/2004-3/2005. The percentages reflect the parents who knew the definition of ETS and some of the health effects it may cause.

Results

Phase 1 – Parents' knowledge, attitudes and practices regarding passive smoking

A total of 524 families with young children (272 in Nicosia and 252 in Larnaca) responded to the questionnaire. 50% of the responding parents were university graduates and 30% had at least secondary education.

Knowledge and attitudes

71% of the parents knew the definitions of ETS and passive smoking and 96% knew tobacco smoke is harmful to children's health. 81% knew that ETS may affect asthma development and worsen its symptoms and 83% knew that it increases the risk for cancer. However, 64-80% of the parents were ignorant of other adverse effects of ETS exposure, such as SIDS, middle ear infections, pneumonia and behavioural changes (Figure 1). 30% of parents had the erroneous perception that they protected their children adequately if they smoked in a different or in a ventilated room, or near a window. Generally, smokers gave more wrong answers and were less willing to support the adoption of protective or anti-smoking measures than non-smokers.

Practices

From the investigation done in 2005, in 42% of the homes (Figure 2a) at least one parent was an active smoker and in 6% of the homes both parents smoked. 68% of the smokers smoked at least occasionally in the presence of their children (Figure 2b). This percentage increased to 72% when children were in a different room or outside (Figure 2c). 27% of smokers smoked in the car, even when children were present (Figure 2d).

Phase 2 – Intervention strategy

Based on the evaluation of the questionnaires, a comprehensive but flexible strategy was developed with the goals to:

- create an antismoking culture in children,
- make parents more aware of the negative health effects of ETS exposure of children,
- guide parents to effectively minimize their children's exposure and create "smoke-free homes".

Phase 3 – Experimental measurements of children's ETS exposure

The effectiveness of the intervention strategy was evaluated with experimental measurements of nicotine in the children's homes and the biomonitoring of cotinine in their saliva. 64 households and 71 children participated.

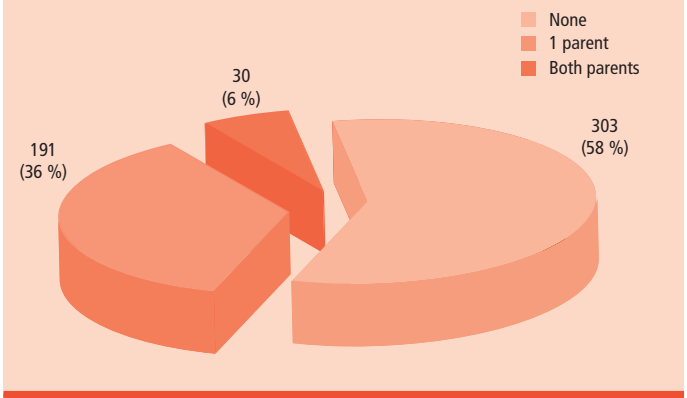
Nicotine in indoor air of households with young children

Nicotine was measured in the indoor air of 39 homes where at least one parent was an active smoker and in 17 non-smokers' homes. Nicotine was detected in 30% of all assayed homes (Figure 3a). In all but one (94%) of non-smokers' homes, nicotine was not detected (Figure 3b). In a single exception, 0.05 μg/m^3 nicotine was found and the parents reported that visitors were allowed to smoke indoors. In 59% of the assayed smokers' homes, nicotine was not detected, in 36% of these homes nicotine levels up to 0.5 μg/m^3 were found and in 6% nicotine was in the range of 1.5-3.1 μg/m^3 (Figure 3b).

Cotinine in children's saliva

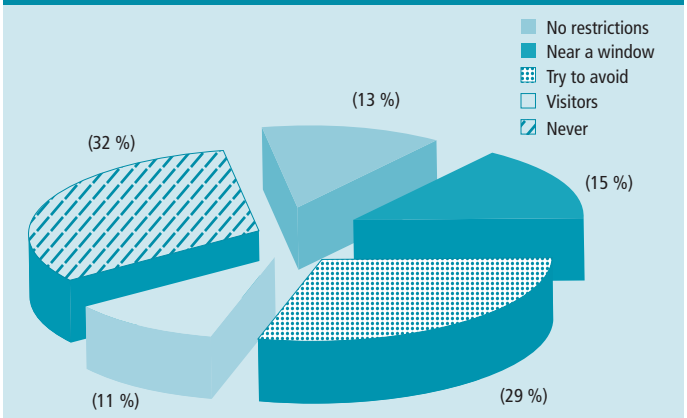
134 saliva samples from 71 children were analysed for cotinine. 32 children (45%) were of non-smoker parents and 39 children (55%) were of parents who smoked. For each child (with the exception of 8 children), two saliva samples were obtained, one on day 1 of the placement of the air pump in the household and one on day 4, when the pump was

Figure 2a **Cypriot parents' smoking practices: Percentage of homes where at least one parent was an active smoker (Cyprius, 2004-2008).**



A total of 524 parents responded to questionnaires in the period 11/2004-3/2005.

Figure 2b **Cypriot parents' smoking practices: Practices of actively smoking parents at home, in the presence of their children (Cyprius, 2004-2008)**



A total of 524 parents responded to questionnaires in the period 11/2004-3/2005.

retrieved. The average of these two measurements was used in the analysis.

Overall, cotinine ranged from non-detected to 19.8 ng/ml. In 97.2% of all participating children cotinine was detected and in 15.5% of the cases the average cotinine values exceeded 7 ng/ml (Figure 4a). Children of smokers had an average cotinine value of 4.0 ng/ml (range: 1-12.4 ng/ml) and 18% had cotinine values that exceeded 7 ng/ml. Children of non-smokers had an average cotinine value of 3.4 ng/ml (range: non-detected to 11.2 ng/ml) and 13% had cotinine values that exceeded 7 ng/ml (Figure 4b). The results were subjected to the t-test, which showed no statistically sound difference in the levels of cotinine found between children of smokers vs. children of non-smokers.

No correlation could be found between air nicotine concentrations in the home and cotinine in children's saliva.

Discussion

The "Protection of Public Health (control of smoking) Law" and "Regulation of 2004" currently in place in Cyprus, conforms to the European Union Directive 2001/37/EC and prohibits smoking in places potentially used by children, as follows: in public-use vehicles, in private-use vehicles when a person under 16 is on board and in areas other than designated smoking areas with proper ventilation in places of recreation [14]. Despite these restrictions, children in Cyprus are widely exposed to ETS both in public places and in homes.

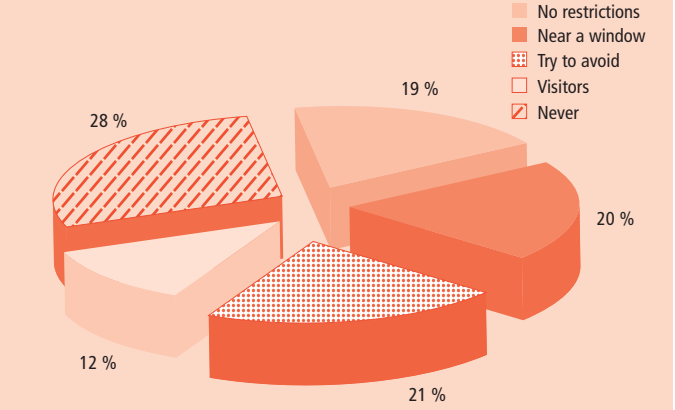
In this study, an intensive anti-smoking campaign was structured on the hypothesis that children's ETS exposure will be most effectively reduced by educating the public on the negative health effects of ETS exposure and the vulnerability of children, by changing people's (especially parents') attitudes and practices with regards to smoking and by creating an anti-smoking culture in children. The campaign ran over a period of 4 years. In the first phase, parents' knowledge, attitudes and practices with regards to children's ETS exposure were evaluated through questionnaires. The most alarming findings were:

- The percentage of homes with at least one active smoker parent was much higher in Cyprus (42%) than in other European countries (25-37%);
- Despite the fact that smoking in a vehicle in the presence of children is prohibited by the current legislation, 27% of active smoker parents smoked in the car in the presence of their children. This implies one or more of the following: (i) people are unaware of this prohibition; (ii) they are unaware of the danger in which they subject their children; (iii) have problems in changing their manners;
- Most parents were unaware of children's vulnerability and of many adverse health effects of ETS exposure;
- Many parents had erroneous perceptions on how they could protect children from ETS exposure;
- Smoker parents gave more wrong answers and were less willing to support the adoption of protective or anti-smoking measures than non-smokers.

The above findings were used to develop an aggressive intervention programme which was implemented in the second phase of the study. The intervention was multi-faced and targeted parents, teachers, the public at large and the children themselves. Considering the high educational level and the high degree of ignorance or wrong perception of parents experienced through questionnaires, it was obvious that the intervention had to move from just providing guidance to:

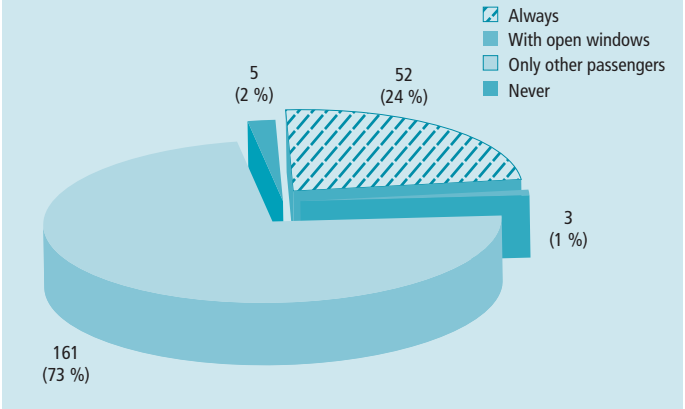
- The development of in-depth understanding of the vulnerability of children and the real, multiple and irreversible consequences of ETS exposure on children's health;

Figure 2c **Cypriot parents' smoking practices: Practices of actively smoking parents at home, when children are in a different room (Cyprius, 2004-2008)**



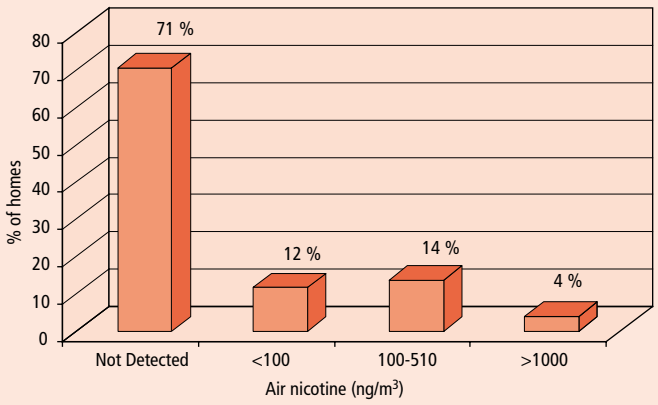
A total of 524 parents responded to questionnaires in the period 11/2004-3/2005.

Figure 2d **Cypriot parents' smoking practices: Smoking practices of parents in the car, in the presence of their children (Cyprius, 2004-2008)**



A total of 524 parents responded to questionnaires in the period 11/2004-3/2005.

Figure 3a **Distribution of nicotine in the indoor air of family homes: Distribution of nicotine for all the investigated homes (N=56) (Cyprius, 2004-2008)**



Smokers' homes N = 39; Non-smokers' homes N = 17. Limit of detection ≈ 20 ng/m³.

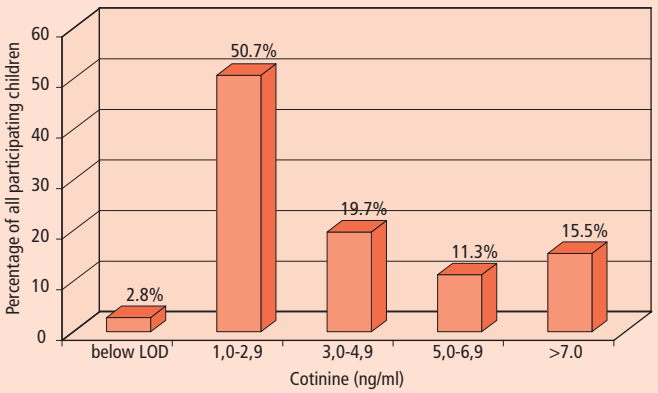
- Making smokers fully responsible and accountable for their practices and to help them understand their responsibility in the future health and development of children;
- Providing guidance on how children can and must be protected from ETS exposure, even when smokers can not quit smoking.

In parallel, an equally important investment was made on children. Primarily, the goal was to create antismoking culture and attitudes from childhood through adolescence. Children were educated on the impacts of active and passive smoking on their health, why and how they should demand their right to smoke-free air and how they could avoid smoking and be protected from passive smoking.

In the last phase of the study, laboratory measurements were performed to evaluate the effectiveness of the intervention programme and to generate experimental data of the children's exposure to ETS, which could then be used in a new campaign. Since tobacco smoke is the primary source of nicotine in air [15], nicotine was measured in children's homes to determine the extent of ETS pollution in the home. Nicotine is metabolised to cotinine inside the human body, which can be measured in saliva, blood, urine and hair and is a very specific and sensitive biomarker for the quantification of the total exposure of the person to ETS [16]. Cotinine was measured in children's saliva to determine their total ETS exposure with the following major conclusions:

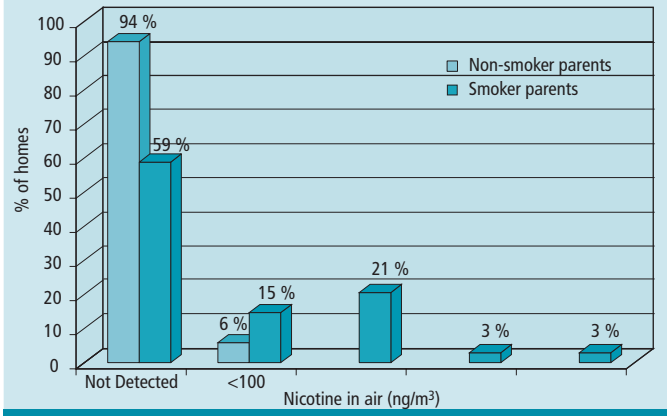
- Generally, nicotine was not found in non-smokers' homes;
- Although nicotine was detected in many homes with actively smoking parents, there was a definite improvement compared to the findings in the first phase of the study. The percentage of smokers smoking in the home dropped from 72% in 2005 to 41% in 2008;
- Despite the reduction of in house smoking, cotinine was found in 97% of participating children, regardless of whether their parents smoked or not. In 15% of the children the cotinine levels exceeded 7 ng/ml, a cut-off value reported in the literature to distinguish passive from active smokers [17]. This is especially alarming, considering that the study group was children aged 4-8 years;
- There was no significant difference in the levels of cotinine found in the children of smokers vs. the children of non-smokers;
- There was no correlation between the levels of cotinine in children's saliva and the levels of nicotine found in the family home, implying that primary exposure must be outside the home;
- It is clearly observed that even the children of non-smoking parents are exposed to ETS, since in only 6% of these children, cotinine was not found.

Figure 4a **Distribution of salivary cotinine in Cypriot children's saliva: Distribution of salivary cotinine in all participating children (N=71) (Cyprius, 2004-2008)**



Children of smokers N = 39; children of non-smokers N = 32. Limit of detection = 1 ng/ml.

Figure 3b **Distribution of nicotine in the indoor air of family homes: Distribution of air nicotine in participating homes according to parents' smoking habits (Cyprius, 2004-2008)**



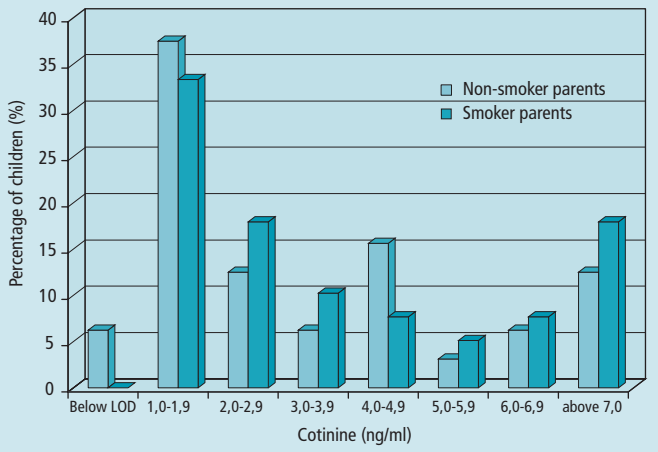
Smokers' homes N = 39; Non-smokers' homes N = 17. Limit of detection ≈ 20 ng/m³.

Conclusions

It is clear that after implementation of the intervention programme there was an improvement in smoking parents' practices at home, which reduced their children's exposure considerably. Nevertheless, children are still substantially exposed to ETS, apparently outside the family home. Measures to prohibit tobacco smoke in public places used by children are urgently needed.

The biomonitoring of cotinine in children's saliva in this work was very important in raising awareness in Cyprus about children's exposure to tobacco smoke and the need to restrict it. It provided complementary information to the environmental monitoring of nicotine in the air of the children's home and presented useful insights on the contributing routes of children's exposure (home vs. public places). It was also very effective in confronting parents with indisputable evidence on the impact

Figure 4b **Distribution of salivary cotinine in Cypriot children's saliva: Distribution of salivary cotinine in children according to their parents' smoking habits (Cyprius, 2004-2008)**



Children of smokers N = 39; children of non-smokers N = 32. Limit of detection = 1 ng/ml.

of their behaviour on their own child. In our experience, biomonitoring may have strong influence on policies and legislation, provided that biomonitoring data are transformed into information for action and are effectively communicated in a structured, problem-orientated strategy. This strategy must be tailor-made to the needs of different actors, mainly the public, NGOs and policy makers.

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Box: A National Biomonitoring Programme in France

The Draft Law for the implementation of the "Grenelle de l'environnement" states that the second National Environmental Health Action Plan (2009-2012) will include a biomonitoring programme that will integrate public health indicators and the state of the environment and help to evaluate policies related to environmental health. The Institut de Veille Sanitaire (i.e. the French Institute for Public Health Surveillance) has started to design this programme, under the umbrella of the ministries of Health and the Environment. The final programme will be presented in full details during the first quarter of the year 2010.

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