Human Biomonitoring

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ABSTRACT

Human biomonitoring is the discipline devoted to the identification of biomarkers useful to measure environmental exposure, to monitor its biological effects and causal relationship with pathological conditions, and to possibly define the genetic susceptibility of the general population. The search for reliable biomarkers, i.e. characteristics that are objectively measured and validated as health or disease requires the expertise of scientists with diversified specializations, who are able to tackle problems of ever increasing complexity using complementary approaches. Within the frame of the PIAS-CNR project "Environment and Health", we have constituted a Biomonitoring network, composed by highly qualified scientists from CNR, and external teams. This action aims at promoting a scientific strategy to develop and validate biomarkers of effect, exposure and susceptibility.

1. Introduction

1.1 Background

Chemicals are present in the air, ingested food and water, at the workplace as well as at home; the exposure to them can occur through inhalation, cutaneous contact, and ingestion. The evaluation and measurement of the impact of chemicals on human health is achieved by "Human Biomonitoring" (HBM), defined as the "systematic standardized measurement of substance or its metabolites in body fluids (blood and urine) of exposed persons" (1-4). The different steps of HBM ranging from the exposure step to the health impairment (and eventually disease occurrence) are schematized in Figure 1. The estimation of the dose really taken up after the exposure (internal dose) can be addressed by qualitative and quantitative assays able to detect chemicals and/or metabolites in biological fluids; this action provides basic information to identify *exposure biomarkers.*

The HBM survey is further extended to the analysis of biochemical and biological effects: as illustrated in Figure 1, biochemical effects could be monitored through the reaction of reactive substances (or their metabolites) with biological macromolecules, such as DNA and proteins. The study of biological effects induced by chemicals implies cellular, cytogenetic, genetic, biochemical, metabolic and immunological approaches. This strategy aims at defining a panel of *effect biomarkers.* Furthermore, it is widely recognized that *biomarkers of susceptibility* may influence each element in the exposure-to-disease paradigm (Figure 1). This class of biomarkers refers to individual factors (e.g. genetic or metabolic) that influence the sensitivity to hazardous compounds. Markers that are measurable at low exposure or dose, have the greatest potential utility to prevent disease, while later markers are

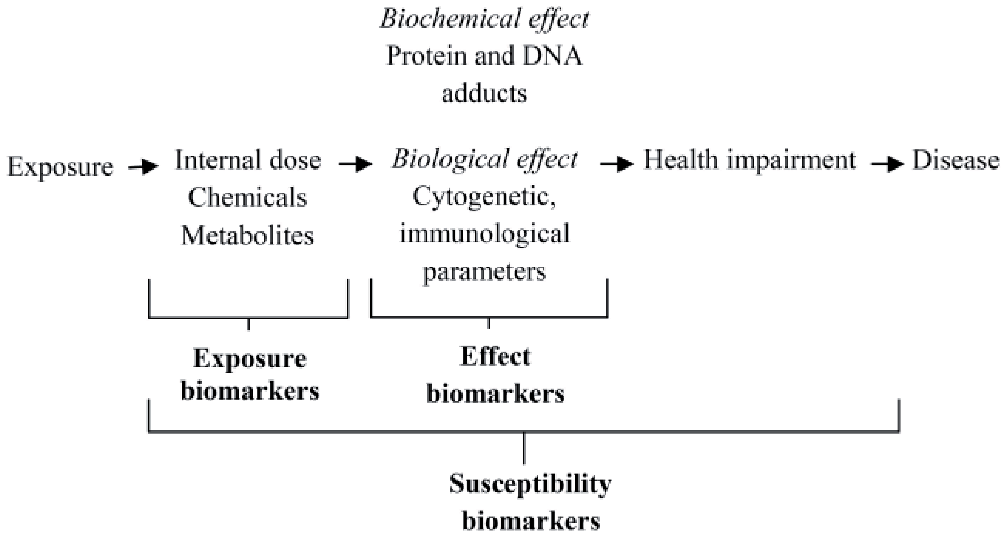

Figure 1: The exposure-to-disease paradigm.

most closely related to the endpoint of risk exposure, that is disease development (5).

As discussed in the special issue of the Bulletin Epidémiologique Hebdomadaire (6), HBM can help set priorities for public health and regulatory follow-up. In fact, periodic measurement of biomarkers in the population reveals how body burdens of chemicals vary from season to season, year to year and decade to decade; by comparing the results over the time it is possible to evaluate the trend of people's exposure to environmental chemicals. Large biomarker studies can highlight exposure differences among racial, geographic or socioeconomic groups. As specified in the above document (6), it is urgent to define the association between the level distribution of chemicals in humans and geographic/social/demographic parameters, in order to generate exposure maps and to promote better-targeted risk assessment and risk management actions. A number of examples demonstrated that a policy change can reduce people's exposure to pollutants, e.g. the decision of way out leaded petrol because of its established association with blood lead, the information campaigns about the link between blood mercury and dioxin and fish diet (6). However, to better exploit these results, a harmonised strategy allowing comparisons between countries is required.

The selection of chemicals allowed to enter a biomonitoring program is very complex. The Centre for Disease Control and Prevention (CDC) has identified a number of important variables able to influence the so-called process, including the evidence of exposure, the presence and significance of health effects after a given level of exposure, the development of assays for accurately measuring biological concentrations of the chemical agent, available specimens, in particular, blood and/or urine samples, and cost-effectiveness.

As reported in the literature, many chemicals released into the environment can disturb the development of the endocrine system and of the organs that respond to endocrine signals and can interfere with the correct functioning of endocrine organs. Such chemicals are generally named "Endocrine Disruptors" (EDs) (7). EDs causing abnormalities and impaired reproductive performance in some species, are associated with changes in immunity behaviour and skeletal deformities and are responsible for apparent changes observed in human health patterns over recent decades, including an increased incidence of certain types of hormone-sensitive cancers (7,8).

1.2 State of the art

The exposure of the general population to xenobiotics through different routes is a matter of growing concern. To face this problem, the European Commission adopted in 2003 a Strategy on Environment and Health (9). Then, the EU launched the "2004-2010 Environment and Health Action Plan", designed to give the EU the scientifically grounded information to reduce the adverse health impacts of certain environmental factors and to endorse better cooperation among actors in the environment, health and research fields (10,11). The final goal of the Action Plan is to promote and integrate environment and health information, and to identify emerging issues, reviewing and adjusting risk reduction policies and improving communication. Of course, this action implies the development of a HMB policy aiming at monitoring activities in human beings, using biomarkers, which focus on environmental exposures, diseases and/or disorders and genetic susceptibility, and their potential relationships (12).

The mid-term review of the action plan pointed out the difficulty of collecting and comparing HBM data from different countries, given that implemented methodologies and sample collection protocols differ (13). In 2007, the EU Council invited the Commission to ensure adequate funding for the EU pilot project on HBM, to pay attention to the existing regulatory frameworks and, most important thing, to demonstrate the added value of HBM as policy tool and support to public health interventions (14). Despite the big effort of the EU action, experiences of HBM programmes at country level reveal the need for harmonisation. In this respect, last December, in Brussels, 35 partners coming from 27 European countries and including governments, research institutes, the Health and Environment Alliance (HEAL) and the European Chemical Industry Council (CEFIC) established a consortium to perform human biomonitoring at European scale (COPHES) (15). This consortium aims at developing a functional framework that contributes to the definition, organization, and management of a coherent approach towards HBM in Europe, including strategies for data interpretation and integration with environmental and health data. A further priority of COPHES is to regulate data communication and dissemination and to provide key information to all stakeholders from the public to the policy markers. The *WHO 5th Environment and Health Ministerial Conference* (Parma, March 10-12, 2010) is the next milestone in the European environment and health process, now in its twentieth year. Focused on protecting children's health in a changing environment, the Conference will drive Europe's agenda on emerging environmental health challenges for the years to come (16).

HBM aims at estimating people's exposure to pollutants, thus providing information about possible health effects and options of policy measures to reduce exposure. Persistent organic pollutants (POPs) are chemical substances that persist in the environment, bioaccumulate through the food, and pose a risk of causing adverse effects to human health and the environment. Priority POPs include pesticides (such as DDT), industrial chemicals (such as polychlorinated biphenyls, PCBs) and unintentional by-products of industrial processes such as dioxins (PCDDs) and furans (PCDFs). Pesticides, PCBs, PCDDs, PCDFs, and other emerging substances have been detected in foodstuffs and are potentially toxic to human health. In addition to the diet, indoor and outdoor air pollutants are possible sources of health risk; however, available data are scarce and inconclusive. POPs are transported across international boundaries far from their sources, even to regions where they have never been used or produced. For most of these chemicals, we simply do not know how they pass through the environment, whether they are accumulated, dispersed or transformed, and how they affect living organisms at different concentrations (17). Consequently, POPs pose a threat to the environment and to human health (18). Among POPs, some trace elements (TEs) e.g. cadmium, arsenic, lead, mercury and nickel, are very dangerous for health because they tend to bioaccumulate, thus enhancing their concentration in a biological organism over the time. TEs may enter the human body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, or residential settings. Human exposure to TEs has risen dramatically in the last 50 years as a result of an exponential increase in their use in industrial processes and products. In general, TEs are systemic toxins with specific neurotoxic, nephrotoxic, and teratogenic effects; they can induce impairment and dysfunction in excretive organs (colon, liver, kidneys, skin), endocrine and energy production pathways, enzymatic, gastrointestinal, immune, nervous, reproductive, and urinary apparatus (19).

A special class of toxicants is represented by Endocrine Disruptors (EDs). In 1991, at the Wingspread Conference, expert scientists at a work session on endocrine-disruptors concluded that "Many compounds introduced into the environment by human activity are capable of disrupting the endocrine system of animals, including fish, wildlife, and humans. Endocrine disruption can be profound because of the crucial role hormones play in controlling development" (20). EDs interfere with the normal function of endocrine system and can exert adverse effects on the reproductive and other, indirectly related, physiological systems.

EDs are natural or synthetic compounds, derived primarily from anthropogenic activities, ubiquitous in the environment. Many of them are resistant to biodegradation, due to their structural stability, and persist in the environment. EDs present in the environment include a variety of potent human and veterinary pharmaceutical products, personal care products, nutraceuticals and phytosterols. Substantial research has been carried out on the mechanisms and effects of EDs. Nevertheless it is still unclear to what extent and/or in what situations and population subgroups EDs may represent a significant, long-term health risk.

To monitor the potential environmental and health impacts of EDs, the EU adopted a Communication to the Council and European Parliament on a Community Strategy for EDs in December 1999 (21). In the proposal for a new policy for chemicals (Registration, Evaluation, Authorisation and Restriction of Chemicals, REACH), EDs are covered by the authorisation procedure for substances of very high concern. Despite the joint efforts of many organizations, there is still a need for agreed test methods that can confirm whether or not "identified candidates" (more than 500 compounds) are real EDs. As stated also by the American EPA (Environmental Protection Agency), validated methods able to evaluate specific effects of EDs are still being developed (22). Remarkably, studies on environmental/occupational exposure to EDs and reproductive risks have been recently carried out, aiming at dissecting the impact of these chemicals on fertility. Exposure *in utero* at critical developmental periods may modify the normal path of reproductive and genito-urinary development (23,24), and induce hypospadia that is the most frequent genital malformations in the male newborn and results from an abnormal penile and urethral development (25).

2. From basic to applied research

HBM fits with the scope of translational research given that the expected results include the validation of conventional/new effect and exposure biomarkers, the elucidation of the biomolecular mechanisms of action of selected toxicants, the identification of genetic susceptibility markers in the Italian population, and the definition of the criteria leading to the evaluation of real exposure to toxicants. In the early 1960s, powerful analytical techniques allowed to measure very low concentrations of chemical substances in biological tissues caused by environmental exposure. Due to the improvement of these techniques essentially through the effort of basic research, it is now possible to detect very low concentrations of agents (parts per trillion and parts per quadrillion) with a high degree of accuracy and precision. This general consideration implies that measurement procedures need a continuous validation and that up-to-dating of basic knowledge of biological effects of chemicals could help in the development of new procedures of risk assessment evaluation. The analysis of benzene exposure, for instance, has taken advantage from the old evidence that *trans,trans*-muconic acid represents the urinary metabolite, thus prompting scientists to develop an *ad hoc* assay (26).

Many disciplines acquired a growing and growing relevance in biomonitoring, e.g. biophysics, whose contributions have gone well beyond the mere application of physical techniques to the study of living systems. Biophysics plays a crucial role in the development of new methodologies and establishes closer links with other frontier areas of the biological and medical sciences (structure-function relations in biological molecules, molecular biology, bioenergetics, bioinformatics). This evolution has widened the range of skills required by the individual researcher and has increased the need for teams with diversified specializations.

Given that biomonitoring is not confined to the exposure to toxicants but covers also the identification of effect and susceptibility markers, the final goal of HBM is to define a toxicogenomic approach, considered as an integration of genomics (transcriptomics, proteomics and metabolomics) and toxicology. This scientific field investigates how the genome is involved in responses to environmental stressors and toxicants. It combines studies of mRNA expression, cell and tissue-wide protein expression and metabolomics, to understand the role of gene-environment interactions in disease. One of the important aspects of toxicogenomic research is the development and application of bioinformatics tools and databases in order to facilitate the analysis, mining, visualizing and sharing of the vast amount of biological information being generated in this field. This rapidly growing area promises to have a large impact on many other scientific and medical disciplines as scientists could now generate complete descriptions of how components of biological systems work together in response to various stresses, drugs, or toxicants. Of course, this approach requires the joint effort of a panel of experts, who accumulated a solid experience through basic research, and can transfer it to health applications. This is the case of the teams involved in the PIAS-HBM project, which are characterized by complementary expertise and capability to cope basic and translational research.

3. The CNR specific expertise: qualified teams and external collaborations

3.1 CNR Institutes

The coordination of the HBM network has been entrusted to the Institute of Molecular Genetics (CNR-IGM, Pavia). CNR-IGM research activity covers a wide range of biological, biochemical and genetic topics, and ensures the participation of most CNR-IGM scientists to the HBM project. This feature, i.e. the potential commitment of a whole CNR Institute to the Environment and Health Inter-departmental project PIAS, has rendered CNR-IGM especially suitable for the coordination of the HBM group. The HBM coordinator (Giuseppe Biamonti, the present Director of CNR-IGM, then replaced by A. Ivana Scovassi) was in charge to identify CNR Institutes with the expertise required to join CNR-IGM in order to establish a PIAS-HBM network based mainly on the analysis of the effects of EDs (see below).

CNR-IGM is mainly devoted to basic research on the control of cell proliferation, DNA replication and apoptosis in human cells; viral replication; analysis of hereditary genetic disorders with defects in DNA damage repair pathways, chromosome X-linked diseases and muscular dystrophies; post-transcriptional regulation of gene expression during cell response to stress treatments and tumor progression, and analysis of the genetic structure of human populations. Long-lasting interactions and collaborations within the CNR-IGM team members are attested by joint peer-reviewed publications. The scientific excellence is completed by a qualified translational research in collaboration with SMEs, which originated a number of patents. CNR-IGM researchers are active in the development of new techniques, protocols and instruments as well as in the identification and characterization of new compounds with therapeutic properties. Considerable effort is put in the training of undergraduate, graduate and post-doctoral students. A group at IGM was recently investigating the biological effects of toxic metals and demonstrated the activation of stress response mechanisms after cadmium administration (27-29).

The Institute of Biophysics (CNR-IBF, Genoa) covers a wide range of research fields, sharing as a common feature the application of typical methodologies and techniques of the physical sciences to develop interdisciplinary approaches to the study of the structure and functions of biological systems. A relevant interest concerns physico-chemical investigations of the impact of anthropic and non-anthropic environmental factors on ecosystems. CNR-IBF devotes much effort to the training of young people for research in the fields of Biology and Biophysics, in close collaboration with local universities. The team involved in PIAS-HBM has a solid research experience in electrophysiology and ion channel biophysics in nervous and endocrine culture cells investigated by patch-recording and voltage-clamp techniques, and intracellular calcium dynamics, monitored by fluorescent probes. The group studies heavy metal accumulation and toxicity in mammalian cells and modulation of neurotrasmitter-gated ion channels. The effect of acute and chronic treatment with toxic metals (lead, cadmium and nickel) on cell survival and maturation of neurons in culture is currently analyzed by functional and viability tests and apoptosis/necrosis measurements (30-36).

The Institute of Biomedicine and Molecular Immunology (CNR-IBIM, Palermo) is involved in research activity, technological transfer and training in the following areas: molecular, cellular and morphological study of early embryo development and mechanisms involved in the differentiation and in the degenerative mechanisms of eukaryotic cells; molecular study of proteins involved in the allergic reaction; synthesis and characterization of bioactive molecules; pathophysiology of the cardio-respiratory system, lung diseases, systemic hypertension and renal failure insufficiency; organ transplantation; bioeffects of magnetic fields; epidemiology.
An CNR-IBIM team developed the concept that sea urchin embryos as a new friendly model for ecotoxicological studies (37-41). Indeed, marine organisms are highly sensitive to many environmental pressures, and consequently, the analysis of their bio-molecular responses to different stress agents is very important for the understanding of putative repair mechanisms and for application in environmental studies. Sea urchin represents a simple though significant model system where to test: 1) the impact on the biology of development in association with gene expression on embryos, and 2) the effects on gene expression and DNA damage on adult immuno-competent cells, which are contained in the coelomic cavity of the adult sea urchin, generically called coelomocytes, studied since many decades, but only recently used as bio-indicators of stress. Due to the capability to respond to injuries, host invasion and cytotoxic agents, coelomocytes have been regarded as the immune effectors of the sea urchin.
Another CNR-IBIM group investigates the impact of environmental substances both on hormone metabolism and nervous system, focusing on the identification of molecular mechanisms at the basis of neurodegenerative diseases, included some retinopathies. The group carries out *in vitro* studies to elucidate the effect of target chemicals on neurosteroidogenic pathways and the interactions of these chemicals with ER and AR receptors. On the other hand, *in vivo* analyses using animal models (mice) could be helpful in elucidating the effects in age-related neurodegenerative disorders. RT-PCR, receptor binding assay, recombinant mammalian and yeast cell based transcription assay, western blot, immunocitochemical and histochemical, proteomic and functional genomics are currently used.

The Institute of Clinical Physiology (CNR-IFC, Pisa), the largest biomedical institute of the CNR's Department of Medicine, whose mission is "Innovation for better patient care", is involved in the study of systemic, neuroendocrine and metabolic factors implicated in many diseases. Molecular medicine, clinical biology, and clinical biochemistry are devoted to the study of experimental physiology, and to the relevant diagnosis and treatment. CNR-IFC is a leader institute in the field of clinical and environmental epidemiology, population registers, and research on health services. CNR-IFC researchers established a network of scientific collaborations with many Italian and international Institutions. CNR-IFC was the first public body in Italy to achieve the status of pharmaceutical developer ("Officina farmaceutica") with its own production site for injectable sterile radiodrugs with Good Medical Practice certification. This achievement was made possible through close collaboration with a leader Company in the field of biomedical technology and diagnostic imaging, which signed a contract for the production and distribution of radiodrugs for diagnosis in Positron Emission Tomography.

The Epidemiology unit works on epidemiological surveillance, air pollution and health, waste and health and is involved in the national strategic programme "Environment and Health", funded by the Ministry of Health and coordinated by ISS. Human biomonitoring represents the priority research area of this unit (42,43). IFC has a long-standing experience in the field of surveillance and research on congenital malformations, as coordinator of the EUROCAT and ICBDSR "Tuscany Registry of Birth Defects". To investigate the correlation between EDs exposure and reproductive dysfunctions, malformations such as hypospadia or cryptorchidism, reduced sperm counts, testicular cancer and endometriosis, are studied (44).

3.2 External collaborations

Each CNR team collaborates with a number of Italian and international Institutions, including Universities, the Scientific Institute for Research, Hospitaization and Health Care (IRCCS), the National Institute of Health (ISS), the World Health Organization (WHO) and SMEs operating in the biomedical/biotechnology field. To increase the competences of the CNR Biomonitoring team, external collaborators, i.e. Salvatore Maugeri Foundation (FSM, Pavia), Perrino Hospital (Brindisi) and National Institute for Occupational Safety and Prevention (ISPESL, Rome) have been added to the CNR network on the basis of their pre-existing cooperations with CNR Institutes and taking into account their complementary expertise.

FSM is a leading institution in the field of the occupational health and prevention, including biological and environmental monitoring of exposure, reference values setting and prevention of occupational risks. The prevention of occupational risks activity is supported by the Laboratory for Environmental and Toxicological Testing, which has been working for decades on environmental monitoring, with respect to occupational exposure to both organic and inorganic substances (risk assessing and sampling techniques), and on the development of new biomarkers and analytical techniques aiming at the evaluation of occupational and environmental exposure to xenobiotics. This laboratory is fully equipped to develop and validate analytical methods for the determination of trace elements (ICP-MS), organochlorinated compounds such as PCB and DDTs (HRGC-MS), phthalate metabolites (HPLC-MS/MS), and other emerging substances in biological and environmental matrices, including foodstuffs. The Unit has recently validated reliable methods for the determination of EDs in biological fluids and in foodstuffs (45-48).

ISPESL is a technical-scientific body in the National Health Service and reports to the Ministry of Health as regards all aspects of

Table 1: PIAS Human biomonitoring network.

CNR INSTITUTE (DEPARTMENT)	Group leader	Expertise
IBIM-CNR, Palermo (Medicina Scienze della Vita) http://www.ibim.cnr.it	Patrizia Guarneri	Impact of EDs on steroid hormone metabolism and neurodegenerative conditions
	Valeria Matranga	Validation of biosensor methods for the analysis of the effects of stress conditions on marine invertebrates
IBF-CNR, Genova (Materiali e Dispositivi) http://www.ibf.cnr.it	Carla Marchetti Gianfranco Prestipino	Identification of the mechanisms of action of toxic metals in mammalian nervous cells
IFC-CNR, Pisa (Medicina) http://www.ifc.cnr.it	Anna Pierini Fabrizio Bianchi	Epidemiological studies on the impact of chemicals on human health
IGM-CNR, Pavia (Medicina Scienze della Vita) http://www.igm.cnr.it	Ivana Scovassi	Identification of effect biomarkers (*in vitro* and *in vivo* approaches)
OTHER INSTITUTIONS		
Fondazione Salvatore Maugeri, Pavia http://www.fsm.it/	Claudio Minoia	Biomonitoring of toxicants and their metabolites in biological fluids from exposed people; total diet studies
Ospedale Perrino, Brindisi http://www.asl.brindisi.it/	Giuseppe Latini	Biomonitoring of phthaltes: impact on infants and human fertility
ISPESL, Roma http://www.ispesl.it/	Elena Sturchio	Model systems: *C. elegans*; DNA biosensors; miRNA platform

occupational safety, health and prevention. Among the various activities carried out by ISPESL, of interest are research, analysis, experimentation and drafting of criteria and methodologies for the prevention of accidents and professional diseases, identification of safety criteria, prevention against chemical, physical and biological exposure risks at work, standardization of test to evaluate occupational safety and health risk assessment.

The ISPESL Department of Production Plants and Anthropic Settlements (DIPIA), carries out research, experimentation, consultation, assistance to the enterprises, proposal of rules, laboratory controls, standardization of methods and procedures of evaluation, analysis of the systems for purposes of safety and environmental compatibility connected to the interaction between the production premises and the external environment. DIPIA is also concerned with the complex problems arising from biotechnologies, particularly with the safety and risk assessment of contained use of genetically modified microorganisms and the evaluation of genetically modified organisms safety and their traceability in food and feed products. DIPIA staff carries out many projects supported by Ministry of Health on eco-genotoxicity studies, biomonitoring of polluted sites with toxicity testing, and molecular analysis; it focuses on indicators to detect the state of environmental health after a release of pollutants from "fall-out". To develop potential biomarkers of susceptibility, toxicity tests and plant and animal models are employed, including the nematode C. elegans, where toxicant effects on phenotype, reproduction, apoptosis and micro RNA profiles are tested. About this latter point, the analysis of miRNAs for the detection of early indicators in various diseases is a distinctive feature of the PIAS-HBM team.

The Director of the Neonatology unit at the Perrino Hospital (Brindisi) has recently joined the EU pool of experts of risk assessment. His main scientific interest is the study of the effect of phthalates, to whom general population is exposed through consumer products, as well as diet and medical treatments. In fact, animal studies showing the existence of an association between some phthalates and testicular toxicity have generated public and scientific concern about the potential adverse effects of environmental changes on male reproductive health. In addition, prenatal exposure to phthalates seems to play a relevant role in determining these adverse effects given that human exposure has been demonstrated to begin during the intrauterine life. A link between antenatal phthalate exposure and abnormal fetal development exists, thus justifying the need of therapeutic tools to fight the adverse effect of this exposure. Numerous maternal lipophilic compounds are eliminated into milk during lactation, their concentrations reflecting fetal in utero exposure. The reported effects of the exposure to phthalates through breast milk in infants confirm that human milk may represent an additional potential source of phthalate exposure in a population at increased risk (49-51). This research is made in collaboration with CNR Institutes.

The members of the "PIAS-HBM network" are listed in Table 1, which reports also the specific (and unique) expertise of each team.

4. Relevant findings

The CNR-IBF team has depicted the molecular mechanisms of nickel and lead toxicity in neural cells. Focusing on the inhibition of N-methyl-D-aspartate receptor (NR) channel in a voltage-dependent manner, the group recently identified specific heavy metal interaction with NR channels (30) and defined the relevance of NR composition in modulating the effect of toxic metals (31). The survey of lead effects represented the first structural work addressing the location of lead interaction site on NMDA receptor channel, thus providing original electrophysiological data (30); the new results obtained for nickel allowed the characterization of its involvement in synaptic currents and transmission (31).

At CNR-IBIM, it has been established the suitability of sea urchin as a sentinel organism for the assessment of the macro-zoobenthos health state in bio-monitoring programmes. A recent survey of sea metal contamination around Pianosa and Caprara Islands revealed that sea urchin coelomocytes might be used as biosensors of environmental stress (52,53). This observation further supports the evidence that sea urchin as well as marine invertebrates are useful as bioindicators of environmental stress.

The SEBIOMAG project represents a good example of an integrated approach to HBM. SEBIOMAG (Studio Epidemiologico Biomonitoraggio Area di Gela) was promoted by World Health Organization, coordinated by Fabrizio Bianchi (CNR-IFC), and carried out in collaboration with the Laboratory for Environmental and Toxicological Testing at FSM (Pavia). The project was conceived as the biomonitoring

Table 2: Published monographs of relevant EDs.

ED	Authors	HBM teams	Reference
Bisphenol A	Minoia C, Leoni E, Turci R, Signorini S, Moccaldi A, Imbriani M	FSM	[55] G Ital Med Lav Erg 2008; 30:214-24.
PFOS, PFOA	Minoia C, Leoni E, Sottani C, Biamonti G, Signorini S, Imbriani M	FSM IGM	[56] G Ital Med Lav Erg 2008; 30:309-23.
Arsenic	Sturchio E, Minoia, Zanellato M, Masotti A, Leoni E, Sottani C, Biamonti G, Ronchi A, Casorri L, Signorini S, Imbriani M	FSM IGM ISPESL	[57] G Ital Med Lav Erg 2009; 31:5-32.
PCDDs	Turci R, Minoia C, Leoni E, Sturchio E, Boccia P, Meconi C, Zanellato M, Signorini S, Benzoni I, Mantovani A, La Rocca C, Bianchi F, Imbriani M	FSM IFC ISPESL	[58] G Ital Med Lav Erg 2009.; 31:325-70.

of the exposure of target population living in Gela (Sicily). The study consisted of the evaluation of toxicant levels in biological fluids, focusing on trace elements (antimony, arsenic, beryllium, cadmium, mercury, lead, copper, selenium, thallium, vanadium) and organochlorine compounds (PCB, aldrin, dieldrin, DDT, chloroesane, chlorobenzene). Trace elements and organochlorine compounds were measured by inductively coupled plasmadynamic reaction cell-mass spectrometry (ICP-DRC-MS) and gas chromatography coupled to mass spectrometry (GC-MS), respectively. The target population consisted in 184 subjects aged 20-44 years living in Gela (116), Niscemi (39) and Butera (29). The analysis revealed that the levels for organochlorine compounds, antimony, selenium, thallium, beryllium and vanadium were comparable to control population. The most relevant finding was the peculiar profile of widespread exposure to arsenic recorded in blood, plasma and urine samples, where the values were higher than reference values of non-exposed subjects. In conclusion, the results underlined a widespread exposure to arsenic, and recommended the evaluation of different forms of arsenic (inorganic arsenic, arsenobetaine, arsenocholine) to better understand the potential sources of exposure (environmental, seafood intake) in this crucial area. The results of this study have been presented in Gela, on July 16, 2009, at the SEBIOMAG meeting.

Remarkably, this analysis represents an added value to a previous survey conducted on the same geographical area and providing environmental data about contaminants present in water, earth and air. These data are reported in the special issue of the journal Epidemiology & Prevention devoted to “Environment and health in Gela (Sicily): present knowledge and prospects for future studies” (54). The joint effort to evaluate the environmental contamination of an industrial area, and the consequent exposure of the general population, is an example of an efficient strategy to combine clinical and research competences for improving the experimental approaches useful to depict the effects of environmental risk on human health.

Of note, some members of the network cooperate for preparing toxicological information profiles of specific chemicals. Based on a huge amount of literature data, these reviews provide relevant scientific information actually on EDs, including bisphenol A, perfluoroalkyl agents (i.e. PFOS/PFOA), trace elements (i.e. arsenic) and dioxins (i.e. PCDDs) (55-58). The papers, listed in Table 2, are published on the "Giornale Italiano di Medicina del Lavoro e Ergonomia", that is the official Journal of FSM. Each paper includes general aspects of exposure source, chemical and physical properties, metabolism, toxicological and carcinogenic potential, food intake and diet exposure, mechanism of action, genetic susceptibility, analytical procedures and general population levels. This body of information represents a very useful tool not only for clinicians but also for researchers in the environmental and occupational context. In fact, even if extensive toxicity data for a chemical are available, they are almost always in a form that is difficult to combine with biomonitoring-generated exposure values to assess risk (15).

An example of scientific literature that aims at making attractive and understandable to the general public a so complex topic as Environment&Health is represented by the recent book "Ambiente e salute: una relazione a rischio. Riflessioni tra etica, epidemiologia e comunicazione" by the scientists from the CNR-IFC Epidemiology unit (59). The book uses a number of case studies to define the concept of epidemiology, population study, genetic susceptibility, risk complexity, scientific communication, and future perspectives in risk assessment. The hot topic "Environment&Health" is discussed at different levels, that is ethics, epidemiology and communication also in a recent publication (60).

5. Future perspectives and developments

In the last years there has been a call for increased epidemiological and experimental research to substantiate the disruptive effects of environmental chemicals in humans. This is currently recognized as an important issue of concern in the protection of human environmental health.
Human biomonitoring is an important tool to evaluate the internal exposure through the environment and to provide early warning indicators for possible long-term adverse effects. Biomonitoring has two main goals: i) determination of the levels of toxicants in biological fluids from a general population; ii) search for new exposure, effect and susceptibility biomarkers.

The here described PIAS-HBM network combines solid clinical expertise with advanced research work. With an adequate funding, it could act in a coordinated way to address the following crucial points:

1. To handle the Environment&Health problem with a multidisciplinary approach, combining medical tools with a biological approach based on biochemistry, biophysics, cell and molecular biology, bioinformatics, molecular genetics and genomics. This goal could be achieved through a strict cooperation between the different members of the network, sharing the respective competences and working in tight association.
2. To validate conventional/new *exposure* biomarkers. The growing number of chemicals potentially toxic for human health implies a continuous effort to develop new specific assays and to

validate them; in parallel, conventional procedures have to be periodically checked for their efficacy by different laboratories. As for exposure markers, it could be of interest to share biological samples collected at Hospitals and IRCCS in order to increase the number of selected biomarkers. As a new perspective, it could be useful to evaluate the real exposure of the general population through the diet, which is considered to be the main source of body burden of several contaminants. This approach, based on a validated protocol that estimates the dietary intake of compounds, could contribute to face the lack of data on the real exposure to several contaminants and to develop *ad hoc* protocols to measure their levels in food. Quantification of the risk by the ingestion of pollutants in food is complex and depends on many factors (species, diet composition, duration of exposure, efficiency of pollutant absorption, subsequent metabolism, sensitivity of target organs and stage of development). While the effects of high doses of single chemicals are proven, dietary exposure generally involves prolonged, low-level exposure to a large number of compounds, each of which has different chemical characteristics, different biological effects and is present at varying concentrations.

3. To validate conventional/new *effect* biomarkers. The search for effect biomarkers implies a panel of approaches, spanning from cellular to molecular biology, focusing on the effects of chemicals in terms of cell proliferation, cell death, signal transduction, and neurodegeneration. The PIAS-HBM team possesses the ability to focus on the dissection of the biochemical steps of basic biological processes, such as DNA replication and repair, transcription, translation and post-translation. *In vivo* assays on cell lines of different origin (e.g. neuroendocrines, neurals, fibroblasts, keratinocytes, transformed) may allow the identification of the effect of chemicals on different cellular (adhesion, cell cycle, proliferation and death, motility) and biochemical (DNA damage, electrophysiology, inflammation, mitochondrial metabolism, neurotoxicity, ionic transport, replication and repair, oxidative stress) parameters. Also the analysis of the impact of chemicals on different classes of receptors (e.g. ER alpha/beta, AR, PR, GR, ThRs, retinoic acid, aryl-AhR, pregnane-X-R) is appealing. The measurement of the activity of a number of reference enzymes (e.g. acetylase, 5-alfa reductase, aromatase, kinase, DNA polymerase, phosphorylase, ligase, nuclease, PARP, protease, telomerase, topoisomerase, reverse transcriptase, sulfatase, sulfotransferase) represents an original tool to elucidate the biomolecular mechanisms of action of selected toxicants and to identify new effect biomarkers to be tested in the population through validated protocols.
4. To identify genetic *susceptibility* markers in the Italian population. The search for susceptibility markers in the Italian population is a priority issue. In addition to cytogenetic and genetic assays, new molecular biology tools such as microRNA and epigenetic regulation of gene expression are useful to obtain predictive markers of noxious effects of pollutants

correlated to the genetic background of individuals. The final goal is to identify toxicogenomic markers in the Italian population.

In addition to the above action plan, which is strictly based on the specific know-how of the single teams, other hints emphasize the power of our network:

a) Human biomonitoring is directed to human beings. However, basic research on organisms other than humans could speed up the process of biomarker identification and limit the use of human material. The investigations at ISPESL aiming at validating *C. elegans* as model organism for the analysis of the impact of toxicants on living organisms could be very useful. In fact, the fast growth and reproduction time of this animal as well as the exhaustive knowledge of this genome renders *C. elegans* an extremely easy model. As underlined in the 2002 Nobel prize award (61), the identification of key genes regulating organ development and programmed cell death in *C. elegans* was instrumental to the knowledge that corresponding genes exist in higher species, including man. Similarly, the body of evidence accumulated at CNR-IBIM on the use of sea urchin as biosensor could have more applications than the actual ones. Since the publication of the sequence of this marine invertebrate organism (62), new experimental procedures have been developed to test the effect of chemicals both on whole marine organisms and on coelomocytes. This is a new and original tool.

b) It is widely agreed that exposure *in utero* to toxicants may modify the normal path of reproductive development and cause genital malformations (hypospadia, cryptorchidism). In the same scientific area, it has been shown that prenatal exposure to pollutants and/or the exposure through the maternal milk have adverse effect of infant health. The incidence of congenital malformations may represent an early biological indicator for environmental and/or occupational exposure to contaminants. The search for factors affecting the maternal-fetal environment is currently addressed at CNR-IFC and Perrino Hospital.

c) A correlation between neurological disorders and environmental risk has been found. The expertise of CNR-IBF and IBIM in the field of the study of the effects of pollutants on the nervous system strongly supports an in-depth examination of the problem of genetic and environmental factors that modulate the occurrence of such diseases.

d) There is an increasing need for epidemiology studies, to develop a strategy of protection of human environmental health. Within our PIAS-HBM network, the expertise of CNR-IFC guarantees a correct approach to the problem.

e) The compilation of reviews on the toxicological profile of pollutants is extremely useful to the scientific community. Further work is required to increase the number of considered chemicals; to this purpose, the involvement of all the members of the PIAS-HBM team is desirable. This activity will facilitate clinicians and researchers.

In conclusion, it is clear that in order to investigate the relationship between environment and health, the most important prerequisite is the availability of an appropriate panel of biomarkers as indicators of individual exposure to environmental chemicals. This attempt requires a cross-interaction among toxicology, cellular and molecular biology in order to identify a potent body of biomarkers of exposure, effect and susceptibility. In this respect, our PIAS-HBM network fits with the purposes of modern HBM, which has expanded beyond its origin in occupational medicine to cover a wide variety of diagnostic procedures and assessments of environmental pollution, leading to the identification of potentially hazardous exposure before adverse health effects appear and to establish exposure limits for minimizing the likelihood of significant health risk methods. The network we established can be enlarged to other Italian teams providing additional competences, thus ensuring a multifaceted research approach, possibly under the aegis of CNR.

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That each member of the PIAS-HBM team has been/is granted to work in the risk assessment field is an evidence of the qualification of the scientists belonging to the network. However, to establish a coordinated and multidisciplinary work strategy, a global grant from a public agency, covering the expenses of an integrated project, is absolutely required.

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