Cross-Mediterranean Environment and Health Network

CROME-LIFE

Deliverable B.3.1

Concept document on HBM and environmental campaign design

TASK: 3.1
Environmental and HBM campaign design

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Introduction

In deliverable B.1.2 "Methodology report outlining the ways to link environmental, human biomonitoring and health status data to derive environment-wide association and integrated health impact assessment" selected case studies for the demonstration of the project methodology were described:

- **Cross-Mediterranean study** which includes Greece, Italy, Slovenia, Spain and Croatia
- **National targeted environmental studies:**

  Case study Greece - Human Biomonitoring for Cr6+ in Greece

  Case study Greece – Health effects of urban biomass combustion

  Case study Slovenia - Human Biomonitoring in Slovenia

  Case study Italy - Human Biomonitoring in Italy

  Case study Spain - Human Biomonitoring in Spain

Deliverable B.3.1 is a concept documents on HBM and environmental campaign design. This document deals with subtasks environmental and HBM campaign design of each demonstration site of the project. With regard to the Cross-Mediterranean study we plan to use the already existing HBM data collected from within the PHIME project, involving Slovenia, Croatia, Italy, Greece and within the INMA Project (Environment and Childhood) will be followed up at 6-8 years of age (14 years in some cases in Spain) in the CROME Cross-Mediterranean study.

The available environmental pollution data are limited in all demonstration sites of the project. They will be enhanced by targeted campaigns, which will focus on the "hot spots" revealed from the residence of the individuals with higher biomarker values. The latter will be derived after examination of the existing HBM data. Samples of environmental matrices will be collected, stored and analysed with the appropriate analytical techniques, including primarily AAS and ICP-MS (for metals) and GC-MS, HPLC-MS/UPLC-MS (for organic contaminants such as organochlorine compounds and PBDEs). The environmental matrices will comprise top soil, surface and groundwater, and ambient air. Some most consumed food items at local area will be also included in the analysis. These analyses will support the derivation of overall exposure patterns of the local population when analysed in combination with the existing environmental and dietary data found in the databases set. In addition, questionnaire-based surveys of behavioural and dietary habits and time-activity diaries of the local population (the individuals for who HBM data exist in the demonstration sites) will be used to derive complete personal exposure profiles for the study participants. Final goal is to assess personal exposure profile as complete as possible.

The obtained environmental monitoring data will be coupled with HBM and epidemiological observations using physiologically-based toxicokinetic (PBTK) and toxicodynamic (PBTD) models. These would allow us to mechanistically associate the observed concentrations of contaminants in environmental media with HBM data already existing within the consortium and collected through targeted field campaigns. Via reverse modelling human exposure to the chemical substances will be reconstructed. These estimates will be used as indices of population exposure and of the environmental health burden due to the anthropogenic pollution in the project demonstration sites. The CROME-LIFE approach will show the feasibility of environment-wide association studies by inter-operably linking environmental, biomonitoring and health status data. Causal associations between the observed health outcomes and the measured/estimated markers of exposure will be derived by means of advanced statistical models and causal diagrams.

Cross-Mediterranean study

Introduction

Children from the pre-existing Mediterranean cohorts established (1) within the PHIME project, involving Slovenia, Croatia, Italy, Greece and (2) within the INMA Project (Environment and Childhood) will be followed up at 6-8 years of age (14 years in some cases in Spain) in the CROME Cross-Mediterranean study.

PHIME was the largest study ever conducted in the general European population on the impact of mercury and other trace metals through food consumption. Children of the PHIME Mediterranean cohort were tested for neurodevelopment (Bayley III test) at 18 months of age. Mother hair, cord blood, cord tissue and meconium have been sampled at birth, breast milk and mother's hair 1 month after birth. Hair samples have been analysed for mercury, cord blood and breast milk for mercury, cadmium, lead and arsenic, as well as for essential elements (selenium, zinc, copper). The results of the PHIME Mediterranean cohort have already been evaluated, particularly in relation to methyl mercury (MeHg) exposure. Mercury in mother´s hair and in cord blood did not predict Bayley scores but a moderate beneficial effect of fish consumption in pregnancy was observed. Other chemical elements were not associated with the outcomes (PHIME 2006). However, epidemiological studies have demonstrated that the developmental neurotoxicity is associated with prenatal MeHg exposure (Grandjean and Landrigan 2006), but susceptibility to MeHg toxicity may be, among other factors, modified by genetics (Grandjean and Julvez 2013). Adverse associations among genetically susceptible groups were discovered in analyses that were stratified by the single nucleotide polymorphism (SNP) allelic variants.

Objectives

In the *follow-up study*, we would like to find those genetic polymorphisms which could modulate the detrimental effects of metals, particularly Hg, at low exposure in the Mediterranean cohort. In this regard, we will focus on gene polymorphisms of glutathione-related genes (*GSTM1*, *GSTT1*, *GSTP1*, *GSTM3*, *GPX1*), metal binding protein genes (*MT2A*, *MT4*, *SEPW1*, *SEPP1*), genes involved in scavenging of ROS (*CAT*, *SOD1*, *SOD2*, *GSR*), and genes implicated in brain development (*PON1*, *BDNF*, *PGR*) as previous literature has shown that variants in these genes might enhance Hg/MeHg developmental neurotoxicity.Children will be tested at 6-7 years for neuropsychological performance using *Wechsler Intelligence Scale for Children (WISC III*). Examination will include collection of urine, blood and hair samples for analyses of metals and collection of saliva samples for genotyping.

In Spain the priority tasks are related to the assessment of the mechanisms of transfer of environmental pollutants into children at the early age stages. For this purpose, it is important to clarify how the physiological changes of the mothers have an influence in this transfer during the foetal period of children and, at the same time, what are the body burdens of pollutants such as organochlorine and organobromine compounds and metals in the pregnant women.

Existing environmental data

The data available from the PHIME and INMA projects is summarized in the Deliverable B.1.2.

Environmental data that is available from the detailed questionnaire database from the PHIME project is the following:

- Smoking behaviour throughout the pregnancy,
- dental visits and new/replaced dental fillings throughout the pregnancy,
- detailed nutritional data: consumption frequency of different types of food including different fish species and origin of a specific food item (locally grown/caught or bought in the supermarket/local market).
- residential area: vicinity of industry, highways and different roads, railway station, airport;
- potential occupational exposure to lead, mercury, gold and other metals, chromium, pigments, PCBs, solvents, radioactive isotopes, amalgams
- general data on occupation, with particular emphasis on: dentistry, jewellery, working with photography, chlor-alkali industry, paints, production of electric appliances, paper industry, pharmaceutical industry, printing industry, traffic, agriculture

Other data available from the questionnaires:

- pregnancy history: mother's age at delivery, BMI before pregnancy, weight gain increase, health status and health records, type of delivery and delivery implications if any;
- data on child: sex, birth weight and length, weight and length at 18 months, breastfeeding history (any vs. none), duration and exclusiveness up to 4 months, daycare attendance at 18 months, duration of the intake of fresh and homogenized fish up to 18 months; health implications;
- socio-economic status data (home area <50, 50-100, >100 m^2); home ownership; parental education (the higher of the two); number of children in the family; marital status of the mother at delivery.

Relevant food data:

Mercury, selenium and PCBs content is available for fresh and canned fish available on the Slovenian market:

Miklavčič, Ana, Stibilj, Vekoslava, Heath, Ester, Polak, Tomaž, Snoj Tratnik, Janja, Klavž, Janez, Mazej, Darja, Horvat, Milena. Mercury, selenium, PCBs and fatty acids in fresh and canned fish available on the Slovenian market, 2011. Food chem. vol. 124, issue 3, p. 711-720.

There is data on trace element content available also for different food items (vegetables, mushrooms and game) from the Hg-contaminated area, where a subset of PHIME study subjects had been sampled (Miklavčič et al, 2013).

Ana Miklavčič, DarjaMazej, Radojko Jaćimović, TatjanaDizdarević, MilenaHorvat, 2013. Mercury in food items from the Idrija Mercury Mine area. Environmental Research 125, 61–68.

In Greece data on fish consumption have been obtained from questionnaires. However there are no data on fish contamination on heavy metals or other organic compounds.

Valent, F., Horvat, M., Sofianou-Katsoulis, A., Spiric, Z., Mazej, D., Little, D., Prasouli, A., Mariuz, M., Tamburlini, G., Nakou, S., Barbone, F. (2013). Neurodevelopmental effects of low-level prenatal mercury exposure from maternal fish consumption in a Mediterranean cohort: study rationale and design. J. Epidemiol. 23, 146-52.

Limited data on environmental contamination with POPs have been described in the following study:

Lammel, G., Audy, O., Besis, A., Efstathiou, C., Eleftheriadis, K., Kohoutek, J., Kukučka, P., Mulder, M., Přibylová, P., Prokeš, R., Rusina, T., Samara, C., Sofuoglu, A., Sofuoglu, S., Taşdemir, Y., Vassilatou, V., Voutsa, D., Vrana, B. (2015). Air and seawater pollution and air–sea gas exchange of persistent toxic substances in the Aegean Sea: spatial trends of PAHs, PCBs, OCPs and PBDEs. Environ Sci Pollut Res. 1-13.

Data concerning environmental pollution in the Friuli Venezia Giulia Region and the Trieste area where the children participating in the study live are collected by the Regional Enviornmental Protection Agency (http://www.arpa.fvg.it/) as for monitoring of air quality, drinkable water quality, contaminants in soil, and pollution in the two main contaminated sites (Trieste and Marano/Grado Lagune) of the Friuli Venezia Giulia Region.

No updated indications concerning the levels of metals in food items in the areas where the population under study lives are available. A report on the national levels of some metals in fish and seafood product in Italy has been published in 2012: Pastorelli AA, Baldini M, Stacchini P, Baldini G, Morelli S, Sagratella E, Zaza S, Ciardullo S.

Human exposure to lead, cadmium and mercury through fish and seafood product consumption in Italy: a pilot evaluation. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2012;29(12):1913-21.

In Spain, there is information on mercury content in fish collected by the Spanish Ministry of Agriculture and Environment in the Balearic Sea. The most common dietary fish species were investigated.

Data on the composition of organochlorine compounds in Mediterranean fish has been described in the followings works:

M. Sole, C. Porte, J. Albaiges. 2001 Hydrocarbons, PCBs and DDT in the NW Mediterranean deep-sea fish Mora moro, Deep-Sea Research 48, 495-513.

J. Albaigés, J. Gallifa, J.O. Grimalt and M. Soler. 1984. Hydrocarbons in biota samples from the Western Mediterranean. Journees des Etudes des Pollutions CIESM 5, 215-218.

Garcia, LM; Porte, C; Albaiges, J. 2000. Organochlorinated pollutants and xenobiotic metabolizing enzymes in W. Mediterranean mesopelagic fish. Marine Pollution Bulletin 40, 764-768.

Pastor, D; Boix, J; Fernandez, V; Albaiges, J. 1996. Bioaccumulation of organochlorinated contaminants in three estuarine fish species (Mullus barbatus, Mugil cephalus and Dicentrarcus labrax). Marine Pollution Bulletin 32, 257-262.

Porte, C; Albaiges, J. 1994. Bioaccumulation patterns of hydrocarbons and polychlorinated-biphenyls in bivalves, crustaceans, and fishes. Archives of Environmental Contamination and Toxicology 26, 273-281.

Sanchez, J; Sole, M; Albaiges, J. 1993. A comparison of distributions of pcb congeners and other chlorinated compounds in fishes from coastal areas and remote lakes. International Journal of Environmental Analytical Chemistry 50, 269-284.

Albaiges, J; Farran, A; Soler, M; Gallifa, A; Martin, P. 1987. Accumulation and distribution of biogenic and pollutant hydrocarbons, pcbs and ddt in tissues of western Mediterranean fishes. Marine environmental research 22, 1-18.

Missing environmental data and filling the gap

Relevant food data:

In connection to the questionnaire data on consumption of different fish species, fish species that were commonly bought and consumed by the study subjects have been sampled in the Slovenian and Italian market. The samples will be analysed for total and methyl mercury as well as other trace elements to link with the exposure as assessed through measurements in blood of study subjects.
For the Greek population, selection of most frequently consumed fish species is still missing and the sampling of identified common species followed by trace metal analyses is to be done within the CROME framework.
For the Greek population, selection of most frequently consumed fish species is still missing and the sampling of identified common species followed by trace metal analyses is to be done within the CROME framework.
For the populations in Valencia and Menorca (Spain) the composition of mercury and organohalogen compounds (organochlorine and organobromine compounds) in the diet items is still missing. The missing information also includes fish because most of the data available concern fish specimens related with environmental studies not specifically diet.

Other environmentally relevant data:
Within the follow-up of the children form the PHIME and INMA studies (described in the Deliverable B.1.2), additional questionnaire will be applied to obtain information relevant for the study objectives and also other general data: age at WISC III assessment, health status, living environment, food frequency data, potential exposure (i.e. amalgam fillings, broken thermometer, and passive smoking), and socio-demographic data.
For the populations in Valencia and Menorca (Spain) questionnaire information is available. However, environmental data concerning pollutant composition in air, water and soils is still missing.

National targeted environmental problems

Case study Greece - Human Biomonitoring for Cr6+ in Greece

Introduction

A major local environmental issue in Greece is related to the presence of hexavalent chromium Cr(VI) in drinking water of the Oinofyta municipality, within the wider area of Asopos basin and the related cancer mortality. The Oinofyta municipality (Figure 1) is situated 50 km North of Athens, Greece, and it includes four villages that were initially rural but transformed into industrial areas in the early 1970s. In 1969, a ministerial decision gave permission for depositing processed industrial waste in the Asopos river, which runs through Oinofyta. This decision, furthered by a presidential decree in 1979, permitted free disposal of processed liquid industrial waste into the river. According to the Technical Chamber of Greece (TCOG 2009), in the 80s there were about 700 industries operating in the Oinofyta area, of which 500 generated liquid industrial waste. After protests from citizens who complained about the discoloration and turbidity of their drinking water in 2007 the Ministry of Environment, Regional Planning and Public Works of Greece imposed fines on 20 industries for disposing industrial waste with high levels of hexavalent chromium into the Asopos river. Official limits on total chromium have been set by both the United States Environmental Protection Agency (US-EPA), equal to 100 μg/l, and the European Union (Council directive 98/83/EC), equal to 50 μg/l. However, as of yet, there are no limits set by any international body for Cr(VI). In 2009, the California Environmental Protection Agency proposed a public health goal level of 0.06 μg/l for Cr(VI) in drinking water (OEHHA 2009).

Figure 1: Asopos Basin and Oinofyta municipality

Because areas characterized by high Cr(VI) concentrations in drinking water are relatively uncommon, human epidemiologic studies are scant; the study carried out by Zhang and Li (1987) is one of the most cited and controversial studies analyzing the effects of oral exposure to Cr(VI) on population cancer mortality rates conducted near a chromium smelting plant in the Liaoning Province, China.

In order to examine the potential effects of elevated oral exposure to hexavalent chromium, an ecological mortality study (Linos et al. 2011) was performed in an industrial area of Greece where the water consumed by the population was contaminated with hexavalent chromium (maximum levels ranging between 41 and 156 μg/l in 2007-2009, and presumed exposure for at least 20 years). The goal of the study was to examine the cancer mortality in an area of Greece, historically satisfying its potable needs with a Cr(VI)-contaminated aquifer.

Existing environmental data

To the best of our knowledge, there are no systematic measurements of Cr(VI) before 2007. Since 2007, three independent sets of hexavalent chromium measurements are available for the Oinofyta area. These include:

a) a study of the Institute of Geology and Mineral Exploration (IGME 2008) during the period November 2007 to February 2008, which detected 35 samples (out of 87) taken from different wells in the same area, where levels above 10 μg/l with a maximum value 156 μg/l were detected;

b) a study conducted by the faculty of the Geology and Geo-environment department of the University of Athens (Vasilatos et al. 2008) during the period September 2008 to December 2008, in which Cr(VI) levels ranged from 41 up to 53 μg/l in three samples taken from the public drinking water supply of Oinofyta; and

c) repeated measurements by the Oinofyta municipality in the public drinking water supply during the period July 2007 to July 2010, in which there are 13 measurements with levels above 10 μg/l and with a maximum value of 51 μg/l

According to official Oinofyta municipality authorities, in early 2009 the main drinking water supply of Oinofyta was diverted to receive water from Mornos lake (reservoir) which is part of the drinking water supply network of the city of Athens. Therefore, more recent measurements made by the Oinofyta municipality (June 2009- July 2010) record relatively lower levels of Cr(VI) (<0.01-1.53 μg/l).

Furthermore, in the study carried out by Economou-Eliopoulos et al. (2011), groundwater samples from the Asopos aquifer showed a wide spatial variability, ranging from <2 to 180 ppb Cr total content [almost same to the Cr(VI)-values] despite their spatial association. The presence of Cr(VI)-contaminated ground water at depths >200m is attributed to a direct injection of Cr(VI)-rich industrial wastes at depth rather than that Cr(VI) is derived from the Asopos river or by the interaction between water and Cr-bearing rocks.

Missing data and filling the gaps

Filling the gap

Human biomonitoring data are the critical data missing for the application of the CROME methodology in Asopos basin. Given that environmental concentrations and actual exposure have been decreased after 2007, we need to identify recent, as well as past exposure. For this purpose, a combination of biomonitoring data will be applied, including urine samples (for assessing current exposure levels), as well as hair samples for assessing exposure burden from the past. For the purposes of the analysis, a sample of 20 residents will be collected. Details on the analytical techniques that will be followed are given below.

Study design

50 individuals age stratified (4 to 65 years old) will be recruited so as to evaluate current as well as past and cumulative exposures to Cr(VI). For this purpose, both urine and hair samples will be taken. Population will cover the wider basin of Asopos area, reflecting the variability of exposures related to the different levels of Cr(VI) sampled in the environmental campains mentioned above.

Methods

Electrothermal Atomic Absorption Spectroscopy (ETAAS) for assessing Cr(VI) in hair

The analytical method is based on the described by Afridi et al (2006) where biological samples were collected from a total of 56 long-term exposed steel production workers (PW), 35 quality control workers (QCW) and 75 unexposed normal controls (all male, age range 25 55 years). The working solution of Cr will be prepared daily from certified standard solutions of all analytes under study in 2M nitric acid. All solutions will be stored at 4^oC. Hair samples will be collected from the occipital region of the head. The samples of hair will be obtained using stainless steel scissors from the nape of the neck. The hair samples will be cut into approximately 0.5-cm pieces in length and mixed to make a representative hair sample. In the case of each person, hair strands will be washed with diethyl ether-acetone (3+1) mixture, non-ionic detergent solution (distilled water) and ultrapure water, respectively. After washing, the hair samples will be dried at 80^oC for 6 h. Hair samples for each participant will be placed in separate plastic envelopes, which indicated the identification (ID) number of the participant. Duplicate 0.5 mL of each certified urine and blood samples, while 0.2 g of human hair samples BCR 397, will be placed into 50-mL Pyrex flasks. A 5-mL volume of a freshly prepared mixture of concentrated HNO_3 -H_2O_2 (2:1, v/v) is added to each flask, and the solutions will be heated on an electric hot plate at 80^oC for 2-3 h, until the clear transparent digests is obtained. Final solutions will be made up to 10 mL with 2M HNO_3. The final solutions will be collected in polyethylene flask for determinations of Cr by ETAAS. Blank digestions will also be carried out. Duplicate samples of QCW, PW and normal controls will be treated as described above. Detailed instructions on the operation of the Perkin-Elmer model 4110 ZL are described in the operator's manual. The sample (calibration blank, standards, reagent blank, and control sample) and matrix modifiers will be introduced to the furnace by an auto sampler. The calibration will be periodically verified by analyzing the standard at the frequency of 10 readings. A microwave-assisted digestion procedure will be carried out in order to achieve a shorter digestion time. For digestion of biological samples, duplicate samples of dried scalp hair (200 mg) three replicate samples of CRM 397, will be weighed into Teflon PFA digestion vessels directly, to which 2 mL of HNO_3 and 1 mL of 30% H_2O_2 will be added and left to stand for 10 min, then the vessels will be sealed and placed in a PTFE reactor. This will be then heated following a one-stage digestion programme (250 W, 15 min for hair samples). After cooling the digestion vessels in an ice bath for 20 min before opening, the resulting solution will be evaporated almost to dryness to remove excess acid, and then diluted to 10.0 mL in volumetric flasks with 2M HNO_3. Blank extractions (without sample) will be carried through the complete procedure of both methods. The concentrations will be obtained directly from calibration graphs after correction of the absorbance for the signal from an appropriate reagent blank.

Urine Samples - Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

The methodology to identify Cr(VI) in urine samples is based on the one proposed by Scheepers et al. (2008). Spot urine samples are stored at 4 ^{0}C in the dark and transferred to a laboratory for further storage at −18 ^{0}C. The analysis of Cr is performed according to Lewalter et al. (1985). Urine will be diluted with a solution of magnesium nitrate with Triton-X and sulphuric acid (matrix modifier). Cr levels will be determined at 357.9nm using electro thermal atomic absorption spectrometry, AAS (Solaar M, Thermo Analytical) with Zeeman background correction. The LOQ is 0.10μg/L (corresponding to 0.10μg/g creatinine if a creatinine value of 1 g/L is assumed) of urine and the coefficient of variation is 7.1% at 0.31μg/L. Creatinine concentrations in urine samples will be determined using the Jaffe reaction.

Case study Greece – Health effects of urban biomass combustion

Introduction

Over the last couple of years, the use of biomass as heating source was allowed in Greece as a CO_2-neutral means of space heating in the large metropolitan areas of Athens and Thessaloniki affecting more than half of the country's population. At the same time the use of light heating diesel was heavily taxed. In the same period Greece faces a financial crisis with significant repercussions on the average household income. That combination of parameters resulted to increased use of biomass for residential heating in year 2012, followed by a significant increase of ambient air, indoor air and exposure to PM_{10} and $PM_{2.5}$. In this case study, we aim to quantify the health effects related to that shift from light heating diesel to biomass burning, as well as to evaluate alternative scenarios of residential heating energy share.

Existing environmental data

Preliminary data for the verification of the case have been delivered by Sarigiannis et. al (2013). Interesting conclusions are given by the comparative study of daily values for the same dates between the years 2011 and 2012.

During the warm period of early October-early November 2011 at the traffic station, PM_{10} concentrations were 59.8 and $PM_{2.5}$ 47.0 μg/m^3, while during the cold period, amounted to 82.9 and 68.3 μg/m^3 respectively. During the warm period of 2012, the respective concentrations remained lower, being 53.1 and 29.5 μg/m^3 for PM_{10} and $PM_{2.5}$, while a significant increase occured during the cold period (76.5 and 59.7 μg/m^3 for PM_{10} and $PM_{2.5}$ respectively). Since average wind speed was similar in both the seasons, the reduced concentrations observed in 2012 at the traffic station are attributed to reduced traffic emissions, which in turn are due to the reduced traffic load by 30% in 2012, as evidenced by *in situ* traffic measurements carried out.

Moreover, while in 2011 the ratio of $PM_{2.5}/PM_{10}$ remained almost constant (~ 0.8) in the two periods (warm-cold), in 2012 it increased significantly during the cold period (from ~ 0.55 up to 0.78); the latter indicates that additional contribution beyond traffic sources was becoming important. Instead, at the background station, whereas concentrations of PM_{10} and $PM_{2.5}$ were higher during the warm period of 2011 (41.4 and 31.1 μg/m^3), with respect to the ones measured in 2012 (30.6 and 19.4 μg/m^3 respectively), during the coldest period of 2012 PM concentrations were significantly higher (73.1 and 62.7 μg/m^3 for PM_{10} and $PM_{2.5}$ respectively) than the ones in 2011 (53.1 and 43.5 respectively).

The increase of concentrations in year 2012 was accompanied by a sharp increase in the ratio $PM_{2.5}/PM_{10}$ (from 0.63 to 0.86), in contrast to 2011, where the corresponding change was smaller (from 0.75 to 0.82). Between the two years, there was a significant variation among the emission patterns, where the traffic component appears to be reduced in 2012, while during the colder period the component associated with the biomass heating was dramatically increasing. Given that temperatures during the cold season of 2012 are close to the ones of 2011 (daily average 11.1 ^{0}C for both years), these differences cannot be attributed to increased need for domestic heating. During the last winter (2013-2014), the pattern of traffic and biomass burning emissions seems to be similar to the previous year, thus, the problem of biomass burning still remains.

The contribution of biomass burning to PM air pollution was verified by levoglucosan analysis of PM, which is considered the most specific tracer of biomass burning (Belis et al. 2013; Perrone et al. 2012; Zhang et al. 2008) and the empirical function proposed by (Caseiro et al. 2009), according to which:

$$\text{wood smoke PM (in } \mu g/m^3) = \text{Levoglucosan (in } \mu g/m^3) \cdot 10.7$$

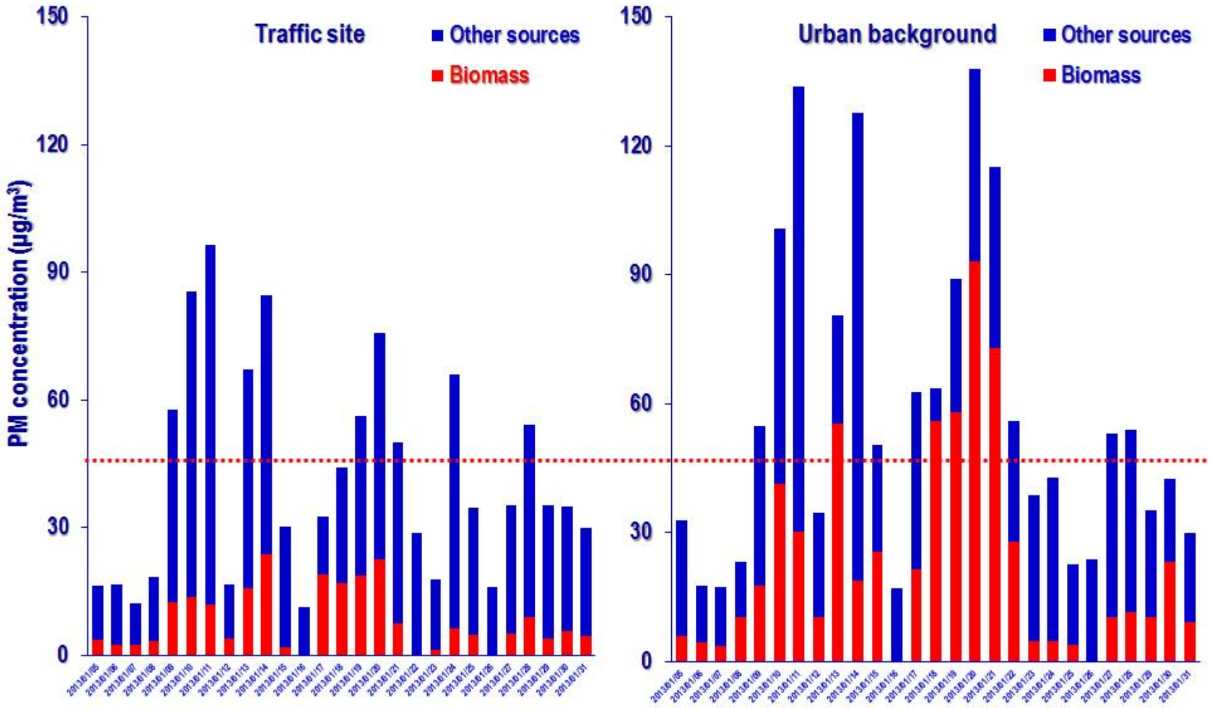


Figure 2: Biomass burning contribution in two distinct sites (traffic and urban background) in the city of Thessaloniki

Increased biomass emissions, are linked to elevated exposure to PM, as well to toxic compounds adsorbed on them, PAHs (IARC 2010), being the ones of highest concern among them. It is also interesting that higher concentrations of PM due to biomass burning, where accompanied by equally high concentrations of PAHs; the latter shows that toxic potency of biomass emitted PM is at least equally toxic to traffic emitted PM (Figure 3).

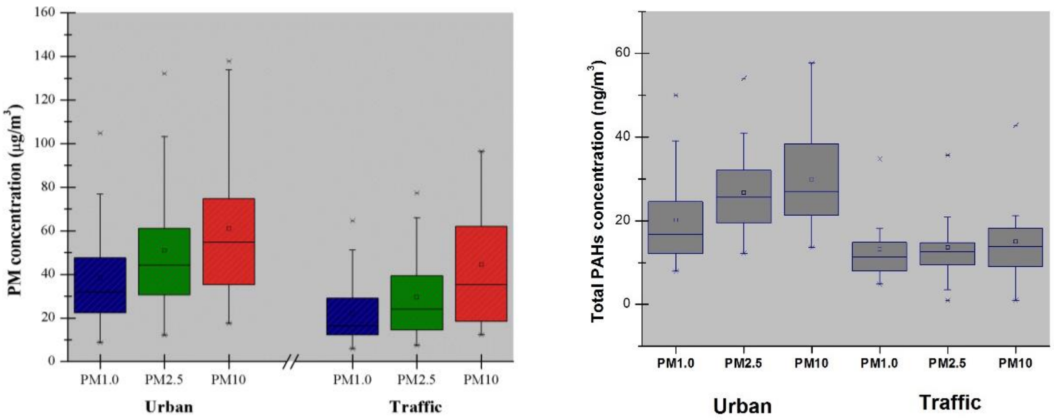


Figure 3: PAHs concentration between urban and traffic site for the different PM fractions

Missing data and filling the gaps

Filling the gap

There are plenty of detailed environmental data related to PM concentrations and chemical speciation. This data incorporated to advanced modelling framework described above will allow the accurate assessment of external and internal exposure to PM. Exposure to PAHs and consequently to PMs, will be verified by PAHs metabolites measurements in urine. Health data for the endpoints mentioned in **Error! Reference source not found.** will be retrieved by he Hellenic Statistical Authority and the hospitals of the wider Metropolitan area of Thessaloniki.

Study design

In order to fill the biomonitoring gaps, a biomonitoring sampling campaign took place in winter of 2014-2015 in Thessaloniki, aiming to capture the effect of biomass burning in PM and PAHs exposure. Overall 50 of non-smoker individuals aged 8 to 64 were sampled, covering a wide spatial distribution of the city. This was necessary, since differences in exposure were expected to be found based on the levels of the performed activities, the ventilation conditions (Karakitsios et al. 2014; Sarigiannis et al. 2011), as well as the respective distribution of PM and PAHs levels in the city (Sarigiannis et al. 2015). The locations of the 50 individuals sampled in Thessaloniki are illustrated in Figure 4**Error! eference source not found.**.

Methods

Within the CROME-LIFE project, 1-OH pyrene in urine was used as a metric of internal exposure to PM, which is a well validated (Bouchard and Viau 1999; Miao et al. 2014) major metabolite traced after exposure to PAHs. The straightforward HPLC method we followed is a good basis for the analysis of 1-OHP in human urine. It is generally sensitive, specific and can be made very reproducible with some minor modifications (Bouchard and Viau 1999). Urine samples were collected in standard polyethylene tubes and of a small amount of thymol was added so as to prevent bacterial growth. Tests conducted in our laboratory have further shown that storing of urine samples (collected over thymol) at room temperature, 4 °C or –20 °C causes no loss of the analyte for a period of several weeks.

Figure 4. Location of the 50 residential sites

Urine samples

Preservation

- At least 10 ml of urine were collected in polyethylene tubes (Roos et al. 1997).
- Samples were kept in the dark (Roos et al. 1997).
- Label for storage were put on the polyethylene tubes.
- The bottles were stored immediately at -80 °C until analysis; 1-OHP is stable for at least 3 days at 4 °C in urine adjusted to pH 5 and buffered (Quinlan et al. 1995).
- Urine samples are stable for at least 6 months at -20 °C (Boos et al. 1992).
- Always record exact time of urine collection, centrifugation and storage to -80 °C (on a worksheet), and always record any deviation from the procedure.

Materials

- Solvents: HPLC-grade acetonitrile and methanol from Sigma and and water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).
- Materials: b-glucuronidase / aryl sulfatase (type H-2: from *Helix pomatia*: b-glucuronidase activity 100 000 units /mL and sulfatase activity, 5000 units / mL) were from Sigma (St. Louis, MO, USA), Waters Sep-Pak C_{18} (Waters, Mil ford, MA, USA)
- Evaporative Concentrators: Rotary evaporator, dry-nitrogen "blow-down" device, or equivalent.
- Analytical balance: capable of accurately weighing 0.0001 g. The balance must be properly calibrated and maintained.
- Pipettes or equivalent: Electronic or mechanical pipettes with capacities from 10 to 1000 µL. Pipettes must be calibrated and maintained according to relevant standard operating procedure (SOP).

Sample Preparation

Hydroxylated metabolites of PAHs in urine are determined using a slight modification of the method of Jongeneelen (1997).

1. Prior to performing the procedure, Waters Sep-Pak C_{18} (Waters, Mil ford, MA, USA) cartridges are primed by performing two cycles of filtering 5 ml of HPLC grade methanol followed by 10 ml of Milli-Q water.
2. The frozen samples of urine are thawed in a warm water bath while stirring.
3. Once thawed, 10 ml of the samples are transferred to 50 ml conical tubes and adjusted to pH 5.0 (with ± 0.05 error) using 0.1M HCl. Once properly adjusted for pH, 15 µl of β-glucuronidase/ aryl sulfatase (1655/63 units) and 20 ml of 0.1 M acetate buffer are added to each sample.
4. After the deuterated internal standard (*1-OHP-d_9*) (Chetiyanukornkul et al. 2002) is added the reaction mixtures are placed and incubated at 37ºC for 2 hours.
5. After incubation, primed Sep-Pak cartridges are added to 10 ml syringes with the plungers removed and 60% of the samples are poured in the syringes. The samples are then slowly pushed through the cartridges at a rate of 2.5 ml per minute to allow the 1-OH-P to be collected on the Sep-Pak.
6. Once the syringe is empty, the rest of the samples are added and the step is repeated to finish loading the cartridges. Once loaded, the cartridges are washed with 8 ml of Milli-Q water by slowly pushing it through the Sep-Pak.
7. After all contaminants are removed from the cartridge, 10 ml of HPLC grade methanol is added to the syringe and the 1-OH-P is eluted at a rate of 2.5 ml per minute into 25 ml glass vials.
8. The vials are placed in a warm water bath nitrogen evaporator and placed under a gentle flow of nitrogen until all of the solvent evaporated.
9. The samples are resuspended by adding 2 ml of HPLC grade methanol to each vial.
10. A 3 ml syringe is used to draw up the remaining methanol solution and a 0.45 µm filter was added to each syringe before filtering the sample into 2 ml HPLC vials (Jongeneelen et al. 1985).

Analysis of urinary creatinine

The concentration of urinary creatinine is determined with alkaline picrate using a test kit (Wako) (Schaller et al. 1995).

High-Performance Liquid Chromatography with Fluorescence detection
The HPLC system included a RF German 10AXL fluorescence detector and a C-R7A plus integrator. An analytical column is: an RP-Amide column (Discovery RP-Amide C16, 2503 4.6 mm I.D., 5 mm Supelco, Bellefonte, PA, USA) The mobile phases for the RP-Amide column is acetonitrile–phosphate buffers (pH 7.0) (57:43 and 47:53, v/ v, respectively) at a flow-rate of 1 mL/min. The column temperature is set at 40 ^{o}C. The excitation (l) and emission (l) wavelengths were 240 and 387 nm, respectively (Chetiyanukornkul et al. 2002).

Calibration Standards
Standards is placed every tenth sample to validate the results. A standard calibration curve is determined with solutions containing 1-OHP standard and 1-OHP-d_9 as internal. For the linear regression, r^2 = 0.997 and the detection limit of 1-OHP is 0.1 mg/L (signal-to-noise ratio > 3). The quantification range is from 1 to100 nmol/L.

Recovery
The percentage of 1-OHP recovered is determined comparing the amount of detection of the standards to the expected amount with known amounts of 1-OHP. This recovery percentage is used to adjust the samples accordingly.

Case study Slovenia - Human Biomonitoring in Slovenia

Introduction

Human biomonitoring (HBM) in Slovenia is a shorter term for assessment of exposure to chemicals, identifying sources of exposure and geographic trends and determination of reference values in the Republic of Slovenia. The pilot phase of the HBM was implemented in the time period between 2007 and 2010 and the second phase of HBM was between 2011 and 2014.

Short-term objectives of the HBM programme are to provide data on exposure of the inhabitants to chemicals and related health impact throughout Slovenia, reference (background) values, and spatial differences in exposure including rural, urban environments and contaminated sites. Provision of institutional framework for the implementation of the programme on a long-term basis is also one of the key objectives to be settled.

Long-term objectives include the exposure and risk assessment for health, implementation and monitoring of implemented measures, science based risk evaluation (awareness, case-by-case consulting, risk communication …), time trends of exposure, and providing input for policy making, based on surveillance activities.

The objectives of HBM in Slovenia comply well with the main problem targeted by the CROME-LIFE which is the assessment of the impact on human health due to exposure to chemical agents originating either from environmental contamination (air, soil, water), or from consumer products (food contact materials, construction materials, cosmetics, clothes, etc.) through multiple routes.

Study design: Study population includes lactating women and men from the same area in the age from 20-40 years, 50 women and 50 men from each area (1200 subject all together). Twelve areas (Figure 5) were selected:

a) urban (3)
b) rural (3)
c) contaminated sites (6): selected due to past activities (former Hg mine, former Pb mine, former factory of transformers and capacitors – PCBs pollution) and also present industry (smeltres, cement factory, power plant, glass factory, etc.)

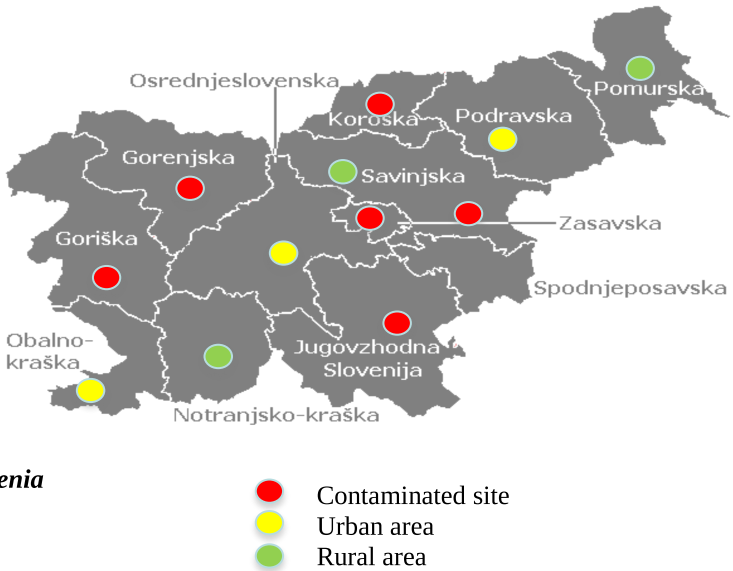


Figure 5: Selected areas in Slovenia

Schematic presentation of study protocol is presented in Figure 6 and measurements and matrix table in Table 1.

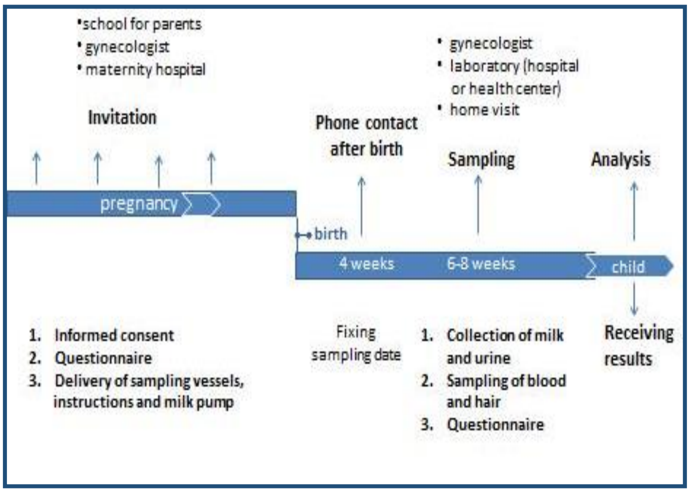


Figure 6: The timelines of the study protocol

Table 1. List of biological samples/matrixes and measurements included in the HBM in Slovenia.

Sample	*Individual samples*	*Pooled samples*
Breast milk	*Pb, Cd, Hg, As, Cu, Zn, Se* *organochlorinated pesticides* *marker PCBs (28, 52, 101, 138, 153, 180)* *triglycerides, cholesterol*	*PCDD,* *PCDF,* *dioxin like PCB,* *PBDE*
Blood - women	*Hemogram* *Pb, Cd, Hg, As, Cu, Zn, Se* *creatinine* *TSH*	
Blood - men	*Hemogram* *Pb, Cd, Hg, As, Cu, Zn, Se* *organochlorinated pesticides* *marker PCBs (28, 52, 101, 138, 153, 180)* *triglycerides, cholesterol, creatinine* *TSH*	*PCDD,* *PCDF,* *dioxin like PCB,* *PBDE*
Urine	*Pb, Cd, Hg, As, Cu, Zn, Se* *Markers of kidney function* *(albumin, alpha-1-mikroglobulin, IgG, NAG)* *Creatinine*	
Hair	*Hg*	

Biomonitoring provide a link between exposure and processes in the body. Collection of as much as possible of exposure information is important to identify pathways of exposure and to eliminate sources. All this data will be used in advanced modelling described in B1.2. Results of this process will allow the assessment of external and internal exposure to selected metals.

Existing environmental data

In Slovenia, availability of environmental data and related spatial data infrastructure was recently analyzed into detail within the EU project EGIDA (Coordinating Earth and Environmental cross-disciplinary projects to promote GEOSS (FP7-ENV-2010), GA 265124, 2010-2012). As most important sources Environmental Atlas (web mat viewer), Inspire GEOportal http://www.geoportal.gov.si/ (hosted by the Surveying and Mapping Administration), GEOportal http://gis.arso.gov.si/geoportal/catalog/main/home.page (hosted by the national Environment Agency), EIONET-SI http://nfp-si.eionet.europa.eu/ (interactive maps, tables, texts or downloadable shape files), GERK http://rkg.gov.si/GERK/viewer.jsp (land parcel identification system hosted by the Ministry of Agriculture and Environment) were identified. Based on the outcome of these analysis, a catalogue with more than 100 indicators identified by DPSIR framework is available along with the data source and provider within different Societal Benefit Areas (natural disasters, health, energy, climate, weather, ecosystems, agriculture and biodiversity) on EU level. The web portal **Environmental indicators in Slovenia** (http://kazalci.arso.gov.si/?lang_id=94) hosted by Slovenian Environmental Agency provides access to over 100 indicators which use graphs, maps and comments to present the environmental trends in Slovenia. The indicators are organized into thematic groups – chapters covering environmental components (e.g. water, air), environmental issues (e.g. climate change, nature protection, loss of biodiversity, waste management) and the incorporation of environmental content in the formulation of sector policies (e.g. transport, agriculture, tourism, energy, instruments of environmental policy). Structure of the portal (only one subgroup is shown as an example):

- Agriculture
- Air
- Climate change
- Energy
- Forestry
- Household consumption
- Human health and ecosystem resilience

    [01] Mortality due to respiratory diseases in children older than one month and under one year of age

    [02] Asthma and allergic diseases in children

    [03] Exposure of residents and children to polluted air due to PM10 particulate matter

    [04] Number of outbreaks of waterborne diseases attributable to drinking water and bathing water

    [05] Access to safe drinking water

    [06] Leaf injuries of indicator plants as indicators of external air pollution by ozone

[07] Biomonitoring of air pollution using epiphytic lichens
[08] Exposure of roe deer to heavy metals (lead and cadmium) and fluorides
[09] Critical loads and exceedances of sulfur and nitrogen for ecosystems
[10] Biomonitoring of deposition of metals and nitrogen
[12] Standardized incidence rate of leukemia in children aged 0 to 14 years
[13] Incidence of melanoma in the population aged less than 55 years
[14] Exposure of children to road traffic noise in the Municipality of Ljubljana
[15] Dietary intake of certain metals
[16] Dioxins in maternal milk
[17] Levels of lead in children's blood in the Upper Meža Valley
[18] Mortality due to respiratory diseases
[19] Number of outbreaks of communicable diseases attributable transmitted by food
[21] Incidence of foodborne
[23] Exposure of children to road traffic noise in the Municipality of Nova Gorica
[24] Proportion of resident population living in a flood plain
[25] Lyme borreliosis

- Industrial production
- Instruments of environmental policy
- Nature and biodiversity
- Sea
- Soil and land use
- Socioeconomic development
- Tourism
- Transport
- Waste and material flow

Statistical data about lifestyle, socio-economics, consumer's habits etc. of general population on selected areas can be found from the website of the Statistical Office of the Republic of Slovenia (http://www.stat.si/eng/index.asp). Data on the website are arranged by 4 major fields of statistics including 29 subject areas and 2 general areas. The same organization of data is applied in the SI-STAT Data Portal.

- Demography and social statistics covers 8 subject areas including population structure, earnings and other labor market data, education data and several socioeconomic indicators.
- Economy is the most extensive field (14 subject areas). The most requested data are inflation, tourism and external trade. Beside that, business, production and services statistics data are available here. The novelty is data on the information society.

- Environment and natural resources covers 5 subject areas; agriculture and fishing is the most important. Relatively new but becoming more and more important are data on environment and energy.
- General brings beside 2 subject areas (elections and administrative territorial structure) also information and news about the Statistical Office and News Releases on special days and holidays.

More specific data about food on Slovenian market are combined in food composition tables:

- SLOVENSKE prehranske tabele. Meso in mesni izdelki = Slovenian food composition tables. Meat and meat products / Terezija Golob ... [et al.]. - Ljubljana : Biotehniška fakulteta, Oddelek za živilstvo, 2006
- Slovenske prehranske tabele - živila rastlinskega izvora, Zaključno poročilo o rezultatih ciljnega raziskovalnega projekta V4-1047, ARRS-CRP-ZP-2012-05/33, 2012

In addition, spatial (geographic) differences in exposure of population due to the specifics of local environment must be taken into account (e.g. locations of past and current industrial activities, spatial distribution of pollutants in different environmental compartments...). Important source about local exposure data are reports on a local level which are mainly in Slovene and in some cases also articles in international journals:

- MIKLAVČIČ, Ana, MAZEJ, Darja, JAĆIMOVIĆ, Radojko, DIZDAREVIČ, Tatjana, HORVAT, Milena. Mercury in food items from the Idrija Mercury Mine area. V: *International Workshop on Mercury in contaminated sites: characterization , impacts and remediation, 10-14 October, 2010, Piran, Slovenia*, (Environmental research, ISSN 0013-9351, vol. 125, 2013). New York: Academic Press, 2013, vol. 125, str. 61-68, doi: 10.1016/j.envres.2013.02.008. [COBISS.SI-ID 26741543]
- KOTNIK, Jože, HORVAT, Milena, DIZDAREVIČ, Tatjana. Current and past mercury distribution in air over the Idrija Hg mine region, Slovenia. *Atmospheric environment*, ISSN 1352-2310. [Print ed.], 2005, vol. 39, str. 7570-7579. [COBISS.SI-ID 19431719]
- FALNOGA, Ingrid, TUŠEK-ŽNIDARIČ, Magda, HORVAT, Milena, STEGNAR, Peter. Mercury, selenium and cadmium in human autopsy samples from Idrija residents and mercury mine workers. *Environmental research*, ISSN 0013-9351, 2000, vol. 84, str. 211-218. [COBISS.SI-ID 15595559]
- PRIMERJALNA ŠTUDIJA ONESNAŽENOSTI OKOLJA V ZGORNJI MEŽIŠKI DOLINI MED STANJI V LETIH 1989 IN 2001, Končno poročilo, ERICo Velenje, Inštitut za ekološke raziskave, 2002
- POROČILO O STANJU OKOLJA V MESTNI OBČINI CELJE – 2008, Inštitut za promocijo varstva okolja, Maribor, 2009
- POROCILO O STANJU OKOLJA V OBCINI ZAGORJE OB SAVI, ERICo Velenje, Inštitut za ekološke raziskave, 2010
- OBČINSKI PROGRAM VARSTVA OKOLJA ZA OBDOBJE 2010 - 2020 ZA OBčINO JESENICE, Marbo, d.o.o. Bled, 2010

- Povzetek ugotovitev meritev onesnaženosti tal in vrtnin v občini Jesenice v letu 2006, Marbo, d.o.o. Bled, 2006

To assess personal environmental exposure of participants questionnaires are also a useful way. Questionnaires used in Slovenian HBM were simple with only a few questions about:

- General data - gender, age, height and weight
- Residence – type, distance from industry, traffic, type of heating, water supply
- Occupation - education, working place, self-reported exposure on a working place
- Hobbies
- health status - drug intake, amalgam fillings, passive smoking
- general dietary habits - food frequency assessment of consumption of vegetables/fruits, milk/milk products, eggs, meat, fish: fresh/frozen/canned, and alcoholic beverages, coffee/tea, mineral supplements .

Missing environmental data and filling the gaps

Existing environmental datasets cover overall exposure of local population. To assess complete personal exposure profile of study participants some more data are needed. According to available datasets in selected sites targeted environmental campaigns are selected to fill these gaps. They will focus on the "hot spots" revealed from the residence of the individuals with higher biomarker values. Hot spots in HBM Slovenia are contaminated sites described in the Introduction part. Targeted environmental campaigns will be the following:

1) Tap water samples will be collected directly from water pipes at households. Collection of samples will be done all over Slovenia with a special focus on hot spots i.e. contaminated sites described in Introduction part. Samples will be analyzed for selected elements by appropriate analytical techniques including primarily AAS and ICP-MS.

2) External exposure assessment will be improved by the use of sensors for outdoor and indoor air monitoring. The indoor environment quality is, however, a very broad term comprising essentially all kinds of factors that people are exposed to indoors. Air quality data parameters that will be monitored on-line by sensors are temperature, relative humidity, CO_2, NO_2, dust, O_3, noise, VOC and radon. In the CITI-SENSE project use of sensors for online monitoring of the indoor air environment will be implemented in a pilot study in just one city. In CROME_LIFE project this will be expanded to more locations.

3) Exposure through food is one of the most important pathways for some elements. Consumption of locally grown vegetables/fruits could be a significant factor on selected contaminated sites. **Vegetables/fruits together with top soil** will be collected from people's gardens and analyzed by appropriate analytical techniques including primarily AAS and ICP-MS. The same methodology as was used in the case of Idrija described in Miklavčič et al 2013(Environmental research, ISSN 0013-9351, vol. 125, 2013) will be applied.

Case study Italy - Human Biomonitoring in Italy

Introduction

In Italy the first national biomonitoring programme of the general population on metals has been launched in 2008. The pre-existing situation was related to the internal dose assessment of metals in population groups, available only for a few geographical areas of the country. In 1990, data for 20 metals in inhabitants of the region Lombardy were published (Minoia et al.,1990), and, after a gap of many years, in 2004, a survey on 26 metals in the region of Latium was conducted (Alimonti et al., 2005a). Moreover, for some metals data were still missing for the Italian general population, and information on internal exposure have not been systematically examined in relation to other characteristics of the population groups in study (i.e. gender, age, smoking or dietary habits, etc.). HBM data production is a time-consuming and expensive process that requires a deep estimation of several analytical and biological factors that could affect the final results, including route of absorption, the presence of sources of environmental pollution in certain residential areas, the physiological variability and lifestyles. The data obtained from these studies are necessary to obtain Reference Values (RV), statistically derived numbers that indicate the upper margin of background exposure to a given substance in a defined population at a given time (Schulz et al., 2007).

The PROBE project ***(****PROgramme for Biomonitoring general population Exposure on metals****)***

was conducted by the Istituto Superiore di Sanità (ISS) in the 2008-2010 period, funded by the Ministry of Health.

Aim

Primary objective of PROBE was to supply representative data on the metals' internal dose in adults in order to highlight the environmental impact on the health of Italian population. The activities carried out were devoted to:

- develop, standardize and validate protocols and methods for samples collection and metal analysis as a basis for their reliability, transferability and comparability;
- establish Reference Values (RVs) for the exposure of healthy adults to environmental metals, including a large metal profile (20 metals);
- examine the possible influences of certain variables (demographics and habits) on the metal level of individuals.

Study design

To achieve these objectives, the project was divided into the following phases:

- identification of the adequate skills in the areas to be enrolled;
- training to enable the harmonization of procedures for collecting, storing, transporting and processing the human specimens;
- development and validation of laboratory methods for the quantification of metals;
- determination of levels of metals in study population blood and serum;
- stratification of results according to age, sex, alcohol and smoking habits, lifestyle, diet, etc.

Impact

Further to provide information about the serum metal levels of urban population living in different geographical areas of Italy, the data obtained by PROBE can be used as support to REACH regulation application in Italy:

- to encourage further investigations (Commission Regulation No. 1907/2006, art. 45, paragraph 5);
- to identify substances of very high concern, persistent, bioaccumulating and toxic or chemicals of equivalent concern (Commission Regulation No. 1907/2006, Annex XIV);
- to assess the efficiency of risk reduction measures or of substitutional choice in authorized substances underlying the minimization requirements (Commission Regulation No. 1907/2006, art. 60, paragraph 10).

Methodological procedures

Population

452 adolescents (13-15 yrs aged) from 4 areas (Roma, Fontenuova, Monterotondo and Viterbo) of Latium region (Italy) were sampled, 210 were males and 242 females. Each donor was also asked to answer a short interview, in the form of questionnaire in order to collect information on:

- general data such as gender, age, height and weight (body mass index), place of residence and job, dental fillings or metal implants (type, number, how long);
- life-styles in terms of alcohol consumption (type, quantity, frequency), smoke (type, quantity, frequency), exercise (type, frequency), traffic at home and at work (type, intensity), home/work distance from potential industrial areas and type of industrial area;
- consumption of fish (weekly amount) and milk and dairy products.
- complete blood count was also carried out on each blood sample by the Center for Study of microcythemia of Rome (ANMI).

Analytes: twenty metals – Sb, As, Be, Cd, Cr, Co, Ir, Pb, Mn, Hg, Mo, Ni, Pd, Pt, Rh, Tl, Sn, W, U and V – were measured in blood of the PROBE participants. The selection of metals was based on a compromise among different requirements:

- gravity of known or suspected health effects subsequent to the environmental exposure to the metal;
- need to assess the effectiveness of public health actions to reduce exposure to a metal;
- availability of adequate sample amounts;
- availability of a multi-elemental analytical technique with adequate accuracy, precision, sensitivity, specificity, and throughput.

Analysis: method based on Sector Field Inductively Coupled Plasma Mass Spectrometry (SF-ICP-MS). The method used for blood analysis was validated by ACCREDIA (the Italian National Accreditation Body, method MI05) and the following validation performances were assessed: linearity; Limit of Detection (LoD) and Limit of Quantification (LoQ); specificity; accuracy (precision and trueness); and robustness (AOAC, 1998; Commission Decision 2002/657/EC; LGC, 2003; NATA, 2009; Thompson et al., 2002).

Results

PROBE provided the Reference Values for metals of the urban Italian population; the higher percentiles (5th, 50th, 95th) provided for each metal, i.e., the upper limits of derived RVs, are helpful for determining whether levels observed in separate public health investigations or other studies are unusual. The GM is preferred because takes into account all values measured and represents the "ideal" measure of central tendency in the event of logarithmic normal distribution, as usual for metals concentration in blood. Table 2 reports, as an example, the RVs for whole blood for the entire population.

Table 2. Reference values (μg/L) for metals in blood of adolescents (n. 452)

Element	GM	50th	5th	95th
As	0.71	0.75	0.13	2.95
Cd	0.29	0.3	0.13	0.6
Co	0.09	0.09	0.03	0.3
Cr	0.31	0.3	0.09	1.25
Hg	0.78	0.83	0.27	2.04
Ir *	6.71	6.84	2.5	15
Mn	7.22	7.46	3.36	16
Mo	1.11	1.1	0.54	2.39
Ni	0.94	1.02	0.18	2.6
Pb	9.6	9.55	4.13	21.6
Pd*	21.3	22.1	7.5	38.6
Pt*	10.9	10.9	5.16	23.9
Rh*	21.2	22.1	7.5	35.6
Sb	0.37	0.39	0.17	0.79
Sn	0.57	0.56	0.18	1.52
Tl	0.04	0.04	0.02	0.09
U*	5.09	4.85	2.26	14.3
V	0.07	0.08	0.03	0.17
W	0.03	0.03	0.01	0.08

(μg/L; when * ng/L)

Linking biomonitoring data to exposure

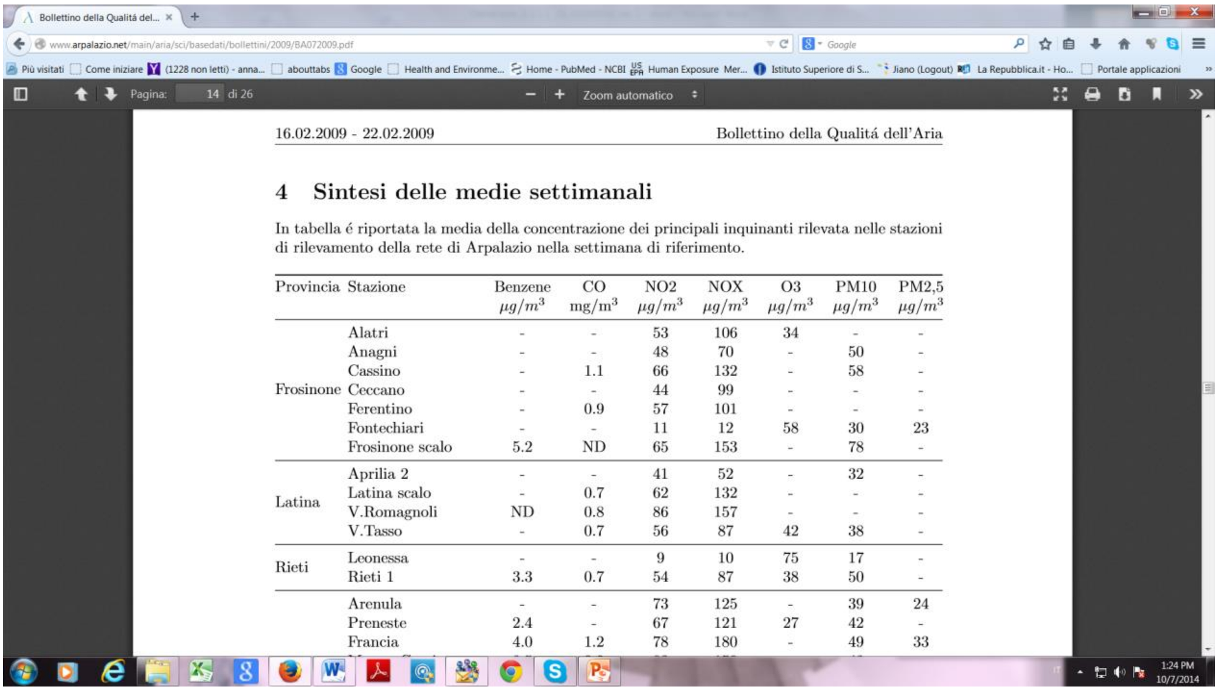
To support an integrated assessment of the environmental exposures in the population of adolescent in study, we carried out a multivariate analysis to explore potential associations inside the database of the HBM-metals results (already collected), considering demographic data, lifestyle factors, dietary habit, hematological profile as well as geo-referenced and environmental data.

In this context geographic differences in exposure of adolescents due to characteristics of the local environment (e.g. presence of industrial plant, air quality, spatial distribution of metals in different areas, etc.) were considered.

Existing environmental data

In Italy the most important sources of environmental data are the Regional Agencies of environmental protection (ARPA); in particular the Latium agency draw up daily, weekly and annual technical reports on chemical data of air and water state of the Region. Our interest was focused on air quality in the area where the adolescents lived (Rome and its province and Viterbo). The environmental chemical data considered were Benzene, CO, NO_2, NO_x, O_3 PM_{10} $PM_{2.5}$. The data are available at:

http://www.arpalazio.net/main/aria/sci/basedati/chimici/chimici.php#



16.02.2009 - 22.02.2009 Bollettino della Qualitá dell'Aria

4 Sintesi delle medie settimanali

In tabella é riportata la media della concentrazione dei principali inquinanti rilevata nelle stazioni di rilevamento della rete di Arpalazio nella settimana di riferimento.

Provincia	Stazione	Benzene $\mu g/m^3$	CO mg/m^3	NO2 $\mu g/m^3$	NOX $\mu g/m^3$	O3 $\mu g/m^3$	PM10 $\mu g/m^3$	PM2,5 $\mu g/m^3$
Frosinone	Alatri	-	-	53	106	34	-	-
	Anagni	-	-	48	70	-	50	-
	Cassino	-	1.1	66	132	-	58	-
	Ceccano	-	-	44	99	-	-	-
	Ferentino	-	0.9	57	101	-	-	-
	Fontechiari	-	-	11	12	58	30	23
	Frosinone scalo	5.2	ND	65	153	-	78	-
Latina	Aprilia 2	-	-	41	52	-	32	-
	Latina scalo	-	0.7	62	132	-	-	-
	V.Romagnoli	ND	0.8	86	157	-	-	-
	V.Tasso	-	0.7	56	87	42	38	-
Rieti	Leonessa	-	-	9	10	75	17	-
	Rieti 1	3.3	0.7	54	87	38	50	-
	Arenula	-	-	73	125	-	39	24
	Preneste	2.4	-	67	121	27	42	-
	Francia	4.0	1.2	78	180	-	49	33

Figure 7: excerpt from the Air quality bulletin from ARPA Lazio

Very scarce data are available on the metal content in local food and their consumption by the adolescents involved in this study. For some food items data are reported in literature, but only for some categories, including the levels of few metals and not always for the areas understudy (Danieli PP, Serrani F, Primi R, Ponzetta MP, Ronchi B, Amici A. Cadmium, lead, and chromium in large game: a local-scale exposure assessment for hunters consuming meat and liver of wild boar. Arch Environ Contam Toxicol. 2012 Nov;63(4):612-27; Selected trace and ultra-trace elements: Biological role, content in feed and requirements in animal nutrition – Elements for risk assessment available on http://www.efsa.europa.eu/it/publications.htm . Levels of metals and other potential contaminants in tap water for the areas involved in the PROBE study are also available in the Latium Environmental Protection Agency website http://www.arpalazio.gov.it/ambiente/indicatori/

Missing environmental data and filling the gaps

Filling the gap

PROBE was a classical HBM campaign devoted to obtain RVs for general population. However, during the CROME-LIFE activities, individual geo-referencing as well as

environmental exposure at the time of blood collection will be implemented in order to obtain a broader meaning of the HBM data in terms of association between external and internal doses. Reanalysis of data sets taking into account life styles and dietary habits is currently under study in the CROME framework.
Preliminary data suggest that the Viterbo area (2) shows higher blood levels of As and Hg than Rome (1), Fontenuova (3), Monterotondo (4), as these metals are known to be associated with sedimentary deposits derived from volcanic rocks.

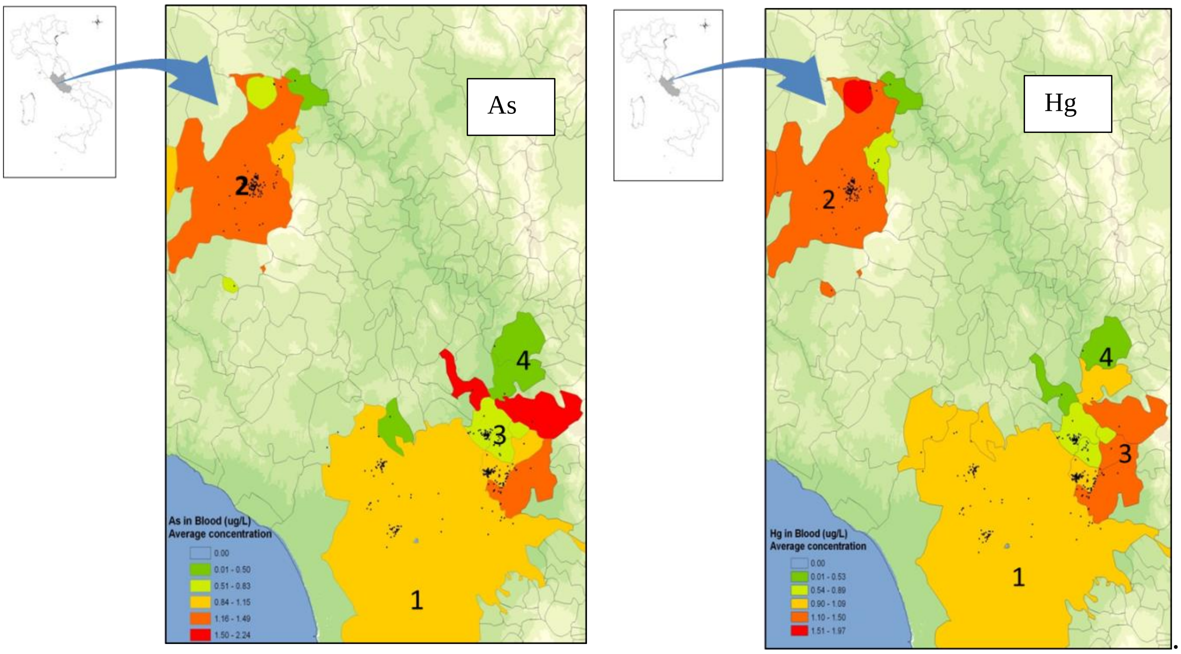


Figure 8: spatial distribution of As and Hg human biomonitoring data in Latium (Italy)

Considering the air chemical data, no association s were obtained with the PM_{10} occurring in the four different sites.

General Linear Models carried out on HBM, diet and land use data show that:

i. blood-Cr has a statistically significant association with diet and with land use both alone and in combination;
ii. Hg has a more complex interaction between diet and human activities; no pathway alone is dominant but the combined effect results in statistically significant associations with blood concentration levels;
iii. Pt and W have an interaction effect between diet and land use;
iv. internal exposure to Ni is mainly driven by diet.

Preliminary remarks

The results obtained so far could be summarized as follows:

i. RV results overlap data of similar surveys and/or international guidelines - although Pb and Hg need particular attention;
ii. is confirmed the association of As with Hg and V, typical elements present in volcanic areas;
iii. there is no association of HBM data with air pollution (PM10);

iv. the internal dose of Ni is mainly driven by dietary pathways (co-exposure to milk and fish);
v. v, Pt is associated to fish consumption, industrial activities and enhanced traffic (even though they fail to meet the statistical robustness test);
vi. Cr is associated with all dietary pathways and land use, i.e., both out-of-region and local sources can be associated with the observed Cr levels in the population;
vii. W can be associated with co-exposure to milk and proximity to industrial activities;
viii. finally, Hg is related with co-exposure to different dietary pathways (milk and fish) coupled to proximity to industrial activities, while other metals have not shown statistically significant associations with dietary patterns and/or land use information.

Table 3: HBM mean concentration vs. land cover (summary)

	As	Cd	Co	Cr	Hg	Ir	Mn	Mo	Ni	Pb	Pd	Pt	Rh	Sb	Sn	Tl	U	V	W	*n*
Urban	1.15	0.36	0.08	0.81	1.03	8.75	8.32	1.22	2.19	11.5	22.9	14.5	24.0	0.50	1.06	0.027	7.01	0.06	0.02	303
Industrial	1.49	0.38	0.15	0.62	1.31	6.93	10.2	1.58	6.71	11.7	26.4	10.5	21.7	0.38	0.86	0.037	6.50	0.12	0.05	28
Agricultural	1.13	0.23	0.07	1.00	1.01	7.50	7.81	1.44	2.94	12.0	25.4	13.1	22.4	0.47	0.69	0.033	5.32	0.06	0.03	111

Next steps

To define the complete personal exposure profile of the subjects future analyses will focus on:

1) Acquiring the As data in water derived from ARPA reports;
2) Adding air quality parameters i.e., NO_X, SO_X, NO_X, CO, Benzene, PM2.5 to better describe the distribution of environmental pollutants and differences between rural and urban areas;
3) Examining the information present in the complete blood count , to find possible associations between metals and e.g. morphological changes in red cells.

Case study Spain - Human Biomonitoring in Spain

Introduction

In Mediterranean countries there is very limited information on prenatal exposure to pollutants (Carrizo et al., 2006; 2007a; Gomara et al., 2007; Lopez-Espinosa et al., 2007). Furthermore, the available information needs to be discussed in terms of geographic variability and possible temporal differences related to the periods of use of compounds such as organochlorine compounds and polybromodiphenyl ethers.

Human exposure to organohalogen compounds has been studied in two Mediterranean Spanish cohorts, Valencia and Menorca (Figure 9). Cord blood serum taken upon child birth is a good medium for description of the in utero exposure to the organohalogen compounds. They provide information of the exposure levels in this first life stage.

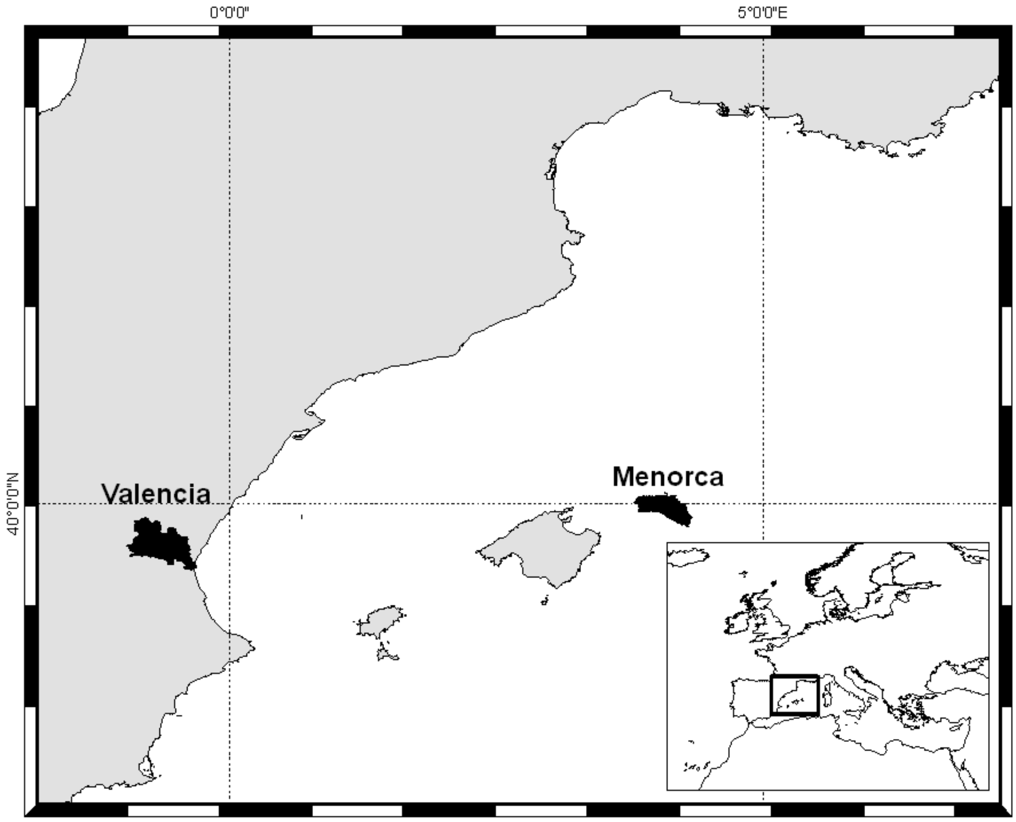


Figure 9: Geographic areas inhabited by the populations selected for study

The island of Menorca is a popular tourist destination with strong agricultural activities. The population is distributed among eight municipalities, Maó (the capital) and Ciutadella (the historical capital) are the main cities (Figure 10). Cattle rising for milk production is important, which has involved an extensive use of the land for pasture (Carrizo et al., 2007b). The island does not contain any large factory. A portion of the inhabitants are fishermen and people have easy access to fish and seafood. The hospital where mothers and children were recruited belongs to the public health service of the Balearic Islands and collects a large proportion of the births in the whole island.

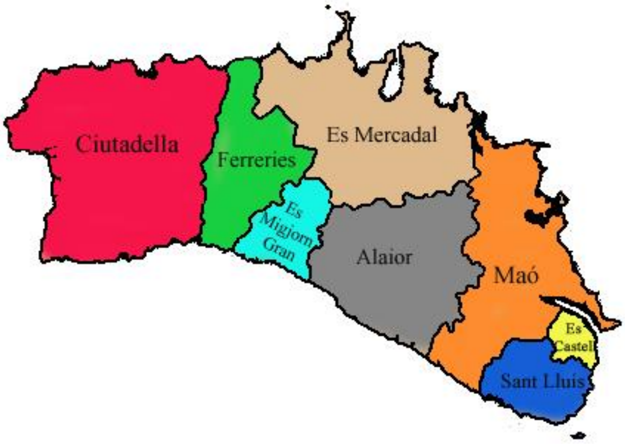


Figure 10: Municipalities of Menorca in which the population of the cohort is distributed.

Valencia is a coastal city located in the eastern shore of the Iberian Peninsula. The cohort was recruited in Hospital La Fe which belongs to the public health system of the Valencian Community. This hospital gives service to an area of 1372 km^2 including 34 municipalities with a reference population of approximately 300,000 inhabitants (Figure 11).

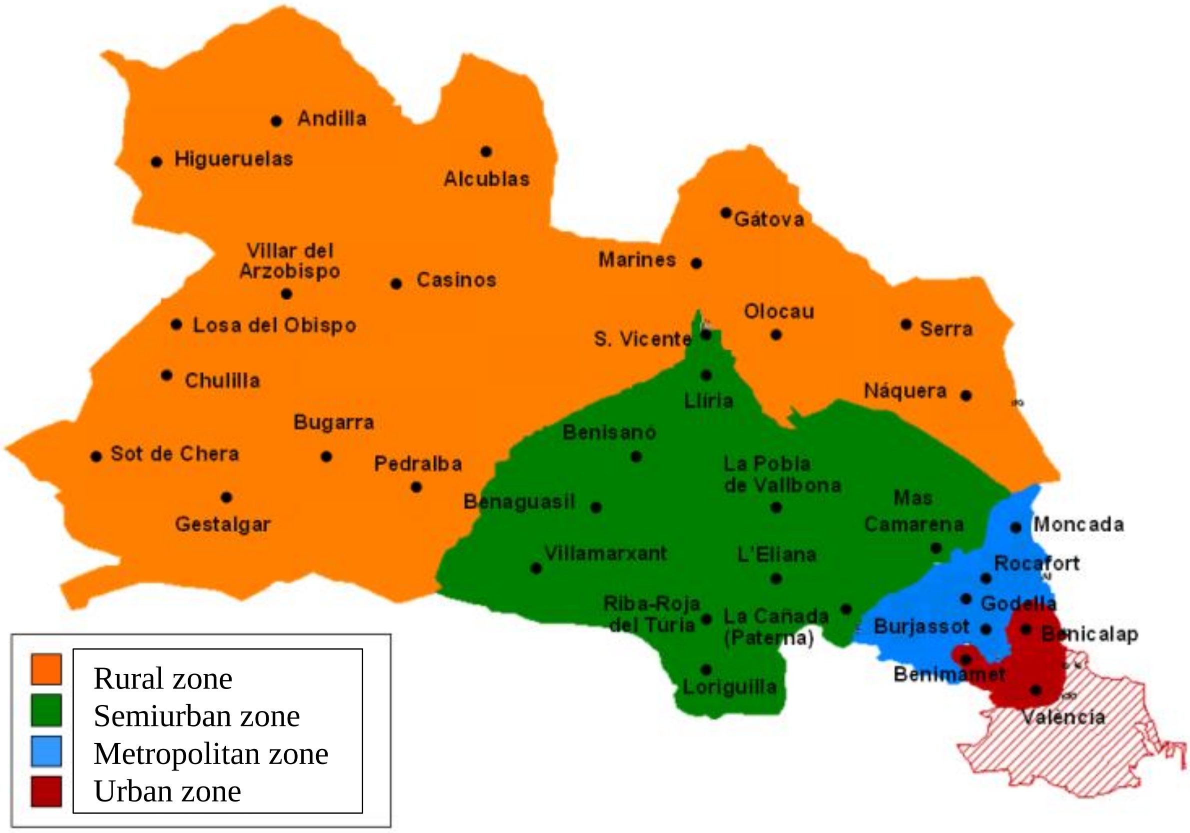


Figure 11: Geographic distribution of the cohort represented by the population recruited in the Hospital La Fe in the city of Valencia

This population has wide socio-demographic and environmental heterogeneity that can be divided into an urban zone (city of Valencia), a densely populated metropolitan zone, a semi-urban zone encompassing industrial and agricultural activities as well as residential areas, and a wide rural zone in which extensive aquiculture is developed. No large factories are located in the area. Fish is also a common constituent in the diet of people belonging to these four groups.

The distance between the two cohort locations is about 400 km. Both communities are situated in typical Mediterranean sites and can be assumed to have similar exposure scenarios. The mothers living in these areas are likely exposed to baseline organohalogen compound levels of western Mediterranean locations.

Existing environmental data

Information on environmental exposure of humans in Menorca and Valencia is available in:

Ballester, F., D Corella, S Pérez-Hoyos, and A Hervás. 1996. Air pollution and mortality in Valencia, Spain: a study using the APHEA methodology. J Epidemiol Community Health 50, 527–533.

Ballester, F., M. Estarlich, C. Iñiguez, S. Llop, R. Ramón, A. Esplugues, M. Lacasaña, M. Rebagliato. Air pollution exposure during pregnancy and reduced birth size: a prospective birth cohort study in Valencia, Spain. Environmental Health 2010 9:6, doi:10.1186/1476-069X-9-6

Basagaña X1, Torrent M, Atkinson W, Puig C, Barnes M, Vall O, Jones M, Sunyer J, Cullinan P; AMICS study. 2002. Domestic aeroallergen levels in Barcelona and Menorca (Spain). Pediatr Allergy Immunol. 13, 412-417.

Castell, N., Mantilla, E., Tellez, L., and Torres, A. L., 2010. Previozono: 10 años de vigilancia del ozono troposférico en la Comunidad Valenciana. Nuevos retos. In: Segones Jornades de Meteorologia i Climatologia de la Mediterrània Occidental. Valencia, 11-12 Marzo 2010.

Castell, N., Tellez, L., and Mantilla, E., 2012. Daily, seasonal and monthly variations in ozone levels recorded at the Turia river basin in Valencia (Eastern Spain). Environmental Science and Pollution Research, 19: 3461-3480.

Coscolla, C., Muñoz, A., Borrás, E., Vera, T., Ródenas, M., and Yusà, V., 2014. Particle size distributions of currently used pesticides in ambient air of an agricultural Mediterranean area. Atmospheric Environment, 95: 29-35.

García Algar O1, Pichini S, Basagaña X, Puig C, Vall O, Torrent M, Harris J, Sunyer J, Cullinan P; AMICS group. 2004. Concentrations and determinants of NO2 in homes of Ashford, UK and Barcelona and Menorca, Spain. Indoor Air. 14, 298-304.

García-Hurtado, E., Pey, J., Borrás, E., Sánchez, P., Vera, T., Carratalá, A., Alastuey, A., Querol, X., and Vallejo, V. R., 2014. Atmospheric PM and volatile organic compounds released from Mediterranean shrubland wildfires. Atmospheric Environment, 89: 85-92.

Gómez, I., Marín, M. J., Pastor, F., and Estrela, M. J., 2012. Improvement of the Valencia region ultraviolet index (UVI) FORECASTING SYSTEM. Computers & Geosciences, 41: 72-82

Muñoz, A., Ródenas, M., Borrás, E., Vázquez, M., and Vera, T., 2014. The gas-phase degradation of chlorpyrifos and chlorpyrifos-oxon towards OH radical under atmospheric conditions. Chemosphere, 111: 522-528.

Muñoz, A., Vera, T., Ródenas, M., Borrás, E., Mellouki, A., Treacy, J., and Sidebottom, H., 2014. Gas-phase degradation of the herbicide ethalfluralin under atmospheric conditions. Chemosphere, 95: 395-401.

Pereira, K. L., Hamilton, J. F., Rickard, A. R., Bloss, W. J., Alam, M. S., Camredon, M., Muñoz, A., Vázquez, M., Borrás, E., and Ródenas, M., 2014. Secondary organic aerosol formation and composition from the photo-oxidation of methyl chavicol (estragole). Atmospheric Chemistry and Physics, 14: 5349-5368.

Rovira, P., Palau, J. L., Millán, M. M., and Alonso, L., 2010. Reconstrucción paramétrica de series climáticas: Espacio tiempo-frecuencia y obtención de armónicos principales mediante análisis T-student. In: Segones Jornades de Meteorologia i Climatologia de la Mediterrània Occidental. Valencia, 11-12 Marzo 2010.

Sunyer, J., C. Puig, M. Torrent, O. Garcia-Algar, I. Calicó, L. Muñoz-Ortiz, M. Barnes, P. Cullinan, AMICS study. 2004. Nitrogen dioxide is not associated with respiratory infection during the first year of life. Int. J. Epidemiol. 33, 116-120.

Vera, T., Muñoz, A., and Palau, J. L., 2012. Pesticide residues in the atmosphere. In: Rathore, H. S. and Nollet, L. M. L., (eds.). Pesticides. Evaluation of environmental pollution. 203-232. CRC Press,

Human biomonitoring has been performed in both sites. Databases describing the following parameters have been generated:

In Menorca, pentachlorobenzene, α-hexachlorocyclohexane, β-hexachlorocyclohexane, γ-hexachlorocyclohexane, δ-hexachlorocyclohexane, hexachlorobenzene, polychlorobiphenyl congeners 28, 52, 101, 118, 138, 153 and 180, 2,4'-DDE, 4,4'-DDE, 2,4'-DDD , 4,4'-DDD, 2,4'-DDT, 4,4'-DDT, polybromodiphenyl ether congeners 17, 28, 47, 66, 71, 85, 99, 100, 153, 154, 138, 183, 190 and 209. These data are available for serum cord blood (n = 410) and serum venous blood of children at 4 years of age (n = 280). Mercury concentration in hair from the same cohort is also available (n = 302). In Valencia, databases for the same parameters are available for serum cord blood children (n = 510) and serum venous blood of their mothers (n = 510).

This information is complemented with data from home location, origin of the mother, number of years living in Menorca or Valencia, maternal age, weight, weight gain during pregnancy, height and BMI, breastfeeding habits, parity. Information on the diet of the mother is also available.

Missing environmental data and filling the gaps

The main information gaps concerns the pollutant composition of the diet items and how they may be relevant for the incorporation of pollutants into the mothers and children of these cohorts. Meet, vegetables, fruits, fish, cereals and dairy products will be collected monthly during two years in markets and stores. Aliquots of the samples collected will be stored and labelled adequately. Pooled samples will be compiled to represent large numbers of food items, e.g. n = 100 each. Among these, special attention should be devoted to fish since Mediterranean diets are characterized by a high consumption of wild fish. Thus, both lean fish and oily fish have been described to be correlated with the body burden of several pollutants both in children (Gari et al., 2013) and adults (Fort et al., 2014). Preliminary data on mercury content in fish from the Balearic Sea shows that the concentrations of this metal are above legal limits (0.5 μg/g wet weight). This is consistent with the known conditions of the Mediterranean Sea as a store of mercury (Covelli et al., 2001; Ferrara et al., 1991; 1997). Fish species of usual human consumption are therefore main items to monitor for assessment of the ways of incorporation of these pollutants into humans.

In addition, the composition of these pollutants in soils, water and air will also be monitored. Collection of representative samples over the whole island will be performed during the sampling period of diet items.

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