Cross-Mediterranean Environment and Health Network

CROME-LIFE

ANNEX 13

Deliverable C.1.1

Main sources of exposure for the population and vulnerable groups and identification of effective countermeasures to limit the impact on human health in each demonstration area

LIFE ENVIRONMENT PROGRAMME

LIFE12 ENV/GR/001040

http://www.crome-life.eu

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Table of Content

Executive summary

This report aims at identifying the main sources of exposure for the population in the four countries and at proposing effective countermeasures to limit the impact on human health in each demonstration area. The CROME-LIFE team believes that the methods the project is based on have a robust and scientifically sound basis as they rely on data, analysis, and methodological approaches published in the peer-reviewed literature. For these reasons the CROME-LIFE partners dedicated a particular attention in preparing a *ad-hoc* mitigation recommendations addressing the different environmental issues covered by the case studies implemented.

The report is subdivided into two main sections. The first one has a main objective the identification of the main sources of exposure for each demonstration area in the Mediterranean basin where the CROME-LIFE methods and tools have been implemented. Based on the results of the first section in the second one a list of potential measures to mitigate or limit the exposure to toxic substances addressing both key stakeholders and the general population are identified.

Identification of main sources of exposure

Greece

Aspropyrogos – PCDDs/PCDFs / recycling

According to the UNEP, in the near future, non-industrial emission sources such as landfill fires and backyard barrel burning will probably become more important and dominate the overall annual emissions of PCDD/Fs in Europe. In this context this case study is related to the an accidental fire in a plastics recycling plant occurred in the Aspropyrgos area (close to Athens, Greece) on June 6, 2015 Figure 1 .

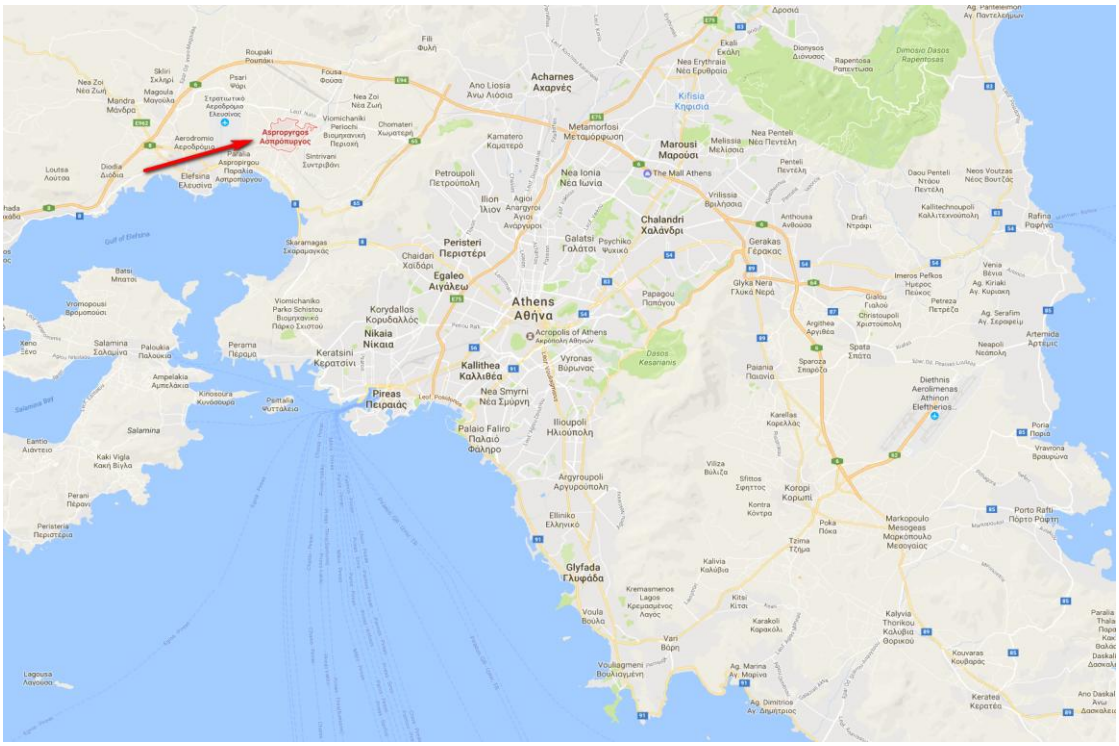

Figure 1. Location of the Aspropyrgos area

The fire resulted in significant particle and gaseous emissions of several compounds related to plastic industry. More specifically, release of dioxins and furans (PCDDs/PCDFs) was a major concern, due to their persistence in environmental and biological matrices, as well as to their carcinogenic potency.

Data on ambient air levels of PCDDs/PCDFs during the accidental event were obtained by various measurements of PM and analysis of PCDDs/PCDFs in the particle and gaseous phase. Air samples collected in the 5th day of the event were found to contain over 1000 pg/m^3 TEQ (toxic equivalent quantity) of dioxin, exceeding background levels by 2,500–25,000 fold (Fernando et al., 2014).

Analysis of ambient air samples (both particle and gaseous phase) showed that the levels of PCDDs/PCDFs in the surrounding area were 1.8 pg/m^3 TEQ WHO (toxicity equivalent concentration in accordance with the methodology of the World Health Organization). These levels are significantly higher than the ones reported in previous studies, where atmospheric background concentration of a typical industrial site in the wider area of Athens was found to be equal to 0.1 pg/m3 TEQ WHO, but in the same order of magnitude to the levels of landfill fires (Figure 2).

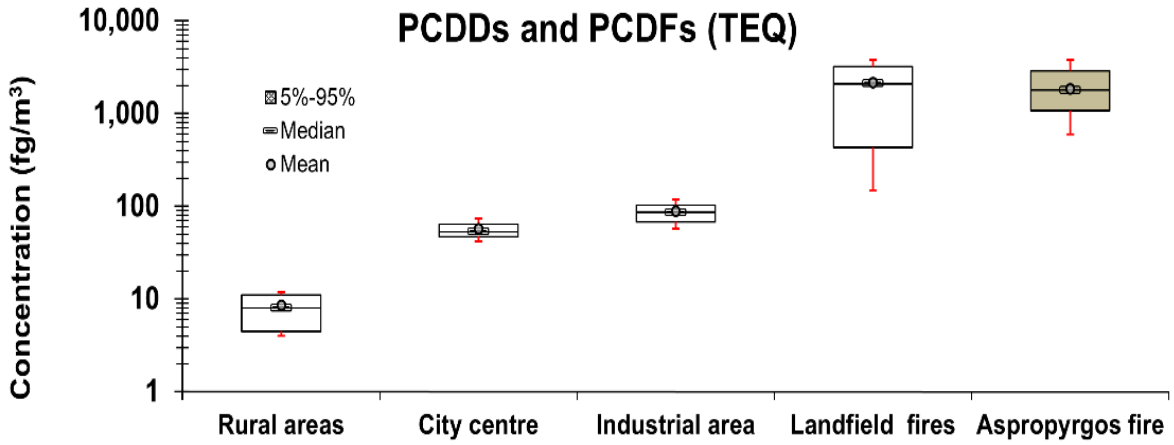

Figure 2. Levels of PCDDs/PCDFs (TEQ) at various Athens sub-areas, as well as during accidental fire events

The PCDDs/PCDFs levels were calculated as the TEQ values by multiplying with the corresponding WHO-TEF for each congener (Van den Berg et al., 1994). Upperbound total TEQ values were calculated for each category of congeners assuming that non-detected individual congener concentrations are equal to their corresponding limit of detection. The results of the analysis of the 60 samples, indicated that exposure to PCDDs/PCDFs fumes resulted in exposure levels that are higher than the ones identified in previous studies, hence indicating that the intake of the population was significant.

The results of the PCDDs/PCDFs levels in blood of the exposed individuals are presented in Figure 3. The red line (at 6.8 pg/g lipid_TEQ) indicates the mean background concentration in adult blood in the area, as identified in a previous study (Costopoulou et al., 2006).

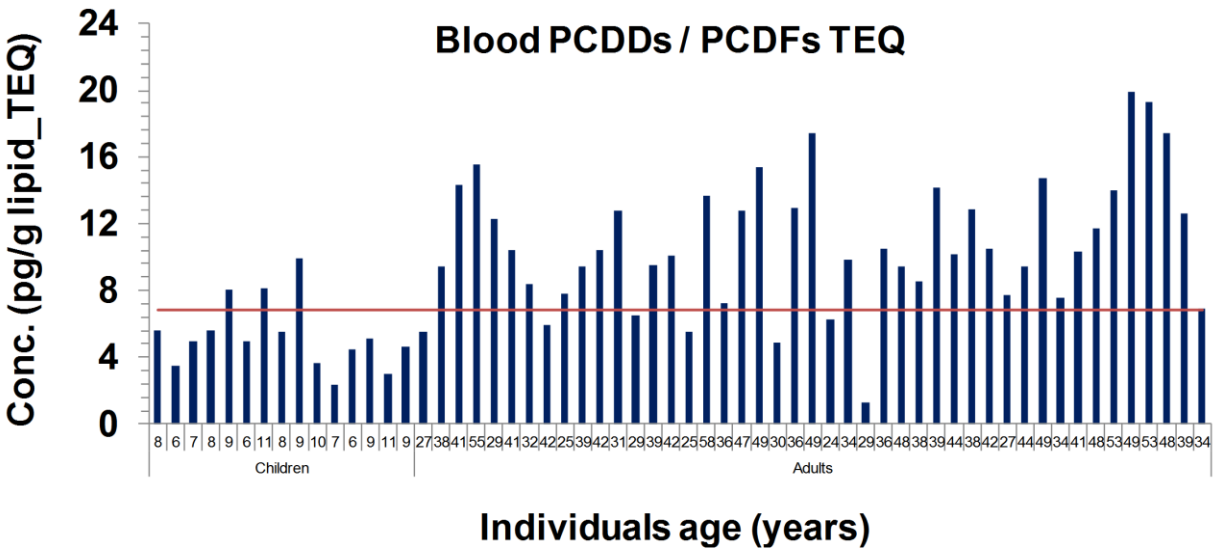

Figure 3. PCDDs/PCDFs blood levels of the Asropyrgos population after exposure to the accidental fire fumes

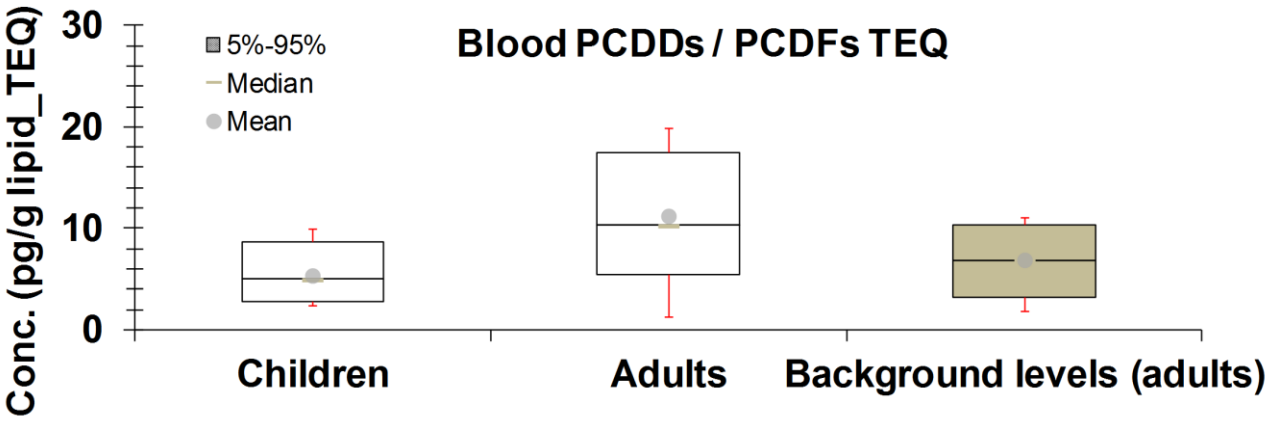

Figure 4. PCDDs/PCDFs blood levels distribution of the Asropyrgos population after exposure to the accidental fire fumes, compared to previous measurements in the area

The distribution of the PCDDs/PCDFs blood levels between children and adults, as well as between exposed and non-exposed population are graphically illustrated in Figure 4. The analysis indicated that the accidental event resulted in significant exposure to the population, raising the blood concentrations of adults to almost twice levels. Although the respective change could not be identified in children due to lack of previous measurements, it is expected that the accidental event would have a higher contribution to children, since newer generations always have lower internal background levels of PCDDs/PCDFs.

Based on the above, an average increase of 13% of cancer risk attributed to PCDDs/PCDFs was estimated population. A key finding of the study was that neonates and breast fed infants face even higher lifetime risks, and also that these risks were quantified (20% and 32% respectively).

Thessaloniki PM-PAHs / biomass combustion

Several epidemiological studies have shown the adverse health effects of airborne particulate matter deposited in the human respiratory tract (HRT) (Kennedy, 2007; Pope and Dockery, 2006). HRT

deposition of a particular particle depends on its aerodynamic diameter (dp). Particulate matter can be divided to coarse particles (dp>2.5 μm), which are mainly deposited in the upper respiratory system, fine particles (0.1<dp<2.5 μm), which are deposited in the tracheobronchial region of the human respiratory tract, and ultrafine particles (dp<0.1 μm) which are deposited in the pulmonary/alveolar region (Lin et al., 2008)). As a result, xenobiotics contained in ultrafine particles can be easily translocated in the human body via systemic circulation.

About 90% of PAHs are emitted by vehicles (Nielsen, 1996). Other sources include industry, biomass combustion, coke and tar production, as well as tobacco smoke (Freeman and Cattell, 1990; Masclet et al., 1987). Including benzo[a]pyrene (B[a]P), the only PAH classified as known carcinogen to humans by IARC, the most hazardous PAHs are mainly distributed in the particulate phase (IARC, 2010).

Give the above context the aim of this study was to quantify the health effects related to the shift from light heating diesel to biomass burning, as well as at evaluating alternative scenarios of residential heating energy share in Thessaloniki.

Field campaigns for collection of concentration data of particulate matter (PM_1, $PM_{2.5}$ and PM_{10}), have been executed in Thessaloniki during the following periods: from October 2012 to April 2013 and from December 2013 to March 2014.

With regard to ambient air measurements, PM10, PM2.5 and PM1 samplers were installed at two locations in the urban area of Thessaloniki to determine the chemical composition of urban aerosols and to correlate their toxicity with biomass combustion as a way of residential heating. The urban background site is located in the Ilioupoli district of western Thessaloniki (40°40` N, 22°55` E), a densely populated area where road construction and elevated buildings do not favor pollutants dispersion. Samplers were placed at the roof of a building at a height of approximately 9 m from the ground. No significant traffic sources were close to the site. The traffic site is located in the campus area of the Aristotle University of Thessaloniki, at a balcony of the School of Engineering, Building D (40°37` N, 22°57` E). The site is crossed by the main highway of Thessaloniki, Egnatia Street, at a distance of approximately 50 m and its distance from residential buildings is as far as 500 m. Samplers were placed at a height of 6 m from the ground.

In addition, data on PM concentration levels from the air quality monitoring network of Thessaloniki were collected.

The levels of the different PM size fractions (as well as main meteorological parameters) are illustrated in Figure 5 and Figure 6 for the urban background and the traffic site respectively.

PM_{10} levels exceed the threshold of 50 $\mu g/m^3$ (as a 24 hour mean, not to be exceeded more than 35 times a year) for the majority of the measurements period. The concentrations start to decline after the end of March as result of lower demand for space heating, since ambient temperature starts to increase.

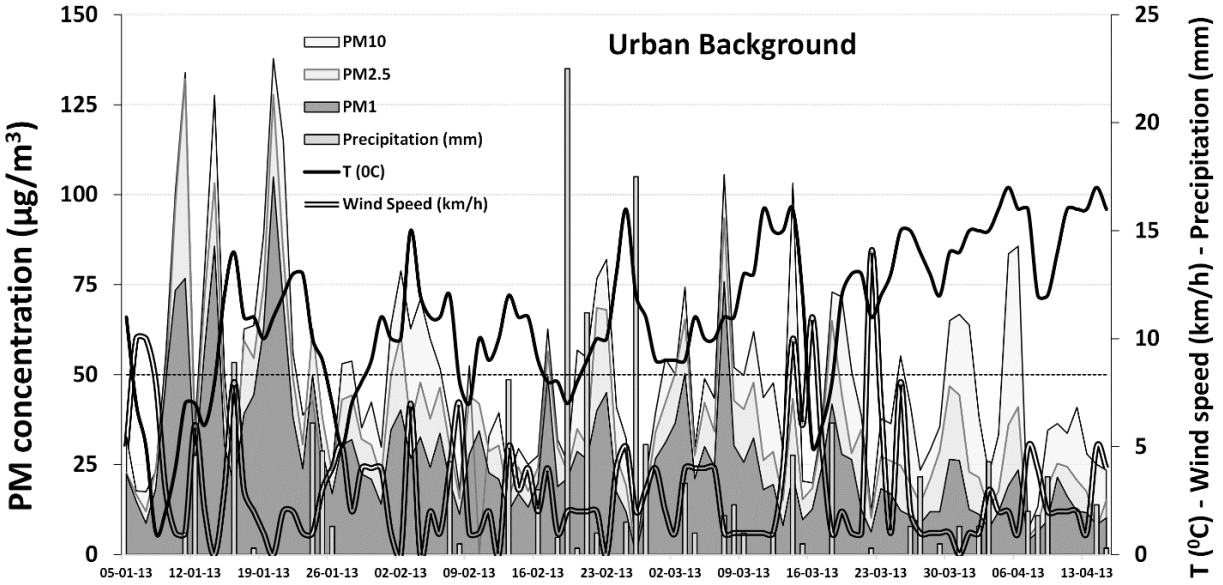

Figure 5. Inter-day variability of the ambient air PM levels at the urban background site

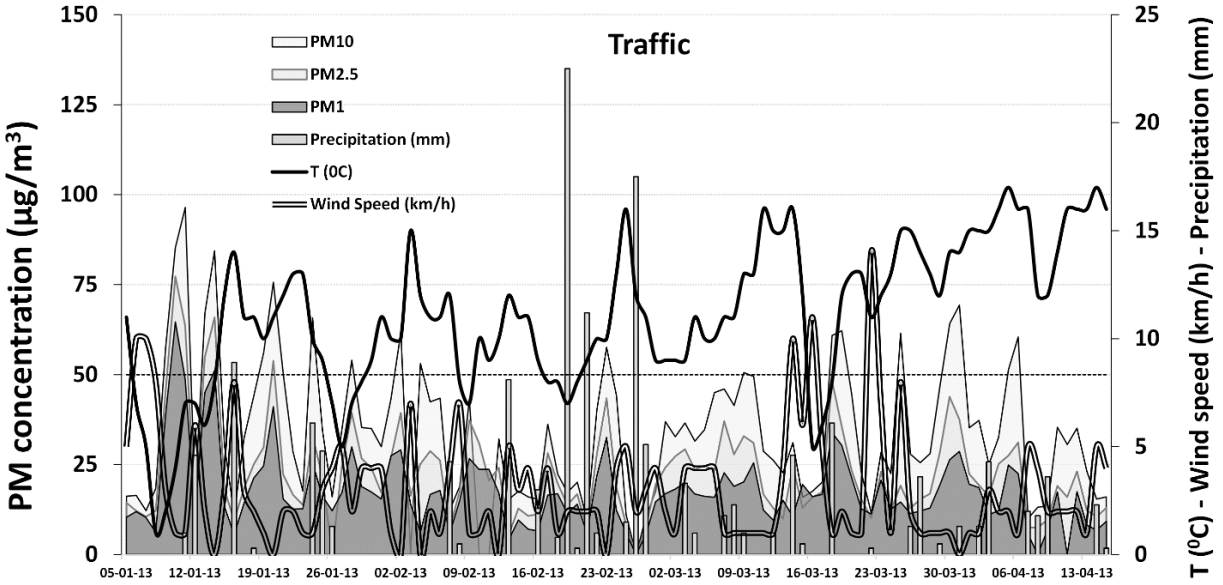

Figure 6. Inter-day variability of the ambient air PM levels at the traffic site

PM_{10} levels exceed the threshold of 50 μg/m^3 (as a 24 hour mean, not to be exceeded more than 35 times a year) for the majority of the measurements period. The concentrations start to decline after the end of March as result of lower demand for space heating, since ambient temperature starts to increase.

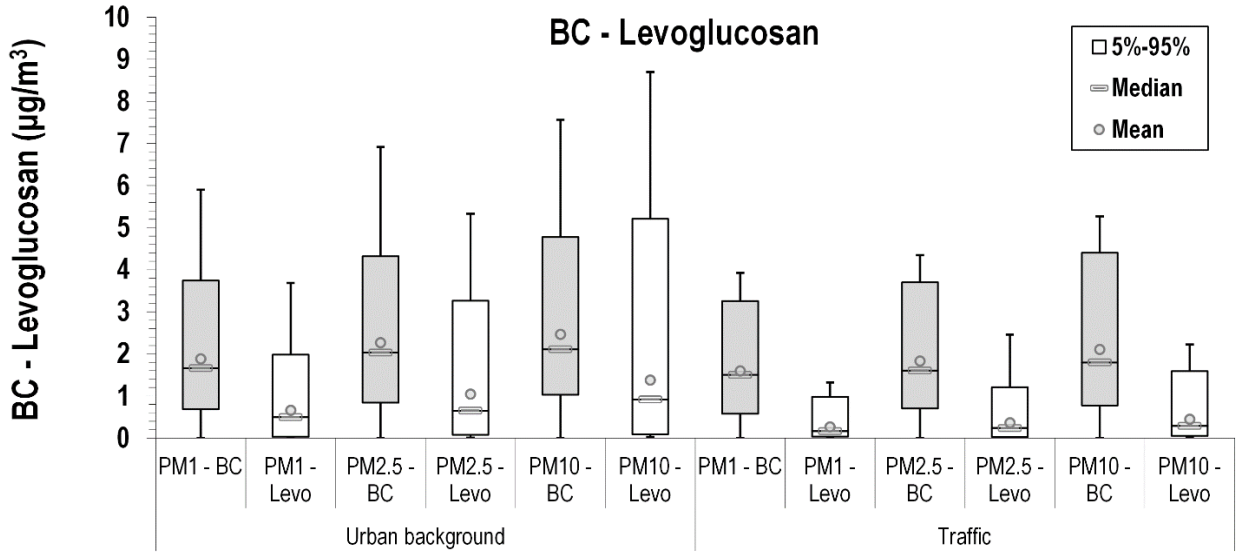

Figure 7. Variability of black carbon and levoglucosan levels for the different size fractions for the two sampling sites

The results of levoglucosan and BC analysis shed light on the relative contribution of the different sources at the two sampling sites; although BC levels (associated to internal combustion sources) did not differ significantly between the two traffic sites, the respective levoglucosan levels were significantly higher at the urban background site (Figure 7).

Levoglucosan is considered the most specific tracer of biomass burning (Belis et al., 2013; Perrone et al., 2012; Zhang et al., 2008). The contribution of biomass burning to PM_{10} levels was determined using the empirical formula proposed by Caseiro et al. (2009), according to which:

Wood smoke PM (in µg/m^3) = levoglucosan (in µg/m^3) ·10.7

Based on the formula described above, the respective biomass contribution for the PM_{10} levels were calculated and the results are given in Figure 8 and Figure 9 for the urban background and the traffic site respectively. For the overall period of measurements, biomass burning accounted for an average of 23.1% of the measured PM_{10}, while the respective contribution for the traffic site was almost 10.5%. However, biomass burning contribution during the coldest days, often accounted for about 60% at the urban background site, considered as the dominant source of PM_{10} in the respective area.

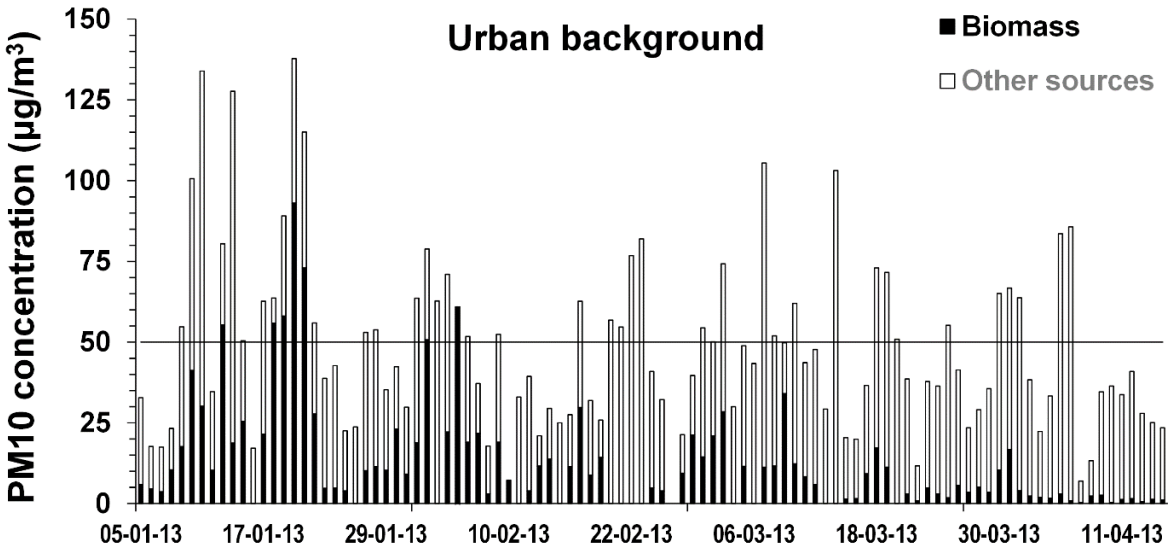

Figure 8. PM10 attributed to biomass burning/other sources for the urban background site

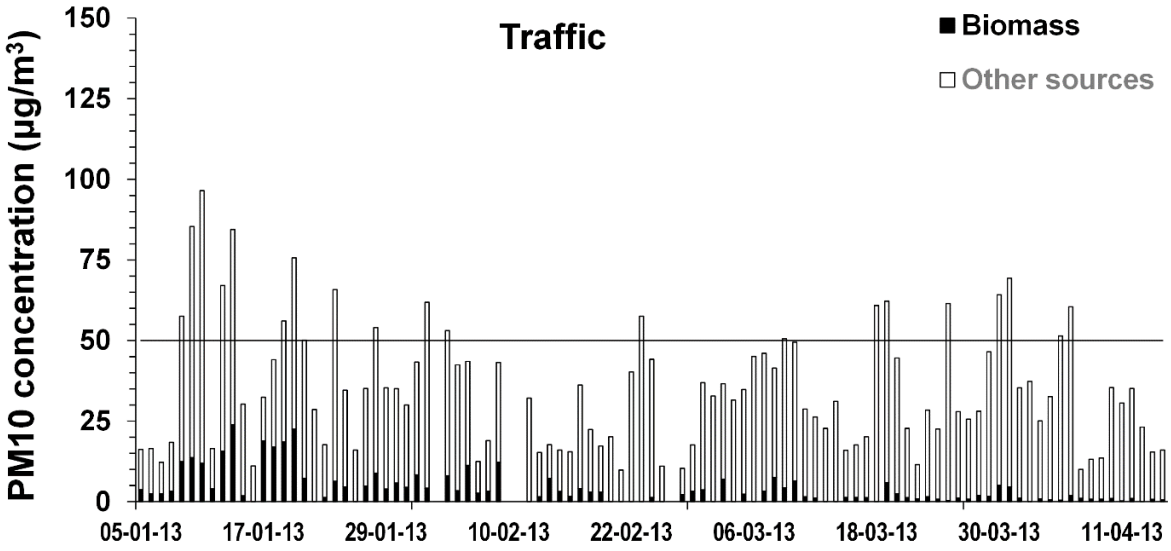

Figure 9. PM10 attributed to biomass burning/other sources for the traffic site

PAH concentrations and TEQ levels

The mean ΣPAHs levels at the urban background site are 14.8, 18.1 and 18.6 ng/m^3 for the PM1.0, PM2.5 and PM10 fraction respectively. At the traffic station, the corresponding levels are 5.3, 7.2 and 7.9 ng/m^3 (Figure 10). Therefore, practically, most of the PAHs are adsorbed in fine particles (PM2.5 and finer). At the urban background site mean TEQs are 3.3, 4.3 and 4.5 ng/m^3 for PM1.0, PM2.5 and PM10; the corresponding values at the traffic site are 1.2, 1.5 and 1.7 respectively (Figure 10). The TEQ at the urban background monitoring station is 3 times greater than the equivalent value found at the traffic station. TEQ/PM ratios at the urban background site are 0.091, 0.083 and 0.066 ng/μg PM for

PM1, PM2.5 and PM10 respectively. At the traffic site, the respective ratios are 0.045, 0.44 and 0.032 ng/μg PM. HRT deposition.

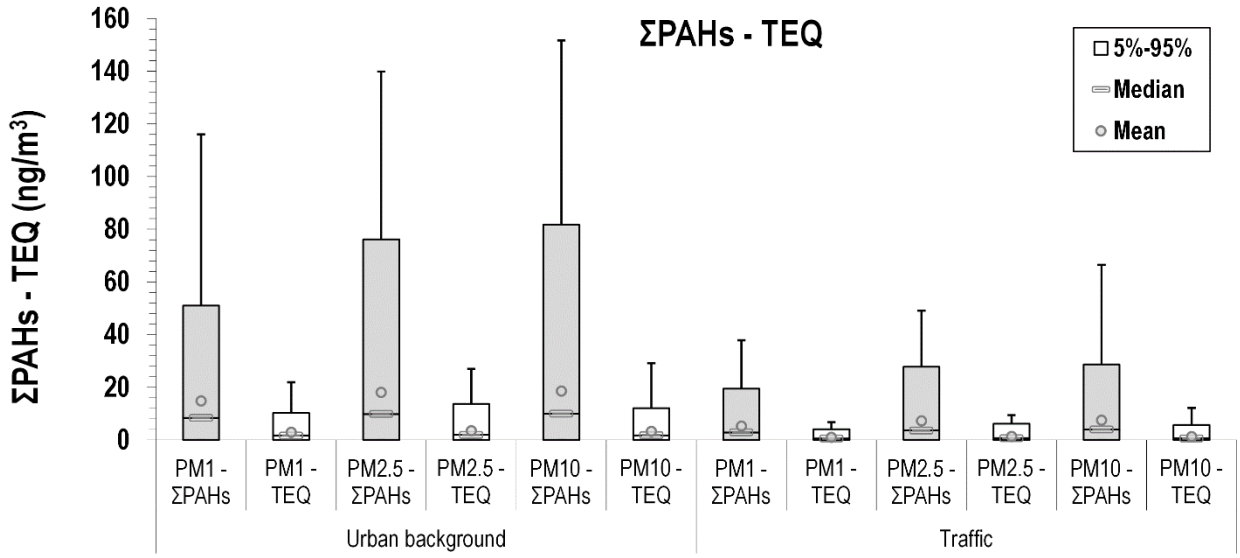

Figure 10. Total PAHs and TEQ per PM size fraction for the two sampling points

At the urban background site mean TEQs are 3.3, 4.3 and 4.5 ng/m^3 for PM1.0, PM2.5 and PM10; the corresponding values at the traffic site are 1.2, 1.5 and 1.7 respectively (Figure 10). The TEQ at the urban background monitoring station is 3 times greater than the equivalent value found at the traffic station. TEQ/PM ratios at the urban background site are 0.091, 0.083 and 0.066 ng/μg PM for PM1, PM2.5 and PM10 respectively. At the traffic site, the respective ratios are 0.045, 0.44 and 0.032 ng/μg PM. HRT deposition.

Oinofoita – Cr(VI) / industrial waste management

The main aim of this case study was to assess the health impact (i.e. cancer mortality) on the local population due to the presence of hexavalent chromium in drinking water of the Oinofyta municipality, within the wider area of Asopos basin (Figure 11).

The Oinofyta municipality 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 11: Asopos Basin and Oinofyta municipality

With regard to the contamination of water 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 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;
- a study conducted by the faculty of the Geology and Geo-environment department of the University of Athens (Loizou 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
- 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 jg/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.

Human biomonitoring data are the critical data missing for the application of the CROMELIFE 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

To evaluate internal exposure to chromium 50 individuals age stratified (4 to 65 years old) where recruited so as to evaluate current as well as past and cumulative exposures to Cr. For this purpose, both urine and hair samples were taken. Population covered the wider basin of Asopos area, reflecting the variability of exposures related to the different levels of Cr sampled in the environmental campaigns mentioned above.

The results of the biosamples analysis for the various individuals (for both urine and hair) are presented in Figure 12, while various statistic metrics per age group and biological matrix are presented in Figure 13.

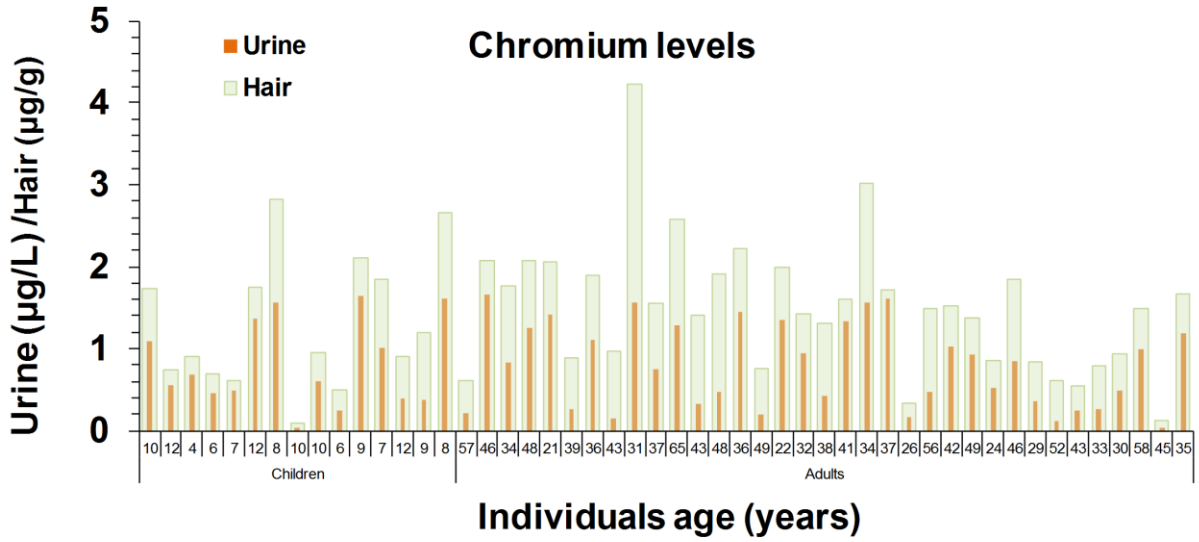

Figure 12. Urine and hair Cr levels for the 50 individuals in the Asopos Basin

Urinary levels range between 0.1 and 1.7 (mean value of 0.7) jg/L, while hair levels vary between 0.1 and 4.2 (mean value of 1.3) jg/g. Slightly higher values were observed in adults, potentially related to higher amounts of tap water consumption. Cr levels in hair showed higher variability that the respective urinary levels, however, this is somehow expected. The lack of highly sensitive methods and the limited weight of the material used (50-200 mg, instead of 2-5 ml for biological fluids such as urine and blood, or even 10 ml, which is the case for breast milk) act as limiting factors for the widespread use of this matrix. In addition, parameters such as external deposition of chemicals, or hair pigmentation have been amended as potential confounders.

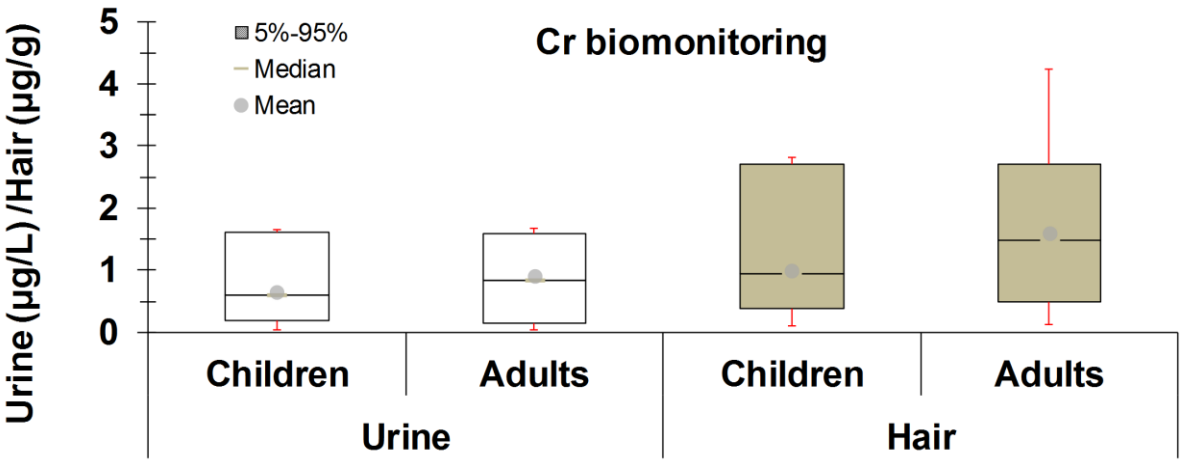

Figure 13. Urine and hair Cr levels for adults and children in the Asopos Basin

Italy

The National Case Study

Within the CROME-project, the ISS team selected as case study the exposure to metals in adolescents. The research was carried out to evaluate, by an extensive human biomonitoring campaign (the PROBE project), the potential exposure of 453 adolescents living in the Latium Region (Rome and Viterbo areas) to 19 metals as As, Be, Cd, Co, Cr, Hg, Ir, Mn, Mo, Ni, Pb, Pd, Pt, Rh, Sb, Sn, Tl, V, and W, through their direct analysis in the adolescents` blood. The exposure assessment was contextualized in an exposome approach considering several determinants related to the subjects (sex, age, Socio-Economic-Status), available environmental parameters and geo-coding of residence address location. To assess the influence of exposure determinants and modifiers on children biomarkers levels we used two independent methodologies. The first makes use of the so-called Environment- Wide Association Study (EWAS) methodology while the second was based on the application of a Generalized Liner Model (GLM) capturing co-exposures to pairs of key determinants. Regarding As, contamination of drinking water is a public health problem in several Italian areas due to the volcanic origins of the territory. In particular, the area of Viterbo province is characterized by the presence of a volcanic system where a continuous basal aquifer flows within Pliocene-Pleistocene sedimentary rocks with very high concentrations of As. The results in the PROBE adolescents` survey, show that children living in Viterbo area are more exposed to As (and also Hg and V). Regarding Hg, the concentration in blood of PROBE adolescents were slightly higher than in those reported in the cross-sectional nationally representative survey (NHANES) for the US population; probably this result is due to a greater consumption of fish in Italian adolescents from the Mediterranean area and to the existence of sub-marine volcanos. Also fish consumption can be considered a source of exposure to As in the PROBE adolescent cohort. Other foods as milk can also be linked, even if in a minor extent, to the exposure to Co. Another interesting item was the higher values for some metals such as Ir, Pt, Rh in adolescents` blood living in urban area (Rome) respect to those living in rural ones; the exposure source of these metals is the road traffic, so PROBE adolescents living in urban areas are more prone to a a higher exposure to traffic-derived metals. Moroever, some metals (as Cr) showed a more complex situation with a co-exposure to different dietary pathways (milk and fish) coupled to proximity of residence to industrial activities. Other sources of exposure to metals are linked

to the adolescents` lifestyle or behavior; for example, secondary smoke (for As and Pd) and use of costume jewelry (Cd and Pt).

Related to health risk, the levels of metals found in adolescents` blood did not indicate a health risk when considering the health reference limits. In any case, a new human biomonitoring campaign should be performed in order assess the present situation in Italian adolescents regarding metals, considering also other potential sources of exposure.

Common Study

The contribution of the Italian Unit to the CROME-LIFE common study consisted in the follow-up of a sample of 200 children aged 7 years and originally enrolled in the Italian Northern Adriatic Birth Cohort II (NAC II). The results of the follow up, described in deliverable D5.2, revealed significant association between metals/metalloids' exposure and neuropsychological scores at school age in children. The size and direction of the effects depend on time window of exposure.

Metals and metalloids assessed in the biomonitoring programme are the following:

- *Mercury:* released into the air/water from coal-burning power plants, mines, volcanos; also found in some medical equipment, main sources through foodweb.
- *Lead is* found in old paint, gasolines, lead pipes and sinkers, toys, jewelry, and other items made of vinyl plastic. Lead can contaminate water, air, soil, dust and food.
- *Manganese, Arsenic, Cadmium:* diet and drinking water are common sources of exposure in non-occupationally exposed individuals. Manganese may be a by-product of iron production. Cadmium is also found in cigarette smoke.
- *Copper, Zinc, Selenium:* important micronutrients determinants for brain development, interact during critical developmental period. Their deficiency of excess may affect brain development

With regard to the source of exposure to the five neurotoxic metals measured in the biomonitoring campaign, the Gulf of Trieste is one of the most mercury-contaminated areas in the Mediterranean Sea. It is characterized by high mercury inputs from the Isonzo River whose tributary, the Idrijca River, drains the mercury mining area of Idrija in Slovenia where extraction activity has taken place for nearly 500 years. Available data show that the processes controlling microbial production and degradation of toxic MeHg in the historically Hg-contaminated Gulf of Trieste are localized in the surface anoxic sediments and strongly influenced by biogeochemical conditions (Faganeli et al., 2014). All sources and forms of Hg contribute to its accumulation in benthic and pelagic food webs with greater accumulation of MeHg in pelagic feeding species. Though extensive contamination of marine biota can be excluded on the bases of the existing data, some MeHg fish levels appears to exceed the limits. However, there is surprisingly little information on MeHg in edible marine species from the Trieste gulf and in this context more future studies should address this issue. The recently published DEMOCOPHES data (Castano et al., 2015) shows that there are significant differences in MeHg exposure across the EU and that exposure is highly correlated with consumption of fish and marine products. Our findings support the relation of Hg levels with fish consumption.

It is possible that environmental sources other than diet, such as air, water and exposure to contaminated dust at home, in gardens and playgrounds (particularly important for infants and children) contributed to metals' levels in the children assessed. Main sources of pollution in Trieste are

represented by the emissions from the iron plant (Ferriera di Servola), vehicle emissions and harbour activities. The Regional Agency for Environmental Protection (ARPA) of Friuli Venezia Giulia Region assures continuous monitoring of air and water. Air concentration of heavy metals, particulate (PM_{10} and $PM_{2.5}$), NO_2 and benzopyrene has been recorded over years by the community air monitoring system, and the exceedance of the threshold has been reported in some occasions. As geolocalization of children enrolled in the study is available, we reconstructed exposure through reverse dosimetry in order to estimate the possible contribution of environmental sources to metals/metalloids levels measured in children during the period of the follow-up. The scarcity of air pollution monitoring in the Trieste municipality did not allow us to differentiate among children exposure levels to air pollutants. Finally, we do not have any information on indoor pollution and content of metals' in the house dust.

We cannot exclude that other food items (vegetables, meat, dairy products) may have concurred to metals' exposure in children (see also https://www.efsa.europa.eu/en/topics/topic/metals-contaminants-food). No updated indications concerning the levels of metals in food items in the areas where the population under study lives are available. A 3-day-diary assessing the quality and quantity of food items consumed by the children in the 3 days preceding the neuropsychological testing has been carried out at the follow-up through specific questions to the mothers and we will possibly have these data analysed within June 2017. This aspect is particularly important as some children presented very high urinary concentration of arsenic (As). Concentration of As may be interpreted in a risk-assessment context differently if As arises from exposure to iAs species (As(III) and As(V)), methylated As (MMA and DMA) or organic As (AsBet and AsChol). Thus the measurement of total As concentration (which contains inorganic and organic As) cannot be used solely to explain the biological and toxic effects of As. From our data, As concentration correlates with Hg and Se concentration at 7 years, and this support the hypothesis that these three metals are associated in fish and marine organism. However, information on other food sources, mainly rice and water consumption is still needed to better understand if other sources might contribute to the concentration of inorganic As in children urine.

Slovenia

Human Biomonitoring in Slovenia – metals and other selected elements

Based on the legislation for the implementation of the human biomonitoring (HBM) programme in Slovenia defined in the Article 49 Act of Chemicals of the Chapter IX Protection of Human Health and the Environment (O.J. RS No. 16/2008), the national HBM in Slovenia was conducted in a period between 2007 and 2015 for the first time. The study was funded by the Ministry of health RS, Chemicals Office of the Republic of Slovenia; the implementing organisation was Josef Stefan Institute Ljubljana and the subcontracting institutions University Medical centre Ljubljana, Regional institutes of public health and regional hospitals and health centres.

Short-term objectives of the HBM programme were 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 potentially 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 namely 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 population and collection of biological samples

The study included lactating *primiparous* women and their partners, 20-45 years of age, from 12 different areas of Slovenia. Among the study areas, there were 6 contaminated (selected due to recent-smelters, cement factory, power plant, glass factory, or past industrial activities - former Hg mine, former Pb mine, former factory of transformers and capacitors – PCBs pollution), 3 urban and 3 rural areas, in each 50 women and 50 men were recruited. The mothers who agreed to participate and were eligible for the study donated 16 mL of venous blood, 50 mL of urine, approx. 1 g of scalp hair and max. 80 mL of breast milk; and the eligible partners 23 mL of blood, 50 mL of urine and 1 g of scalp hair. Sampling was carried out in week 2-8 after the delivery. Along with the sampling, mothers and their partners completed a questionnaire to obtain data on residential environment, nutritional habits and life-style in general, their socio-economic status and potential exposure. In total, samples were collected from 1086 participants (537 females and 549 males) across the country (Figure 14).

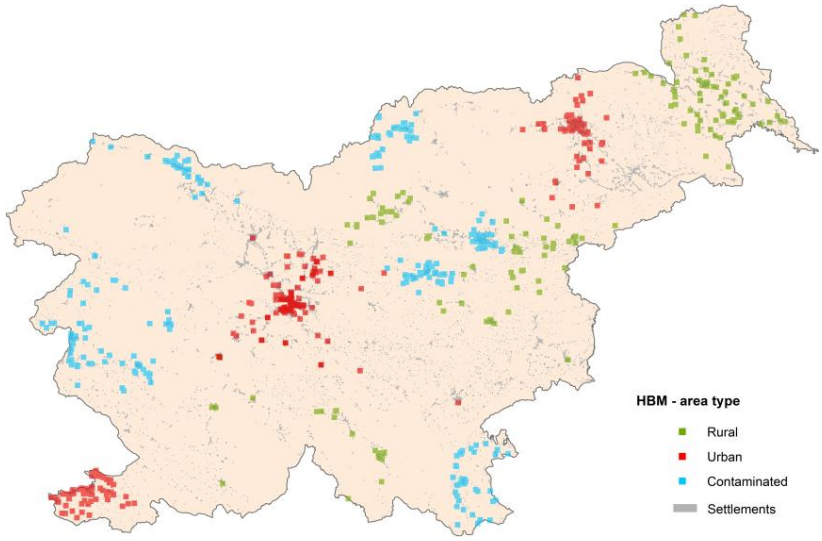

Figure 14: Residential locations of the participants in the Slovenian human biomonitoring, 2007-2015, by the type of areas (rural, urban, contaminated).

Laboratory analysis

Blood, urine and milk samples were analysed for total mercury by CVAAS or CVAFS technique and for other elements (cadmium, lead, arsenic, selenium, copper, zinc) by ICP-MS (Table 1). The results of the first Slovenian HBM are reported by Horvat et al (2015, HBM final report).

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

Sample	*Individual samples*	*Pooled samples*
Breast milk	*Pb, Cd, total Hg, As, Cu, Zn, Se* *organochlorinated pesticides* *marker PCBs (28, 52, 101, 138, 153, 180)*	*PCDD,* *PCDF,* *dioxin like PCB,*

	triglycerides, cholesterol	*PBDE*
Blood - women	*Haemogram* *Pb, Cd, total Hg, As, Cu, Zn, Se* *creatinine* *TSH*	
Blood - men	*Haemogram* *Pb, Cd, total 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, total Hg, As, Cu, Zn, Se* *Markers of kidney function* *(albumin, alpha-1-mikroglobulin, IgG, NAG)* *Creatinine*	
Hair	*Total Hg*	

Questionnaire data

Data obtained from the questionnaire completed by the participants includes the following variables:

(a) general part: age, body weight and height, education, occupation;
(b) basic characteristic of home: type of building, years the house was built, type of type of heating, type of water supply, traffic density;
(c) health conditions: disease if any, medications, number of amalgam fillings;
(d) life-style and nutritional habits: smoking, passive smoking, use of computer, hobbies, consumption of vegetables, fruit, nuts, milk, cheese, eggs, poultry, game, other meat, fish and seafood, tea, coffee, alcohol, type of oil used, daily water consumption, consumption of supplements;
(e) questions regarding pregnancy and lactation period: any problems during the pregnancy, smoking during pregnancy, alcohol intake, breastfeeding, baby`s gender, birthweight, current weight.

Acquisition of environmental and other ancillary data

In order to link internal exposure assessed by measuring concentrations of selected metals and other elements in biological samples with potential sources of exposure, environmental data available in Slovenia was collected within the CROME.

Three major groups of ancillary data were identified and collected: 1) information describing environmental status, 2) information on releases of contaminants from various sources, and 3) data supporting characterization of the built environment in living surrounding of HBM participants. Information gathered is based on variety of national and EU scale sources with different levels of spatial-temporal resolution as described in the following paragraphs and with examples given in the consequent figures.

1) Drinking water quality

Sampling and analysis protocol

Drinking water from public water supply was collected from 108 locations across Slovenia, that have been selected among 1283 locations, where water quality is monitored regularly by responsible institutions (Figure 15). The sampling has been conducted as part of a radon (Rn) survey of tap water in Slovenia and was carried out in year 2014. The locations have been selected based on the following

criteria: (a) number of water supply users, (b) expected Rn concentration, (c) type of water and (d) lithology of the bedrock. The sampling locations have been evenly distributed across the country and more frequent around bigger cities and in areas with relatively higher levels of Rn in air and soil.

Water samples have been collected directly from the pipes after the water was let to run for minimum 10 minutes, in order to collect fresh water. In parallel, tap water was analysed for ^{222}Rn activity concentration using scintillation cell on site. The water samples collected were analysed at JSI for Sb, As, Cu, Ba, Cd, Cr, Ni, V, Pb, Co, Al, Mn, Mo, Fe, Zn, Sn, Sr, Hg and Rb.

Determination of elements in drinking water by ICP-MS

The samples of drinking water were acidified with 1 mL of suprapure HNO_3 per 1 L of sample. The concentrations of selected elements were determined in the samples directly, without any sample pre-treatment, by inductively coupled mass spectrometry (ICP-MS) at optimal measurement conditions. For checking the accuracy SPS-SW1 Quality Control Material for Surface Water Analysis from SPS Spectrapure Standards AS (Oslo, Norway) was used.

Table 2: Determination of elements in Quality Control Material for Surface Water analysis SPS-SW1 (µg L^{-1}) by ICP-MS. The result represents the mean ± standard deviation of three independent analyses.

QCM	Element	Certified value	Determined value
SPS-SW1	As	10.0 ± 0.1	9.4 ± 0.9
	Cd	0.5 ± 0.01	0.49 ± 0.01
	Co	2.0 ± 0.02	1.95 ± 0.04
	Cr	2.0 ± 0.02	1.97 ± 0.02
	Cu	20 ± 1	19.5 ± 0.8
	Fe	20 ± 1	20.3 ± 0.6
	Mn	10.0 ± 0.1	9.8 ± 0.2
	Mo	10.0 ± 0.1	10.0 ± 0.1
	Ni	10.0 ± 0.1	9.7 ± 0.2
	Pb	5.0 ± 0.1	5.1 ± 0.1
	Se	2.0 ± 0.02	2.04 ± 0.06
	Tl	0.5 ± 0.01	0.51 ± 0.01
	Zn	20 ± 1	19.5 ± 0.7

Determination of Hg in drinking water by CVAFS

39,4 mL of a sample was weighed directly in a 125 ml Teflon bottles. After addition of 0,4 ml HCl (30%, suprapur) and 0,2 ml BrCl, the Teflon bottles were closed and left to react on room temperature under the UV light overnight. 15 µL of hydroxylamine hydrochloride was added before being measured. An aliquot was then introduced to the instrument. Mercury in the sample is reduced to gaseous elemental mercury Hg^0 with 3% tin chloride prepared in HCl as described above. Hg^0 is stripped from the aqueous sample by Argon gas via phase separator, and detected by cold vapour atomic fluorescence spectrometry CVAFS, Tekran 2600.

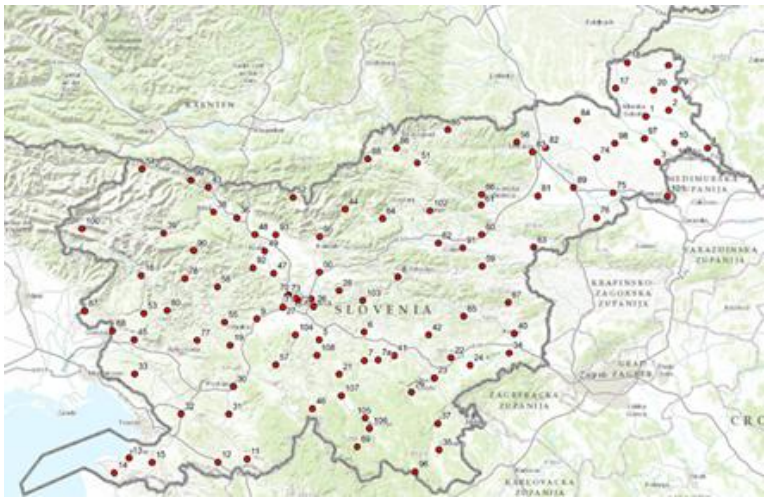

Figure 15: Locations of drinking water measurements.

Results

Dataset comprises results of concentrations of the selected elements at 108 locations in Slovenia (Sb, As, Cu, Ba, Cd, Cr, Ni, V, Pb, Co, Al, Mn, Mo, Fe, Zn, Sn, Sr, Rb, Hg) and Rn level. For each of the sampling sites, number of water supply users, type of raw water for water supply, and information on lithology is available.

2) Bio-indicators of air quality and deposition

Sampling and analysis protocol

Moss species *Hypnum cupressiforme* has been collected from 102 locations in Slovenia according to the EMEP[1] grid (50 km x 50 km) (Figure 16) in years 2010 and 2015. The methodology is described in Heavy Metals in European Mosses: Survey 2010.

Moss samples collected from the selected locations have been carefully cleaned from all dead material and attached litter, homogenized and freeze-dried.

The samples from the 2010 and 2015 campaigns have been microwave digested and analysed for As, Cd, Cu, Pb, Zn, Mn and Se content by ICP-MS. The analysis of total Hg have been performed by thermal decomposition of the samples, amalgamation and CVAAS detection using Direct Mercury Analyser, DMA-80.

[1] Co-operative programme for monitoring and evaluation of the long-range transmission of air pollutants in Europe. http://www.emep.int/

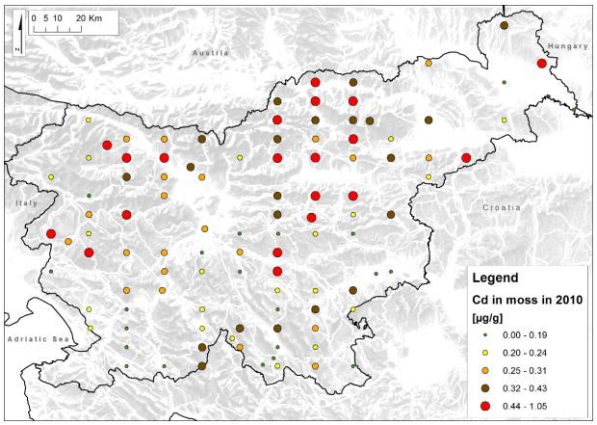

Figure 16: Distribution of elements in moss – an example of Cd concentrations.

Results

Database comprises the results of measurements of As, Cd, Cu, Hg, Pb, Zn, Mn and Se in moss *Hypnum cupressiforme* at 102 locations in Slovenia. For each of the sampling site, land use/cover and topography of the site is available.

3) Geochemical map of Slovenia

Geochemical composition of soil (in ppm) at address location of individual subject was obtained from interpolated geochemical map of Slovenia http://www.geo-zs.si/PDF/Podatki/Geokemicna_karta.pdf. Database comprises geochemical soil maps in raster format with 100 m resolution for the following chemical elements: Al, Ca, Fe, K, Mg, Na, P, Ti, Ba, Cd, Cr, Cu, La, Mn, Mo, Nb, Sc, Sr, Th, V, Y, Zn, Zr, and Hg. Maps covering the whole country were created by Geological Survey of Slovenia, based on geodatabase comprising on average 2500 soil analysis, with the exception of Hg (961 analyzes).

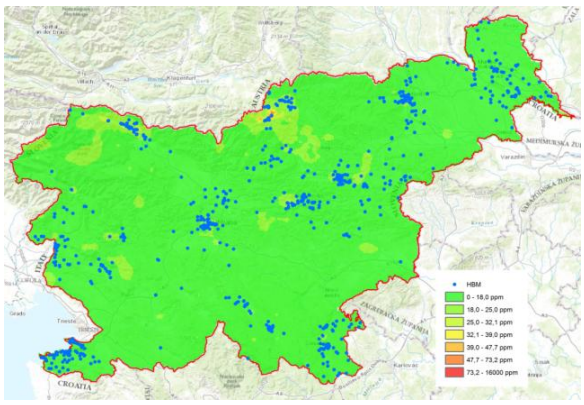

Figure 17: Geochemical map of Slovenia – an example of Pb concentrations in topsoil.

4) Air Quality data – Country scale

AQ monitoring network in Slovenia is relatively scarce with approx. 20 stations operating in the country. Therefore, due to a lack of more detailed spatial information or suitable AQ models, interpolated maps showing air quality in Europe will be used. European Environmental Agency (EEA) provides AQ maps for O3, PM10 and PM2.5 at a resolution of 10 km in the 2006-2012 period. Maps are based on combination of Airbase background station monitoring data EMEP station monitoring data (not

considering industrial and traffic station types), meteorological ECMWF data and EMEP concentration modelling data.

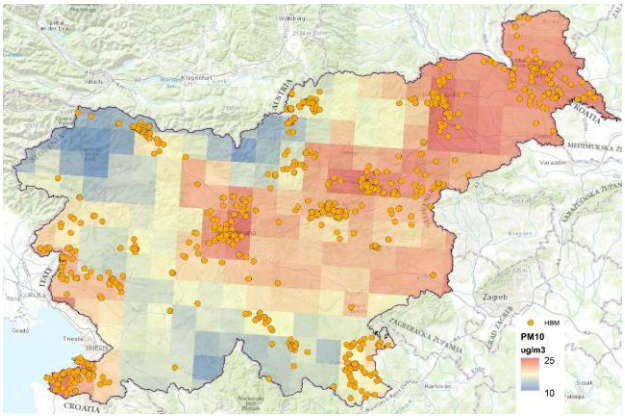

Figure 18: EEA interpolated air quality data – an example of average annual PM10 concentrations for 2012.

Details can be found at ETC/ACM Technical paper 2012/12:
http://acm.eionet.europa.eu/reports/ETCACC_TP_2012_12_spatAQmaps_2010.

5) Air Quality data – pilot scale

Higher spatio-temporal resolution AQ data is available for the area of Ljubljana based on the on-going CITI-SENSE EU project. Therefore, in the case of HBM participants from this region, for the external exposure assessment AQ data collected (PM, NOx, CO, O3, T, RH, AP) within CITI-SENSE monitoring network will be tested.

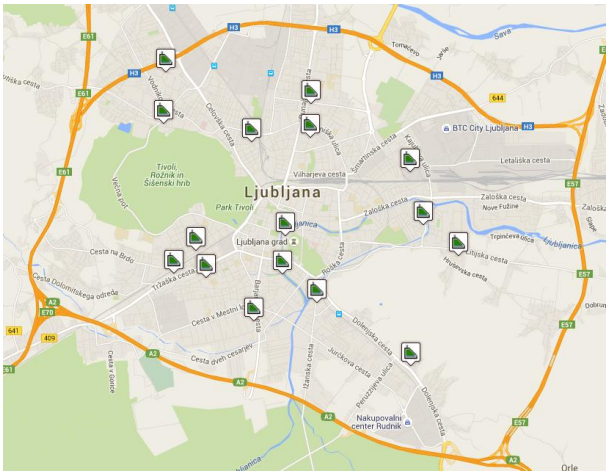

Figure 19: AQ monitoring network in Ljubljana within the CITI-SENSE EU project.

6) Information on releases of contaminants from various sources

Emissions and releases from point sources: In Slovenia there are over 170 facilities reporting releases of various pollutants into the European Pollutant Release and Transfer Register (E-PRTR).

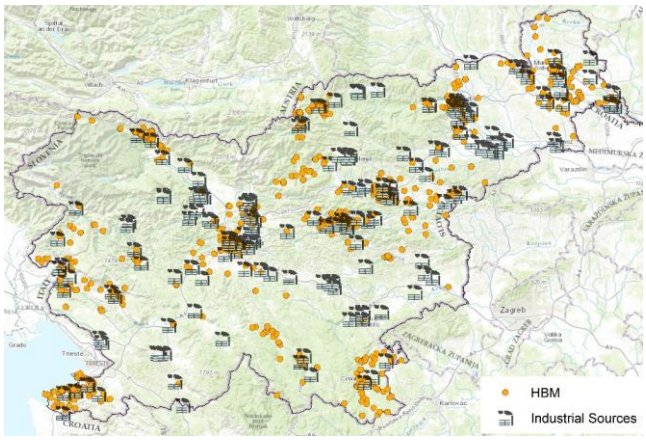

Figure 20: Industrial facilities in Slovenia reporting various pollutant releases to air and water as part of European PRTR.

7) Releases from Diffuse Sources to Air

Within the European Pollutant Release and Transfer Register (E-PRTR) spatial distribution and emission of air pollutants from diffuse sources are also available. The data is based on a scale of 5 by 5 km grid and includes details of NOx, SOx, CO, ammonia NH3, carbon dioxide CO2 and PM10. Diffuse annual emission from industrial sources, residential sources and road in t/grid (5*5 km2), CO2 in kt/grid were considered. Data obtained from http://prtr.ec.europa.eu/#/home.

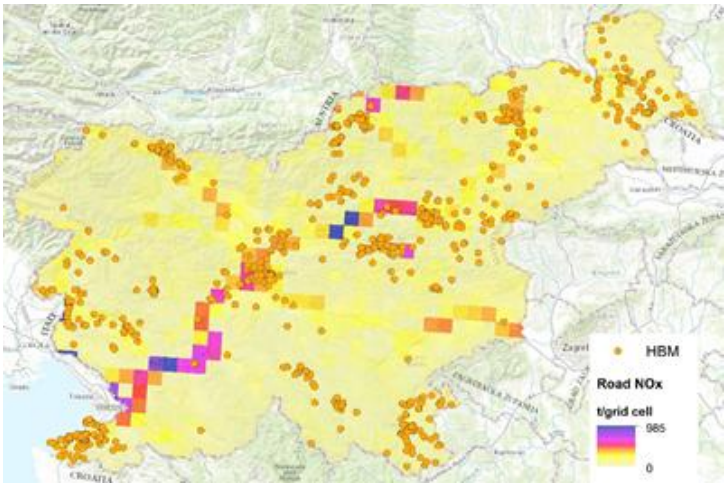

Figure 21: Diffuse sources to air based on E-PRTR – an example of NOx emissions associated with traffic.

8) N and P releases

Modelled nitrogen and phosphorous surpluses were obtained from the Geological Survey of Slovenia. Data covering the whole country of Slovenia is available in raster format with 100 m resolution.

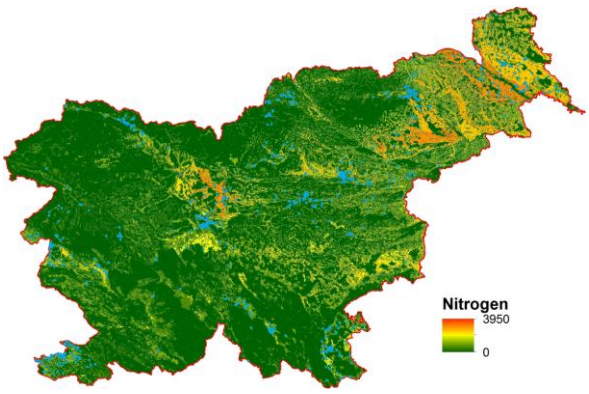

Figure 22: Nitrogen surplus map of Slovenia

9) Build environment and land use

Cadastre of public infrastructure comprises various data on public infrastructure: transport infrastructure, energy infrastructure, utility and water infrastructure. Information on road infrastructure will be mainly used as a proxy for exposure to air pollutants from traffic, especially for regions were detailed information is missing: distances to three types of roads (in meters) according to classification in Slovenia (Highway/Motorway, Main Roads and Regional roads), and length of all roads (in meters) within three buffer zones (50, 100 and 500)

Area (in square meters) of three land use feature in 500 m surroundings of individual subjects address: CLC_3xx (Corine Land Cover classes 3.1+3.2+3.3), CLC_Urban_green (Corine Land Cover classes 2.1+2.2) and Fields_gardens were obtained (based on records of actual use of agricultural and forest land: http://rkg.gov.si/GERK/).

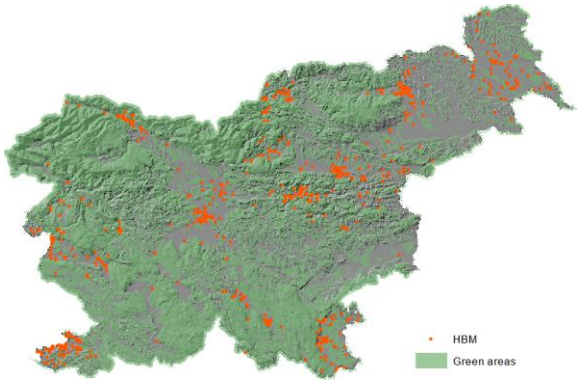

Figure 23: Land use (green vs. urban areas).

Summary of internal exposure

The levels for selected metals measured in the study population are summarized in Tables 3-7. The levels were mainly below the given HBM reference values for toxic elements and within the reference intervals for essential elements.

Mean participants` age was 30 years, 29 for women and 31 for men, and was similar across the study areas. The majority of the participants had finished secondary or high school, among women there was the highest proportion of those with university education. Among the participants, 11% were smokers

(men-current, women-before pregnancy); the highest proportion of smokers was in the PCB-contaminated area of Bela krajina and the lowest in the Pb-smelter area of Mežiška dolina.

Table 3: Levels of toxic metal(loid)s in blood [µg/L] of women and their partners from 12 study areas in Slovenia.

Population group	N	N<LOD	GM	95% CI	min-max	P5	P10	P90	P95
Cadmium									
Total	1085	302	0.28	0.27-0.30	<LOD-4.80	<LOD	<LOD	0.72	1.01
Women	536	71	0.35	0.33-0.37	<LOD-3.08	<LOD	0.14	0.71	0.87
Men	549	231	0.23	0.22-0.25	<LOD-4.80	<LOD	<LOD	0.80	1.22
Lead									
Total	1086	0	18.0	17.5-18.5	3.86-116	9.13	10.3	32.7	41.5
Women	537	0	16.7	16.2-17.3	4.25-71.9	8.82	10.3	28.0	33.2
Men	549	0	19.3	18.5-20.1	3.86-116	9.16	10.4	37.4	46.1
Mercury									
Total	1085	2	1.18	1.12-1.24	<LOD-31.0	0.30	0.41	3.41	4.78
Women	536	1	1.11	1.04-1.19	<LOD-10.2	0.31	0.43	3.09	4.06
Men	549	1	1.25	1.15-1.34	<LOD-31.0	0.26	0.38	3.72	5.13
Arsenic									
Total	1086	1	0.89	0.85-0.93	<LOD-28.9	0.35	0.40	2.62	3.74
Women	537	0	0.96	0.89-1.03	0.20-28.9	0.32	0.39	2.90	5.16
Men	549	1	0.83	0.79-0.88	<LOD-22.4	0.36	0.40	2.12	3.40
Manganese									
Total	812	0	13.8	13.5-14.2	5.69-40.5	8.18	9.02	22.1	26.2
Women	410	0	17.1	16.7-17.7	5.83-40.5	10.4	11.5	25.9	28.0
Men	402	0	11.1	10.5-11.0	5.69-29.8	7.64	8.26	15.0	16.4

Table 4: Levels of toxic metal(loid)s in blood [µg/L] of women and their partners from 12 study areas in Slovenia.

Population group	N	N < LOD	GM	95% CI	min-max	P5	P10	P90	P95
Copper									
Total	1086	0	951	941-961	532-2004	737	766	1200	1272
Women	537	0	1071	1058-1084	657-2004	857	908	1262	1371
Men	549	0	847	839-856	532-1404	708	738	976	1041
Zinc									
Total	1086	0	6607	6550-6665	3010-11733	5150	5518	7809	8298
Women	537	0	6724	6639-6810	3010-11733	5274	5647	8027	8494
Men	549	0	6495	6420-6572	3400-10301	5082	5460	7646	8050
Selenium									
Total	1086	0	105	103-106	53.9-226	74.2	80.5	138	152
Women	537	0	94.7	93.2-96.2	53.9-176	70.7	75.1	119	127
Men	549	0	115	114-117	60.3-226	87.3	91.7	152	166

Table 5: Levels of toxic metal(loid)s in urine [µg/g creatinine] of women and their partners from 12 study areas in Slovenia.

Population group	N	N < LOD	GM	95% CI	min-max	P5	P10	P90	P95
Cadmium									
Total	989	14	0.20	0.19-0.21	<LOD-2.79	0.07	0.09	0.47	0.61
Women	489	12	0.26	0.25-0.28	<LOD-2.79	0.10	0.12	0.58	0.79
Men	500	2	0.16	0.15-0.16	<LOD-1.14	0.06	0.07	0.32	0.42
Lead									
Total	812	249	0.49	0.46-0.51	<LOD-5.31	0.15	0.20	1.19	1.49
Women	410	184	0.49	0.45-0.52	<LOD-3.44	0.15	0.19	1.27	1.57
Men	402	65	0.49	0.46-0.52	<LOD-5.31	0.15	0.22	1.12	1.42
Mercury									
Total	1020	12	0.47	0.43-0.50	<LOD-8.67	0.06	0.11	1.78	2.48
Women	504	10	0.52	0.47-0.58	<LOD-8.67	0.07	0.11	1.92	2.64
Men	516	2	0.42	0.38-0.46	<LOD-7.92	0.06	0.09	1.50	2.19
Arsenic									
Total	812	0	6.70	6.24-7.19	0.37-499	1.71	2.17	26.6	50.5
Women	410	0	7.71	7.02-8.48	0.37-219	2.15	2.53	28.6	48.8
Men	402	0	5.80	5.22-6.43	0.76-499	1.52	1.85	24.5	52.9

Table 6: Levels of metal(loid)s in maternal milk [µg/L] from 12 study areas in Slovenia.

	N	N < LOD	GM	95% CI	min-max	P5	P10	P90	P95
Toxic elements									
Cd	471	262	<LOD	-	<LOD-0.34	<LOD	<LOD	0.14	0.18
Pb	471	161	0.35	0.32-0.39	<LOD-10.6	<LOD	<LOD	1.05	1.81
Hg	471	18	0.14	0.13-0.16	<LOD-3.39	<LOD	0.04	0.51	0.64
As	471	9	0.18	0.17-0.19	<LOD-3.70	0.07	0.08	0.48	0.79
Essential elements									
Cu	471	0	356	345-366	99-954	221	250	525	578
Zn	471	0	1937	1844-2034	207-7904	740	1011	3701	4404
Se	471	0	12.6	12.3-13.0	5.36-38.1	8.13	9.16	17.6	19.4

Table 7: Total mercury in hair [ng/g] of lactating women and their partners from 12 study areas in Slovenia.

Population group	N	N < LOD	GM	95% CI	min-max	P5	P10	P90	P95
Total	947	0	275	258-292	10.0-7068	48.0	79.5	867	1203
Women	503	0	268	248-290	10.0-1947	52.3	82.0	731	993
Men	444	0	282	256-311	11.0-7068	46.0	77.0	984	1396

Determinants of exposure

Among the potentially toxic elements, Cd levels were higher in women compared to men, while Pb, Hg and Mn levels were in general higher in men. Potential sources of exposure to selected elements were examined using multiple linear regression, adjusting for all potential co-variates and confounders. Cadmium levels in blood were determined significantly ($p<0.001$) by gender (higher in women then in men), age (levels increased with age) and smoking. Similarly was observed with urinary Cd, whereat the levels in women were not associated with smoking status during pregnancy. Among the study areas, the

levels (particularly in urine and milk) were higher in the rural areas. Furthermore, in rural areas, milk Cd levels (but not urine or blood) were associated with consumption of game. This association was not observed neither in urban nor in potentially contaminated areas. Vegetable consumption was not associated with Cd levels in neither of the areas. Also the soil Cd levels were not associated with the biomarker levels.

The area-related Cd levels could be associated with artificial fertilizers that potentially contain Cd. For this purpose, HBM based Cd levels were linked to the maps of nitrogen and phosphorous surpluses used as proxy for fertilizer use. No association was discovered, therefore, based on the collected data higher Cd exposure in rural areas of Slovenia could not be associated with the use of Cd-containing fertilizers. Certain significance of regional road proximity to participants homes in Cd exposure was observed by univariate correlations, but the association was insignificant after adjustment for other co-variates.

Cadmium levels in blood or urine exceeded the established HBM I values in smokers only, while in milk none of the observed levels exceeded the HBM level I or II.

Lead levels in blood were determined by gender (higher in men than in women), smoking (smokers having lower levels), and type of water supply used in household: participants consuming water from private water supply had higher blood Pb than participant with public water supply. Furthermore, the levels increased with age and were higher in those with lower educational levels. Among the areas, blood Pb levels were the highest in Pb-smelter area of Mežiška valley (Figure 24). In this area, blood Pb levels were not associated with smoking nor with type of water supply, but were strongly correlated with soil Pb levels ($r=0.44$, $p<0.001$). In other areas of the country, blood Pb levels were not associated with the levels in soil, however the overall correlation was significant (Figure 25).

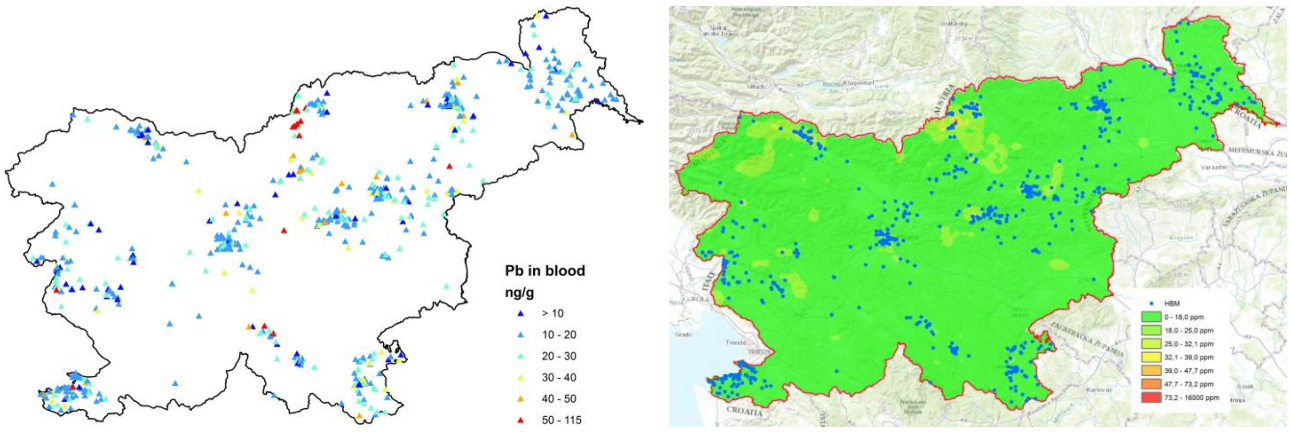

Figure 24: Geolocated Pb levels in blood of HBM study participants (left) and Pb levels in soil (right).

Observing milk Pb levels, the levels were higher in women consuming bottled water than in those using water from public or private water supply and also in those smoking before pregnancy. Milk Pb showed higher levels in urban area in compare to rural and potentially contaminated. Although milk Pb levels were correlated with soil Pb level significantly, similarly as blood Pb levels were, the milk levels were the highest in the capital, Ljubljana, and not in the Mežiška valley. Increased milk Pb in urban vs. other areas might be due to increased emission of particulate matter in urban environment. Indeed, emission of PM10 from residential sources and from road was the highest in Ljubljana and it associated significantly with milk Pb levels in the model adjusted for other co-variates.

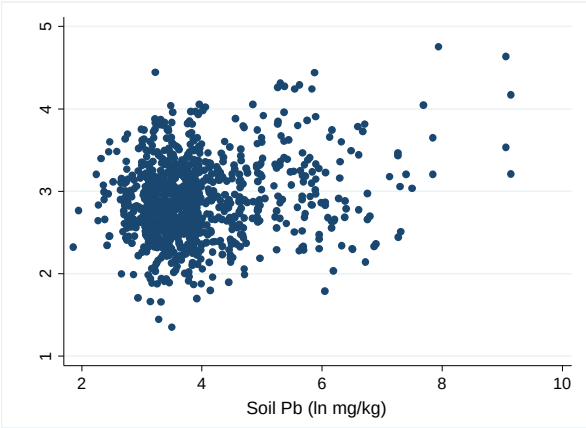

Figure 25: Correlation between Pb in soil and Pb in blood of study population living in Slovenia (r=0.19, p<0.001, n=1043).

In general, the Pb levels observed did not exceed the established reference values, except for blood Pb in few individuals from Mežiška valley, who were occupationally exposed, and milk Pb in one mother from Ljubljana and one from Mežiška valley.

The levels of Hg in blood were dominated by consumption of fresh and canned sea fish and were higher in participants having more amalgam fillings. The levels in blood increased with age and were higher in more educated participants. The levels in hair were associated by consumption of fresh and canned fish and were marginally significantly higher in men than in women. The levels in hair and blood were area dependent, and were the highest in the coastal part of Slovenia (Figure 26), where the people consume sea food more frequently than in other parts of the country. In contrary, the levels in urine were not dependent on the geolocation (Figure 26) and were associated with the presence of amalgam fillings, consumption of fresh and canned sea fish. The levels in milk were associated significantly with amalgam fillings and consumption of fresh sea fish.

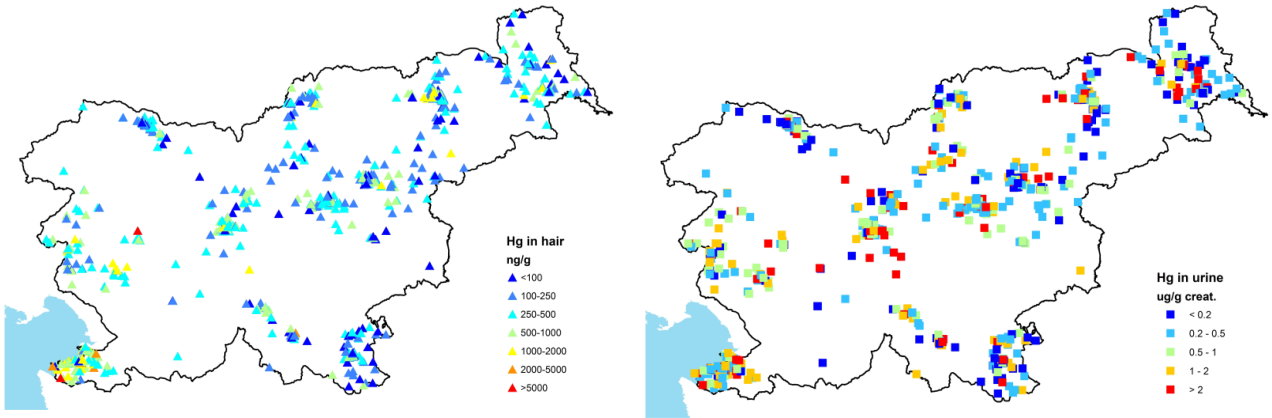

Figure 26: Mercury (Hg) levels in hair (left) and urine (right) of the Slovenian study population.

The Hg levels in blood, urine and hair were in positive correlation with the levels of Hg in soil (Figure 27), the correlation stayed significant also after adjustment for other significant co-variates. The associations observed are predominated by the area of a former mercury mine (the town of Idrija), which is clearly demonstrated from the Figure 27.

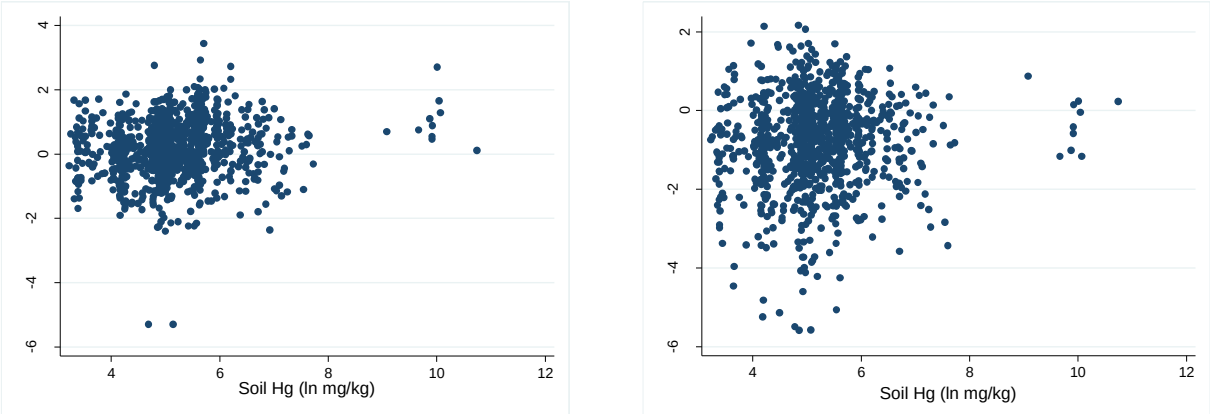

Figure 27: Correlation between Hg in soil and Hg in blood (left) or urine (right) of study population living in Slovenia (r=0.15, p<0.001, n=1042; and r=0.08, p=0.01, n=987, respectively).

In a separate study, exposure was compared between the former Hg mining town of Idrija and control group from rural areas, pregnant women and school-age children were taken into account as susceptible population groups. Although the exposure level of the two study groups living in the contaminated area was significantly higher than in the control rural populations, it is comparable to the urban population of children and is below the critical levels for biological and/or adverse health effects. However, the concentrations and distribution of Hg in the environment surrounding the town of Idrija represent a potential load that could increase exposure to Hg and pose a risk for the most susceptible population groups (Kobal et al., 2017).

Among the participants studied, 26 % were above the normal mean concentration for populations with little or no fish consumption in the US, that is 2 µg/L (NRC, 2000). 4 % of the participants exceeded the HBM I level of 5 µg/L and 4 men exceeded the HBM II value of 15 µg/L. In urine, 8 women and 2 men exceeded the HBM I value of 5.0 µg/g creatinine and none the HBM II value of 20 µg/g creatinine. In hair, 7.2 % of the participants (5 % of women and 10 % of men) exceeded the reference value of 1000 ng/g, established by (EPA, 2001) as a recommended methyl Hg concentration for pregnant women and women in a childbearing age. Most of the increased levels were due to increased frequency of fish consumption.

Arsenic levels in blood, urine and milk were predominated by consumption of seafood, fresh and canned, but not frozen. Higher levels with higher educational status were observed only in men`s blood. The levels of arsenic present in the studied biological samples are mostly the non-toxic form originating from fish consumption and do not pose any health risk for the population.

Manganese levels in blood were significantly lower in participants consuming water from private supply and in those consuming poultry more frequently, but were higher in participants consuming game more frequently. Lower values were observed also with higher BMI. In men, poultry didn`t have any significant influence on the levels in blood. The levels were lower in participants living in contaminated areas than in those living in rural or urban areas. The levels were not associated with concentration of Mn in tap water. The levels observed did not exceed the reference levels and do not pose any health risk.

Conclusions

Internal exposure to selected elements identified in the Slovenian population was a result of 1) life-style determinants, particularly, for Cd (smoking and diet) and Hg (fish consumption and amalgam fillings), and 2) of environmental exposure, which is of most concern in the area of Mežica Valley (Pb) and the former mercury mining town of Idrija (Hg). Linking HBM and environmental databases confirmed geo-dependent environmental nature of Pb and Hg exposure in the two cases, while the rural area related Cd was not associated with activities related to farming, namely artificial fertilizing. Apart of the Pb-smelter related exposure, public water supplies seem to be an important source of Pb (and also Mn). As a possible source of Pb exposure in the urban environment, emission of particulate matter from residential sources and roads was revealed.

Spain

Anthropogenic release of organochlorine compounds (OCs) and mercury into the environment has led to negative effects in human beings and ecosystems (Lamborg et al., 2014; Saeedi Saravi and Dehpour, 2016). The OCs are synthetic products widely used since the 1920s for several applications. Some organochlorine pesticides (OCPs) were extensively used in agriculture due to their insecticidal and fungicidal properties. Polychlorinated biphenyls (PCBs) were used for a large number of industrial applications, such as dielectrics in transformers, coatings, paints and insulating fluids (Wurl and Obbard, 2005).

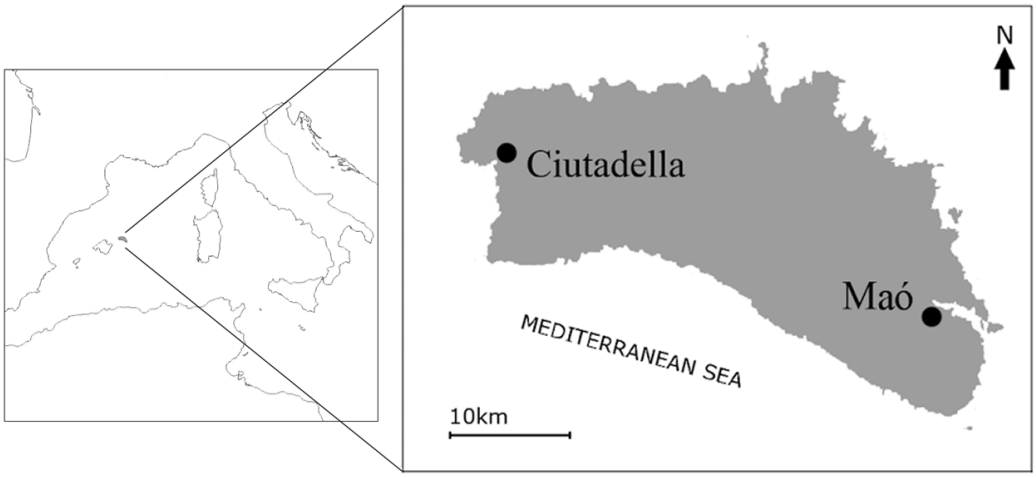

Figure 28: Map of the Menorca Island.

On the other hand, the environmental occurrence of mercury has been enhanced by mining and fossil fuel combustion (Lamborg et al., 2014). Mercury is present in many chemical forms, being methylmercury more toxic than the original metal. In the aquatic environment, mercury is readily transformed to methylmercury by anaerobic bacteria. Most of the metal incorporated into aquatic organisms is in the methylated form (80-90%; (Harris et al., 2003)).

Human exposure to OCs has been related to several health effects including cancer, reproductive defects, neurobehavioral abnormalities and endocrine and immunological toxicity (Mrema et al., 2013).

On the other hand, methylmercury is a potent neurotoxic agent that can cause severe neurological damage to humans (Grandjean et al., 1997).

Despite their different origin and structure, OCs and mercury share common properties such as (I) strong chemical stability and environmental persistence, (II) bioconcentration in living organisms and biomagnification through the food chain due to their hydrophobic character and (III) toxicity for humans and wild animals. Accordingly, the production and use of OCs and mercury has been restricted and/or banned in many countries. However, these contaminants are still found in the environmental compartments, foodstuff and human tissues (Arellano et al., 2011; Gari et al., 2013; Lamborg et al., 2014; Martí-Cid et al., 2010; Olmedo et al., 2013; Vizcaino et al., 2014) .

Dietary intake is a major way of incorporation of these compounds into humans. This is particularly important for children since their organs and metabolism are still under formation and pollutant exposure may have stronger long term effects than in adults (Guxens et al., 2012; Vizcaino et al., 2014).

Assessment of the main routes of exposure to these chemicals in infant populations is needed for implementation of adequate strategies towards minimization of pollutant intake. Previous studies have investigated the main routes of exposure to OCs (Ahmad et al., 2010; Stefanelli et al., 2004; Toms et al., 2016) or mercury (Millour et al., 2011; Olmedo et al., 2013; Rubio et al., 2008) but to the best of our knowledge none of them has performed integrated assessments on both types of pollutants in the same cohort. Concerning the OCs, the present study encompasses both PCBs and OCPs.

Table 8: Organochlorine compounds (ng/g wet weight) and mercury (mg/kg wet weight) in fish from markets of Menorca.

	Site of purchase	HCB	4,4'-DDT	4,4'-DDE	2,4'-DDE	4,4'-DDD	PCB118	PCB138	PCB153	PCB180	DDT/DDE	Hg
Detection Frequency (%)		52%	87%	100%	48%	57%	100%	100%	100%	100%		100%
Angler	Ciutadella	0.049	0.035	0.99	0.065	0.019	0.22	0.42	0.55	0.18	0.035	0.27
Angler	Maó	0.014	0.055	0.57	0.0064	0.0039	0.15	0.26	0.50	0.18	0.095	0.62
Black seabream	Ciutadella	0.014	0.031	0.39	0.010	0.0039	0.27	0.40	0.50	0.15	0.080	0.11
Black seabream	Ciutadella	0.019	0.010	0.79	0.037	0.0039	0.19	0.35	0.38	0.14	0.013	0.76
Black seabream	Maó	0.014	0.043	0.42	0.0064	0.0025	0.18	0.29	0.35	0.12	0.10	0.98
Common dentex	Maó	0.014	0.21	2.1	0.0064	0.017	0.36	0.94	1.3	0.42	0.10	2.0
Common pandora	Ciutadella	0.014	0.0079	0.15	0.047	0.011	0.18	0.24	0.25	0.076	0.054	0.16
Common seabream	Maó	0.083	0.071	0.95	0.0064	0.0025	0.27	0.50	0.77	0.22	0.075	0.69
Dusky grouper	Maó	0.014	0.039	0.54	0.0064	0.0025	0.25	0.49	0.64	0.28	0.071	2.5
European hake	Ciutadella	0.15	0.13	3.9	0.050	0.062	0.37	0.95	1.0	0.47	0.033	1.2
European hake	Maó	0.15	0.40	6.1	0.086	0.15	0.50	1.4	1.8	0.69	0.064	0.56
Forkbeard	Ciutadella	0.37	0.056	2.1	0.047	0.025	0.26	0.53	0.68	0.29	0.026	1.0
Forkbeard	Maó	0.28	0.090	1.7	0.0064	0.0025	0.32	0.64	0.87	0.37	0.052	1.4
Mediterranean moray	Maó	0.014	0.038	0.38	0.0064	0.0025	0.19	0.33	0.46	0.19	0.10	1.1
Red mullet	Ciutadella	0.014	0.010	0.26	0.040	0.0039	0.13	0.20	0.20	0.057	0.040	0.17
Red scorpionfish	Ciutadella	0.019	0.021	0.24	0.056	0.0079	0.27	0.52	0.72	0.26	0.088	1.4
Red scorpionfish	Maó	0.019	0.0079	0.57	0.0064	0.0025	0.17	0.33	0.50	0.17	0.014	0.42
Red sea bream	Ciutadella	0.019	0.033	0.36	0.045	0.0097	0.19	0.30	0.34	0.12	0.091	0.21
Small-spotted catshark	Ciutadella	0.014	0.12	2.3	0.0064	0.0025	0.47	1.6	1.3	1.1	0.050	3.8
Small-spotted catshark	Maó	0.014	0.035	0.36	0.0064	0.0025	0.19	0.36	0.50	0.16	0.095	1.1

Surmullet	Maó	0.044	0.040	0.23	0.0064	0.0025	0.16	0.23	0.26	0.073	0.17	0.39
Thornback ray	Ciutadella	0.014	0.0079	0.54	0.039	0.0039	0.20	0.33	0.37	0.17	0.015	1.7
Thornback ray	Maó	0.054	0.028	0.59	0.0064	0.0025	0.25	0.33	0.54	0.25	0.047	2.1
Mean		0.061	0.066	1.2	0.026	0.015	0.25	0.52	0.65	0.27	0.066	1.1
Min		0.014	0.0079	0.15	0.0064	0.0025	0.13	0.20	0.20	0.057	0.013	0.11
Max		0.37	0.40	6.1	0.086	0.15	0.50	1.6	1.8	1.1	0.17	3.8

Menorca island is located in the central Western Mediterranean (39 47' - 40 05'N, 3 47' - 4 19'E) and has an extension of 702 Km2. This small island represents a relatively isolated Mediterranean environment in which a large proportion of the food consumed by the inhabitants is generated locally. Furthermore, most of the fish consumption depends on the captures by local fishermen. The island has not any industry involving mercury use or production of OCs. Until the emergence of tourism, the main economic activity was agriculture. This island constitutes a representative case of a population living in a Mediterranean environment. High mercury levels are observed in the food-web of this semi-enclosed sea (Gagnon et al., 1997; Heimbürger et al., 2010; Pirrone et al., 2003) despite its anti-estuarine circulation pattern that transfers nutrients and pollutants to the Atlantic Ocean. Identification of the routes of transport of this metal and other pollutants into the population living in sites under Mediterranean influence is important. Menorca can be taken as a model ecosystem of communities living in marine Mediterranean environments in which the main routes of dietary pollutant incorporation of OC and mercury can be assessed.

Table 9: Organochlorine compounds (ng/g wet weight) and mercury (mg/kg wet weight) in seafood and meat from markets of Menorca.

	Site of purchase	HCB	4,4'-DDT	4,4'-DDE	2,4'-DDE	4,4'-DDD	PCB118	PCB138	PCB153	PCB180	DDT/DDE	Hg
Shellfish												
Detection Frequencies (%)		43%	86%	100%	57%	57%	100%	100%	100%	100%		100%
Mussel	Maó	0.014	0.15	0.33	0.060	0.029	1.1	1.2	1.8	0.17	0.44	0.068
Scampi	Ciutadella	0.019	0.033	0.71	0.062	0.0089	0.26	0.35	0.40	0.15	0.046	0.74
Scampi	Maó	0.014	0.028	0.29	0.0064	0.0025	0.16	0.22	0.21	0.060	0.097	0.77
Shrimp	Ciutadella	0.014	0.0079	0.17	0.041	0.0083	0.14	0.15	0.12	0.016	0.046	1.8
Shrimp	Maó	0.014	0.028	0.20	0.0064	0.0025	0.18	0.21	0.16	0.045	0.14	2.3
Squid	Ciutadella	0.053	0.055	0.86	0.061	0.014	0.34	0.67	0.77	0.12	0.064	0.30
Squid	Maó	0.049	0.13	0.67	0.0064	0.0025	0.29	0.63	1.0	0.31	0.19	0.36
Mean		0.025	0.062	0.47	0.035	0.0097	0.35	0.48	0.64	0.12	0.15	0.92
Min		0.014	0.0079	0.17	0.0064	0.0025	0.14	0.15	0.12	0.016	0.046	0.068
Max		0.053	0.15	0.86	0.062	0.029	1.1	1.2	1.8	0.31	0.44	2.3
Meat												
Detection Frequencies (%)		40%	60%	20%	100%	100%	100%	100%	100%	ND		40%
Beef	Ciutadella	0.014	0.021	0.045	0.025	0.0039	0.19	0.17	0.11	0.011	0.46	0.00010
Beef	Maó	0.014	0.022	0.045	0.038	0.0085	0.19	0.21	0.13	0.011	0.50	0.093
Chicken	Ciutadella	0.014	0.0079	0.045	0.034	0.0084	0.10	0.13	0.088	0.011	0.18	0.00010
Lamb	Ciutadella	0.052	0.010	0.26	0.032	0.0082	0.12	0.060	0.0871	0.011	0.039	0.00010
Lamb	Maó	0.048	0.0079	0.045	0.027	0.0092	0.14	0.14	0.11	0.011	0.18	0.11

Mean	0.028	0.014	0.089	0.032	0.0076	0.15	0.14	0.10	0.011	0.27	0.040
Min	0.014	0.0079	0.045	0.025	0.0039	0.10	0.060	0.087	0.011	0.039	0.00010
Max	0.052	0.022	0.26	0.038	0.0092	0.19	0.21	0.13	0.011	0.50	0.11

Food samples

Food samples were acquired in local markets and department stores from the main two cities: Maó and Ciutadella (Figure 28; December 2014, n = 46). The selection was based on Food Frequency Questionnaires (FFQs) from 4 year old children from Menorca cohort (see next subsection below) and interviews with local experts.

The food samples included fish species of several trophic levels and fat contents (Table 8) and shellfish (Table 9), all them captured in the island surroundings. These fish species were representative of the local consumption, encompassing angler (Lophius piscatorius), European hake (Merluccius merluccius), black seabream (Spondyliosoma cantharus), common dentex (Dentex dentex), common pandora (Pagellus erythrinus), common seabream (Pagrus pagrus), dusky grouper (Epinephelus marginatus), forkbeard (Phycis phycis), Mediterranean moray (Muraena helena), red scorpionfish (Scorpaena scrofa), small-spotted catshark (Scyliorhinus canicula), thornback ray (Raja clavata), red mullet (Mullus barbatus), red sea bream (Pagellus acarne) and surmullet (Mullus surmuletus). The selected shellfish species were mussel (Mytilus galloprovincialis), squid (Loligo vulgaris), scampi (Nephrops norvegicus) and shrimp (Aristeus antennatus).

The food samples examined also included meat, e.g. beef, chicken and lamb (Table 9), fruits and vegetables (Table 10), cheese (Table 10) and chicken eggs (Table 10), all them produced locally.

After collection, each food item was dissected in two composite samples for OC and mercury analyses. The former were wrapped in aluminium foil and the second were sealed in plastic bags. The samples were then frozen at -23ºC until further analysis in the laboratory. Only the edible parts of each food item were analysed.

Table 10: Organochlorine compounds (ng/g wet weight) and mercury (mg/kg wet weight) in fruit, vegetables, cheese and eggs from Maó (Menorca).

	HCB	4,4'-DDT	4,4'-DDE	2,4'-DDE	4,4'-DDD	PCB118	PCB138	PCB153	PCB180	DDT/DDE	Hg
Fruit											
Detection Frequencies (%)	ND	ND	ND	100%	100%	100%	100%	100%	ND		ND
Apple	0.014	0.0079	0.045	0.032	0.0039	0.12	0.13	0.041	0.011	0.18	0.00010
Apple	0.014	0.0079	0.045	0.039	0.0039	0.12	0.13	0.041	0.011	0.18	0.00010
Apple	0.014	0.0079	0.045	0.036	0.0039	0.12	0.13	0.041	0.011	0.18	0.00010
Apple	0.014	0.0079	0.045	0.041	0.0039	0.10	0.060	0.041	0.011	0.18	0.00010
Mean	0.014	0.0079	0.045	0.037	0.0039	0.12	0.11	0.041	0.011	0.18	0.00010
Min	0.014	0.0079	0.045	0.032	0.0039	0.10	0.060	0.041	0.011	0.18	0.00010
Max	0.014	0.0079	0.045	0.041	0.0039	0.12	0.13	0.041	0.011	0.18	0.00010
Vegetables											
Detection Frequencies (%)	ND	ND	ND	100%	100%	100%	100%	100%	50%		ND
Courgette	0.014	0.0079	0.045	0.065	0.0039	0.17	0.22	0.15	0.011	0.18	0.00010
Green bean	0.014	0.0079	0.045	0.052	0.0093	0.13	0.22	0.13	0.016	0.18	0.00010

Mean	0.014	0.0079	0.045	0.058	0.0066	0.15	0.22	0.14	0.014	0.18	0.00010
Min	0.014	0.0079	0.045	0.052	0.0039	0.13	0.22	0.13	0.011	0.18	0.00010
Max	0.014	0.0079	0.045	0.065	0.0093	0.17	0.22	0.15	0.016	0.18	0.00010
Cheese											
Detection Frequencies (%)	ND	100%	ND	ND	ND	67%	100%	100%	100%		100%
Semicured	0.014	0.037	0.045	0.0064	0.0025	0.12	0.30	0.24	0.094	0.82	0.239
Semicured	0.014	0.031	0.045	0.0064	0.0025	0.16	0.22	0.19	0.064	0.68	0.154
Semicured 15% fat	0.014	0.024	0.045	0.0064	0.0025	0.04	0.21	0.15	0.068	0.53	0.0966
Mean	0.014	0.030	0.045	0.0064	0.0025	0.10	0.25	0.19	0.075	0.68	0.163
Min	0.014	0.024	0.045	0.0064	0.0025	0.04	0.21	0.15	0.064	0.53	0.0966
Max	0.014	0.037	0.045	0.0064	0.0025	0.16	0.30	0.24	0.094	0.82	0.239
Eggs											
Detection Frequencies (%)	ND	100%	ND	ND	ND	50%	50%	50%	50%		ND
Chicken eggs	0.014	0.054	0.045	0.0064	0.0025	0.20	0.19	0.24	0.016	1.2	0.00010
Chicken eggs	0.014	0.031	0.045	0.0064	0.0025	0.040	0.048	0.033	0.011	0.70	0.00010
Mean	0.014	0.043	0.045	0.0064	0.0025	0.12	0.12	0.14	0.014	0.95	0.00010
Min	0.014	0.031	0.045	0.0064	0.0025	0.040	0.048	0.033	0.011	0.70	0.00010
Max	0.014	0.054	0.045	0.0064	0.0025	0.20	0.19	0.24	0.016	1.2	0.00010

Study population

The Menorca cohort is part of the INMA (Spanish Children`s Health and Environment) project, a research network that focuses on the effects of environmental contaminants during pregnancy and on fetal and child development (Guxens et al., 2012; Ribas-Fito et al., 2006).

Between 2001 and 2002, 4-year old children from this cohort provided serum samples (n=285) and hair samples (n=302) for the determination of OCs and mercury, respectively. These data have been described in Carrizo et al (2006) and Gari et al (2013), respectively. Children`s dietary intakes were assessed through FFQs (Vioque and Gonzalez, 1991; Willett et al., 1985).

All child's parents gave written permission for participation in the study, which was approved by the Ethics Committee of the Institut Municipal d`Investigació Mèdica (Barcelona).

Extraction and clean-up

The methods of extraction and clean-up of OCs in fish, meat, vegetables and fruits were based on previous analytical procedures (Berdie and Grimalt, 1998; Vives and Grimalt, 2002). Briefly, each sample (3-4 g) was homogenized with activated sodium sulphate until a fine powder was obtained and then the mixtures were introduced into previously cleaned cellulose cartridges (6 h in Soxhlet). These mixtures were Soxhlet-extracted with 100 mL of n-hexane-dichloromethane (4:1 v/v) for 6 h. At this step, TBB and PCB 209 were added as recovery standards (50 ng/mL). The extract was concentrated with a rotary evaporator to 2 mL. Three mL of sulphuric acid were then added. After vigorous stirring in a Vortex-mixer (2 min), the mixture was centrifuged (4000 rpm, 5 min) to remove any foam in the interface and the sulphuric acid layer was discarded. This clean-up step was repeated until a colorless transparent acid layer was obtained (3-5 times). The solvent layer was introduced into a chromatographic column packed with 1 gr of sodium sulphate and silica gel (1:1 by weight). The extract was then evaporated to dryness under a gentle stream of nitrogen (10-20 C) and transferred to vials

using 200 μl of isooctane. Before instrumental analysis, the sample was evaporated to nearly dryness under a gentle nitrogen flow and a solution of PCB 142 was added as internal standard (10 ng/mL).

Cheese and eggs samples were extracted following previous analytical methods, with some minor modifications (Kalantzi et al., 2001). Briefly, each sample (1-2 g) was homogenized with activated sodium sulphate (5 g) and then Soxhlet extracted with 50 mL of n-hexane. After cooling, the extract was spiked with TBB and PCB 209 as recovery standards (50 ng/mL) and concentrated with a rotary evaporator to 5 mL. The sample was then introduced into a 1.9 cm i.d. column containing 15 g of acidified silica gel (2:1 silica gel:acid by weight) and eluted with 60 mL of n-hexane. The extract was then evaporated to 1 mL under a gentle stream of nitrogen and introduced into a 0.6 cm i.d. column containing 3 g of activated silica. The sample was eluted with 33 mL of hexane (for PCBs) and 15 mL of hexane-dichloromethane (1:1 v/v; for OC pesticides). Before instrumental analysis, the sample was evaporated to nearly dryness under nitrogen and a solution of PCB 142 was added as internal standard (10 ng/mL).

For mercury determination each sample (5 g) was freeze-dried for 12 h and then introduced (0.1 g) into a digestion Teflon vessel, together with 3 mL of nitric acid and 1 mL of hydrogen peroxide. The mixture was heated in an oven (90 C) overnight. After cooling, the digested sample was dissolved with 40 mL of MilliQ water. Finally, samples were placed in 7 mL glass bottles and stored in a refrigerator until instrumental analysis.

Determination of persistent pollutants and quality assurance

The OCs were quantified using a gas chromatograph with electron capture detection (GC-ECD, Agilent Technologies 7890A, Palo Alto, California, USA) equipped with a HP-5MS capillary column of 60 m length, 0.25 mm internal diameter and 0.25 μm film thickness (J&W Scientific, Folsom, CA, USA), protected with a retention gap. The oven temperature was programmed from 90 C (holding time 2 min) to 130 C at 15 C/min and finally to 290 C at 4 C/min, keeping the final temperature for 15 min. Injector and detector temperatures were 250 C and 320 C, respectively. Injection (2 μl) was performed in the splitless mode, keeping the split valve closed for 30 s. Helium was the carrier gas (1.5 mL/min) and nitrogen was used as make-up gas (60 mL/min).

Structural confirmations were performed by GC (Agilent Technologies 7890A, Agilent Palo Alto, USA) coupled to mass spectrometry (MS, Agilent Technologies 5975C, Agilent Palo Alto, USA) operating in negative chemical ionization mode (GC-NICI-MS). The system was equipped with a HP-5MS capillary column of 60 m length, 0.25 mm internal diameter and 0.25 μm film thickness (J&W Scientific, Folsom, CA, USA), protected with a retention gap. Helium was used as carrier gas (1.2 mL/min). Ammonia was the reagent gas (2.5 mL/min). The oven temperature program started at 90 C which was held for 2 min, followed by a first increase to 130 C at 15 C/min and a final ramp to 310 C at 4 C/min with a final holding time of 10 min. Injector, transfer line and ion source temperatures were 280 C, 280 C and 176 C, respectively. The dwell time was 100 ms/channel.

One procedural blank was included in each batch of samples. Mean recoveries of spiked standards in the samples were 55% and 75% for TBB and PCB 209, respectively. Detection and quantification limits were determined as the average signal obtained from the blanks plus three and five times the standard deviation, respectively. Detection limits ranged between 0.0050 and 0.095 ng/g wet weight.

Total mercury was performed using inductively coupled plasma mass spectrometry (ICP-MS, Elan 6000) operating under standard conditions and using rhodium as internal standard. One procedural blank was included in each sample batch. The detection limit was 0.00010 mg/kg wet weight. Analyses of fish muscle certified reference material, ERM-BB422, ensured that the instrument remained calibrated along the study.

Dietary exposure estimates

The estimation of the daily intake of pollutants through the consumption of selected food items was calculated by multiplying the mean pollutant concentration in the item (expressed in ng/g fresh product) by the average daily consumption rate of that item (expressed in grams/day). Then, total dietary intake was obtained by summing these products for each contaminant.

The daily consumption rates of the 4-year old children living in Menorca were obtained by combining the information from the FFQs (expressed in times/week) and the servings of each food item at the age of 4 years as defined by (AESAN) (expressed in grams; vegetables = 130 g, meat = 53 g, shellfish = 73 g, fish = 73 g, eggs = 50 g, cheese = 27 g and fruit = 87 g). The FFQs from INMA-Menorca were based on a representative sample encompassing 302 children (average body weight = 18.5 kg).

Statistical analysis

The statistical analyses were performed using R (R Core Team, 2015) software and Microsoft Excel (2010). The concentrations of OCs and mercury were expressed in ng/g wet weight (ww) and mg/kg wet weight, respectively. When OCs and Mercury concentrations were under the limit of detection, the values were assumed to be one-half of the detection limits (ND= LOD). Either ww or dry weight (dw) were used for comparison with other studies.

Total HCHs were defined from the sum of α-, β- and γ-HCHs. Total DDT concentrations were the sum of 4,4`-DDT, 2,4`-DDT, 4,4`-DDE, 2,4`-DDE and 4,4`-DDD. Total PCB concentrations were the sum of 28, 52, 118, 138, 153 and 180. HCB and mercury were reported as individual compounds.

Serum OCs and hair mercury concentrations (Carrizo et al., 2006; Gari et al., 2013) were not normally distributed and were transformed into natural logarithms. Multivariate linear regression analyses were used to assess the association of children`s dietary intakes and other covariates (sex, place of birth, breastfeeding, number of siblings, maternal age, and maternal and paternal occupation and educational level), with each compound concentration at 4-year old children. The compound concentrations and food item variables included in the models were standardized.

Food pollution data

The descriptive data of the OC and mercury concentrations in the analysed foodstuffs are shown in Table 8 - Table 10. Non-detected compounds (e.g. PeCB, β- and γ-HCH) or compounds presenting low detection frequencies (e.g. α-HCH, δ-HCH, 2,4'-DDT, PCB28 and PCB52) were not reported. The relative distributions of the main pollutant groups (HCB, ∑HCHs, ∑DDTs, ∑PCBs and Mercury) to the total sum of persistent pollutants (∑P) are shown in Figure 29.

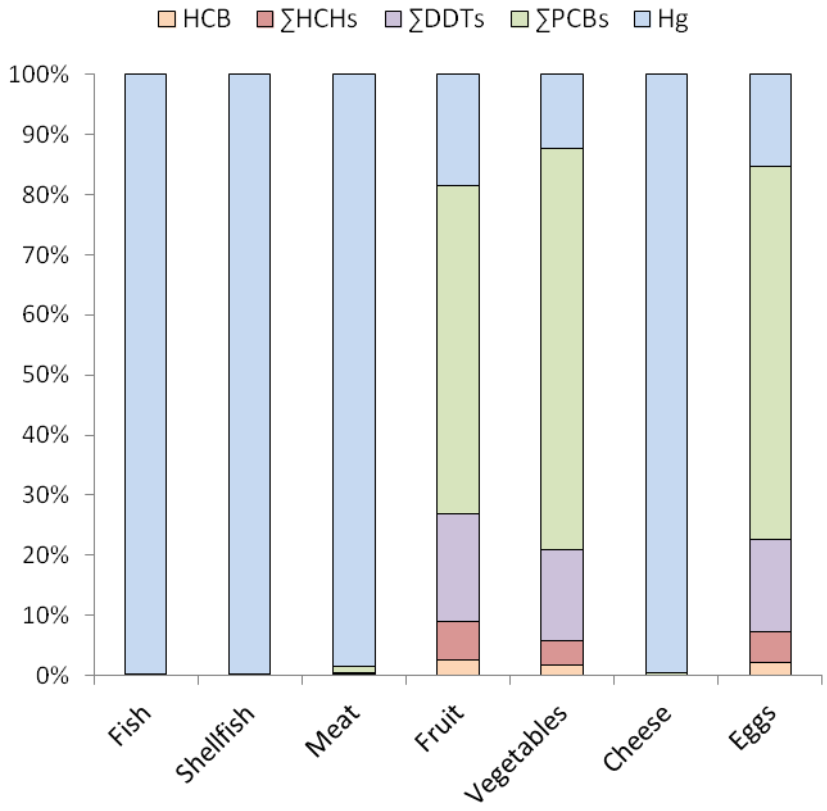

Figure 29: Relative contributions of HCB, ∑HCHs, ∑DDTs, ∑PCBs and Hg to ∑PP in the food items from Menorca.

Fish and shellfish

All fish and seafood samples showed detectable levels of 4,4'-DDE and PCB congeners 118, 138, 153 and 180 (Table 8 - Table 9). 4,4`-DDT and 4,4`-DDD were found in 87% and 57% of the analysed samples, respectively. The other major compounds, e.g. HCB and 2,4`-DDE, were found in 50% of the samples (Table 8 - Table 9). The patterns of DDTs and PCBs in fish and seafood were dominated by 4,4'-DDE and PCB153. The highest OC concentration in fish was found in European hake (6.1 ng/g ww of 4,4`-DDE). The highest concentration in shellfish was observed in mussel (1.8 ng/g ww of PCB153). These two samples were collected in Maó (Table 8 - Table 9).

All fish and shellfish samples showed detectable mercury levels, ranging between 0.068 and 3.8 mg/kg ww (arithmetic means of 1.1 mg/kg ww and 0.92 mg/kg ww for fish and shellfish, respectively; Table 8 - Table 9).

Small-spotted catshark caught in Ciutadella was the species showing highest concentrations within the seafood group (3.8 mg/kg ww; Table 8). As shown in Figure 29 mercury was the major persistent pollutant in fish and shellfish, contributing 99% to the total load.

Meat

All samples showed detectable levels of 2,4'-DDE, 4,4'-DDD, PCB118, PCB138 and PCB153 (Table 9). The other compounds, e.g. HCB, 4,4`-DDT and 4,4`-DDE, were found in less than 60% of the samples and PCB180 was not found in any. The DDT and PCB patterns were mostly dominated by 4,4'-DDE and PCB118, respectively. The highest concentrations were found in lamb bought in Ciutadella (0.26 ng/g ww of 4,4`-DDE; Table 9).

Mercury was also the major persistent pollutant in the meat foodstuff group, contributing to 98 % of ∑P (Figure 29). However, it was only found in beef and lamb samples from Maó (Table 9).

Fruit and vegetables

All fruits and vegetables showed detectable levels of 2,4'-DDE, 4,4'-DDD, PCB118, PCB138 and PCB153 (Table 10). PCB180 was found in half of the analysed vegetable samples but not in any of the fruits. HCB, 4,4'-DDT and 4,4'-DDE were not found in any of the fruit or vegetable samples. The DDT and PCB patterns were dominated by 2,4'-DDE and PCB138 in most of the samples from both groups. The highest OC concentrations found in fruit and vegetables corresponded to PCB138, 0.13 and 0.22 ng/g ww, respectively. Mercury was not found above detection limit in any sample (Table 10).

The PCBs were the major persistent pollutants in both groups of fruit and vegetables followed by DDTs (Figure 29). In the fruit group, PCBs and DDTs constituted 55% and 18% of the ∑P, respectively. In the vegetables, these pollutants represented 67% and 15% of ∑P, respectively.

Cheese

All samples contained 4,4`-DDT, PCB138, PCB153 and PCB180 above limit of detection (Table 10). PCB118 was found in 67% of the samples. HCB, 4,4`-DDE, 2,4`-DDE and 4,4`-DDD were below limit of detection in all cases. The DDT and PCB patterns were dominated by PCB138 and 4,4`-DDT, respectively. The highest concentration was found in cheese from Maó (0.3 ng/g ww of PCB138).

Mercury was found above limit of detection in all samples, ranging between 0.097 and 0.24 mg/kg ww (arithmetic mean 0.16 mg/kg ww). The semicured cheese from Maó showed the highest level (0.24 mg/kg ww; Table 10). This metal was the major persistent pollutant in the cheese group, with a similar relative composition as observed in fish and shellfish (Figure 29).

Eggs

4,4`-DDT was found in all analysed egg samples (Table 10). PCB118, PCB138, PCB153 and PCB180 were found in 50% of the samples. The PCB and DDT patterns were dominated by PCB153 and 4,4'-DDT, respectively. The highest OC concentrations were found in chicken eggs from Maó (0.24 ng/g ww of PCB153). PCBs and DDTs were the major persistent pollutant in this group (Figure 29), contributing to 62% and 15% to ∑P, respectively. No mercury was found above limit of detection (Table 10).

Occurrence of organochlorine compounds in food items

The concentrations of OCs were considerably higher in fish and shellfish than in the other food groups (Table 8 - Table 10.) which is consistent with studies from other European countries, e.g. Sweden (Törnkvist et al., 2011). Fruits and vegetables showed the lowest concentrations of these pollutants.

The PCB congeners found in highest concentrations in all samples were those with highest molecular weights, PCB118, PCB138, PCB153 and PCB180, which involves those with highest hydrophobic properties (octanol-water coefficients, logKow > 6.9). The predominance of these compounds is consistent with their high hydrophobicity and bioaccumulation potential. 4,4`-DDE was the compound of the DDT group showing highest concentrations. The DDT/DDE ratios were low in all food samples, ranging between 0.013 and 1.2, which is indicative of old DDT exposure.

In general terms, the observed concentrations of HCB, total HCHs and total DDTs in meat, fruits, vegetables, eggs, cheese and dairy products from the island were low in comparison to those found in previous studies from other European regions, e.g. Croatia (Kljakovic-Gaspic et al., 2015), Austria

(Mihats et al., 2015), Sweden (Törnkvist et al., 2011), Catalonia (Falco et al., 2004; Llobet et al., 2003; Martí-Cid et al., 2010) and Russia ((Polder et al., 2010); Table 11). The concentrations of PCBs in the food items from the island were in the range of those described elsewhere although the number of cases for comparison was small (Table 11).

Table 11: Arithmetic mean concentrations of OCs in food items of Menorca and in other European sites (ng/g ww).

	Fish and seafood	Meat	Fruit	Vegetables	Cheese and dairy products	Eggs	Location	Reference
HCB	0.053	0.028	0.014	0.014	0.014	0.014		*Present study*
	0.33	–	–	–	–	–	Canary Islands	(Rodriguez-Hernandez et al., 2016)
	0.021	–	–	–	–	–	Croatia	(Kljakovic-Gaspic et al., 2015)
	0.45	0.090	–	–	0.070	0.040	Sweden	(Törnkvist et al., 2011)
	0.22	–	0.005	0.14	–	–	Catalonia	(Martí-Cid et al., 2010)
	0.56	0.82	–	0.020	0.603	0.38	Russia	(Polder et al., 2010)
	0.28	0.17	0.00093	0.0056	1.7	0.18	Catalonia	(Falcó et al., 2004)
ΣHCHs	0.038	0.033	0.035	0.033	0.033	0.033		*Present study*
	0.13	–	–	–	–	–	Canary Islands	(Rodriguez-Hernandez et al., 2016)
	0.32	–	–	–	–	–	Croatia	(Kljakovic-Gaspic et al., 2015)
	0.23	0.040	–	–	0.025	0.015	Sweden	(Törnkvist et al., 2011)
	0.20	–	0.004	0.16	–	–	Catalonia	(Martí-Cid et al., 2010)
	0.28	1.1	–	0.070	1.3	0.90	Russia	(Polder et al., 2010)
ΣDDTs	1.1	0.15	0.098	0.12	0.088	0.10		*Present study*
	0.77	–	–	–	–	–	Canary Islands	(Rodriguez-Hernandez et al., 2016)
	2.8	–	–	–	–	–	Croatia	(Kljakovic-Gaspic et al., 2015)
	3.3	0.24	–	–	0.16	0.10	Sweden	(Törnkvist et al., 2011)
	7.5	–	0.069	2.8	–	–	Catalonia	(Martí-Cid et al., 2010)
	6.4	11	–	0.11	0.81	9.7	Russia	(Polder et al., 2010)
ΣPCBs	1.7	0.43	0.30	0.54	0.63	0.40		*Present study*
	1.3	–	–	–	–	–	Canary Islands	(Rodriguez-Hernandez et al., 2016)
	4.3	–	–	–	–	–	Austria	(Mihats et al., 2015)
	11	–	–	–	–	–	Croatia	(Kljakovic-Gaspic et al., 2015)
	5.1	0.33	–	–	0.10	0.15	Sweden	(Törnkvist et al., 2011)
	11	–	0.036	0.21	–		Catalonia	(Martí-Cid et al., 2010)
	7.4	2.9	–	0.10	2.1	5.2	Russia	(Polder et al., 2010)
	12	0.37	0.0045	0.021	0.67	0.47	Catalonia	(Llobet et al., 2003)

Concerning fish and seafood, the concentrations of HCB, total HCHs, DDTs and PCBs in the specimens captured nearby the island were small in comparison to the fish specimens consumed in these other European countries (Table 11). With a few exceptions, HCB from Sweden (Törnkvist et al., 2011) and DDTs and PCBs from the Canary Islands (Rodriguez-Hernandez et al., 2016), the average concentrations of fish and seafood from Menorca were the lowest observed (Table 11). The differences were particularly significant for the DDT and the PCB groups since the average concentrations found in the fish/seafood group of Menorca were about 6-7 times lower than the average concentrations reported in Catalonia (Llobet et al., 2003; Martí-Cid et al., 2010) or Russia (Polder et al., 2010) (Table 11). In

general, decreasing concentrations in OCs have been observed (Aguilar and Borrell, 2005; Krauthacker et al., 2009), probably as the result from the application of the Stockholm Convention.

The highest OCs concentrations were observed in the European hake from Maó (ΣOCs=11 ng/g ww; Table 8). This is a carnivore species that feeds on small fish and certain cephalopods, thus occupying a high level in the food chain. As mentioned above, most OCs are hydrophobic and have high logKow coefficients which increases their affinity for specimens with high fat content and for those located at high position in the food web. Accordingly, the lower OC levels in shellfish than fish (Table 8 - Table 9) can be related to their lower fat content.

PCBs were the OCs found in highest concentrations in the meat samples, 0.43 ng/g ww (Table 11), followed by DDTs (0.15 ng/g ww), HCB (0.028 ng/g ww) and HCHs (<0.033 ng/g ww). The beef and lamb showed higher levels than chicken, probably due to the mammalian transference of these compounds through placenta and by breast feeding (Table 9; (Shen et al., 2007)).

Semicured cheese samples containing 15% fat showed lower OCs levels than semicured cheese with higher fat content (Table 10). The concentrations of these compounds in cheese and eggs from Menorca were lower than those found in Russia (Polder et al., 2010) and similar to those found in Catalonia ((Llobet et al., 2003); Table 11).

Finally, the fruit and vegetables analyzed in the present study showed the lowest OC concentrations which is consistent with the observed accumulation of these compounds in Catalonia (Falco et al., 2004; Martí-Cid et al., 2010) and Russia ((Polder et al., 2010); Table 9). This low accumulation pattern was not unexpected as these food items are in a low position of the food chain and their fat content is also low.

Occurrence of mercury in food items

Mercury was present in all fish, shellfish and cheese samples and in 40 % of the meat samples. In contrast, it was not found in fruit, vegetables and eggs items (Table 8 - Table 10). The highest mercury concentrations were found in seafood, ranging between 0.068 and 3.8 mg/kg ww. Sixty-six percent of the analysed species had mercury concentrations above the maximum level set forth by the European Union Maximum Residue Limits (MRL) for human consumption, 0.5-1.0 mg/kg ww depending on the species (EC, 2006).

The mercury concentrations observed in the present study were consistent with previous results reported in the same organisms from the Adriatic Sea (Storelli and Barone, 2013; Storelli et al., 2003), Farwa Island (Lybian coast; (Banana et al., 2016)), the Gulf of Lion (Cresson et al., 2014; Torres et al., 2015), the Aegean Sea (Yabanli and Alparslan, 2015) and deep-sea sites from the Mediterranean basin (Koenig et al., 2013; Naccari and Cicero, 2015).

A considerable variation was found in mercury concentrations among species. Biotic and abiotic factors may affect the accumulation of mercury in marine organisms. However, diet and position in the trophic web are determinant (Storelli et al., 2002).

The highest concentration was measured in small-spotted catshark collected in Ciutadella (average 3.8 mg/kg ww; maximum 14 mg/kg dw). In a recent study in fish from the Gulf of Lions (Northern Mediterranean), the same species recorded maximum average values, with an arithmetic mean of 7.1 mg/kg dw, and maximum concentration of 27 mg/kg dw (Cresson et al., 2014). In another study in the

Mediterranean basin lower concentrations were observed (arithmetic mean 1.5 mg/kg ww, range 0.79-2.6 mg/kg ww; (Storelli et al., 2002)).

Small-spotted catshark is a predator with a diet mainly based on consumption of osteichthyes and crustaceans, cephalopods and gastropods. Its trophic level is high (3.93) since it is a top carnivorous predator (Mnasri et al., 2012). The observed high mercury concentrations are consistent with magnification processes due to their high trophic position and feeding behavior.

Concerning shellfish, the highest concentrations were recorded in shrimp from Maó (average 2.3 mg/kg ww). These results were in concordance with a previous study in the Mediterranean basin, with arithmetic mean and the maximum levels of 1.0 and 2.2 mg/kg ww, respectively (Koenig et al., 2013).

Semi-cured cheese was the second food group with highest average mercury concentrations (0.16 mg/kg ww) followed by meat (0.040 mg/kg ww; Table 8). Cheese with low fat content (15%) was the food item with lowest mercury concentration (0.097 mg/kg ww), which parallels the observations on OC concentrations. The mercury concentrations in these food items were higher than those found in other studies in the Canary Islands (Rubio et al., 2008) and Catalonia (Llobet et al., 2003).

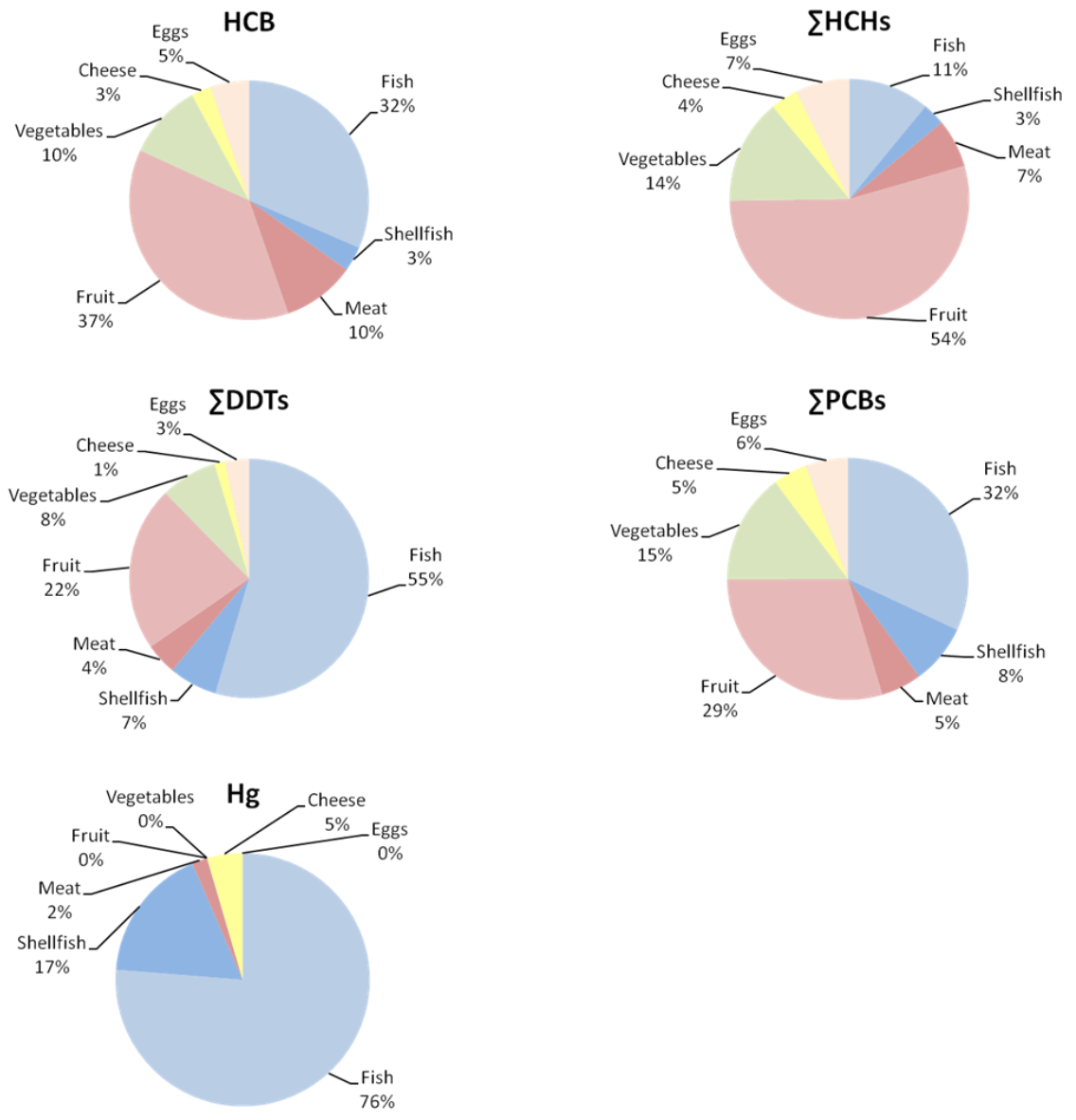

Figure 30: Contribution from each food group to the total dietary intake of HCB, ∑HCHs, ∑DDTs, ∑PCBs and Hg in the children living in Menorca.

Dietary intake of organochlorine compounds and mercury

The contributions of each food group to the total dietary intake of OCs and mercury in the cohort of four-year old children from Menorca are shown in Figure 30. The most important OC sources were consumption of fish (37%) and fruit (29%). However, it is important to note the significant differences among consumption rates of these food items: 107 g/day in fruit and 20.2 g/day in fish (Table 12) involving 47 ng/d and 64 ng/d, respectively. The high significance of the fish contribution to the total dietary OC intake has also been observed in previous studies from other European populations (Llobet et al., 2003; Mihats et al., 2015; Törnkvist et al., 2011). Vegetables and shellfish were also identified as important contributors to the OC estimated daily intakes (EDIs; 13% and 7%, respectively).

Further understanding on the significance of each food item for the OC intake can be obtained by consideration of the Tolerable Daily Intakes (TDIs; (JMPR, 2000)). According to the results of the present study, the EDIs of OCs of the 4 year-old children from Menorca are much lower than the established TDI limits (TDI for HCB=160 ng/(kg bw·d), for ΣHCHs=5000 ng/(kg bw·d), for ΣDDTs=10,000 ng/(kg bw·d), for ΣPCBs=10 ng/(kg bw·d);(JMPR, 2000; Mihats et al., 2015; Rodriguez-Hernandez et al., 2016).

However, in comparison with previous studies, the PCBs EDIS of 4-year old children from Menorca was higher than children ingestion (6-15 years old) from Austria (ΣPCBs=3.37 ng kg-1bw d-1; Mihats et al., 2015). Focusing only on fish products, a recent study in children (average body weight=34.32 kg) from the Canary Islands found higher levels than those from the present study (Rodriguez-Hernandez et al., 2016).

Fish and shellfish were the main contributors to the total mercury intake (76% and 17% of contribution, respectively). Because of its toxicity, the EFSA`s CONTAM Panel established a Provisional Tolerable Weekly Intake (PTWI) of 4 μg/kg for this metal (EFSA, 2012). According to the present results, children from Menorca Island showed an estimated weekly intake (EWI) of 11 μg/kg, which exceeded more than twice the PTWI limits. The worst case scenario of a study in baby foods from the European Union market encompassed an EWI of 18.2 μg/kg in infants (7-8 months old) whose diet consisted on infant formulae of milk-based and fish-based infant solid foods (Pandelova et al., 2012). A study in women (25-44 years old) from Spain has shown that the mercury EWI largely exceeds the PTWI for methylmercury as consequence of consumption of sword-fish and louvar (Herreros et al., 2008).

The specific fish and seafood EDI of Menorca (Mediterranean Sea) can be compared with one from the Canary Islands (Atlantic Ocean) based on the results reported in Rodriguez-Hernandez et al. (Rodriguez-Hernandez et al., 2016) from children having average body weight of 34.32 kg. The EDI identified in this case, 150 ng/(kg bw•d), is nearly ten times lower than 1500 ng/(kg bw·d) that was observed in the four-year old children from Menorca (Table 12).

Table 12: Mean values of the daily intake of each food group by 4-year old children population from Menorca (ng contaminant/(infant·day (± standard deviation)) (average body weight = 18.5 kg).

Consumption rate	Fish (20.2 g/day)	Shellfish (5.30 g/day)	Meat (13.7 g/day)	Fruit (107 g/day)	Vegetables (30 g/day)	Cheese (7.82 g/day)	Eggs (15.1 g/day)	EWI[a] (μg/(kg bw·week)	EDI[b] (ng/(kg bw·d)
HCB	1.2 ± 1.9	0.13 ± 0.095	0.39 ± 0.27	1.5 ± 0.0	0.40 ± 0.0	0.11 ± 0.0	0.20 ± 0.0	0.0015	0.21
ΣHCHs	0.75 ± 0.26	0.21 ± 0.088	0.45 ± 0.00	3.7 ± 0.36	0.97 ± 0.0	0.26 ± 0.0	0.50 ± 0.0	0.0026	0.37
ΣDDTs	26 ± 32	3.1 ± 1.7	2.0 ± 1.3	10 ± 0.41	3.6 ± 0.18	0.69 ± 0.050	1.5 ± 0.24	0.018	2.5
ΣPCBs	34 ± 21	8.7 ± 7.2	5.9 ± 1.5	32 ± 4. 8	16 ± 0.85	5.0 ± 1.1	6.1 ± 5.5	0.041	5.8
Hg	22000 ± 18000	4900 ±4500	550 ± 750	11 ± 0.0	3.0 ± 0.0	1300 ± 560	1.5 ± 0.0	11	1500

[a]EWI: Estimated weekly intake (EFSA, 2012). [b]EDI: Estimated daily intakes (JMPR, 2000).

Associations between pollutant concentrations in children and dietary intakes

The present dietary food analyses can be related to the hair mercury measurements in four-year old children from Menorca (Gari et al., 2013). The infant mercury concentrations show a positive and significant association with the fish and shellfish intake (Figure 31). This result is consistent with those obtained in a previous study in which the contribution of each food source to mercury intake was

estimated from FFQs (Gari et al., 2013). The high mercury concentrations found in the analysed marine organisms (Table 8) and the dietary intake estimations show that 93% of the total dietary mercury intake originates from seafood consumption (Figure 30).

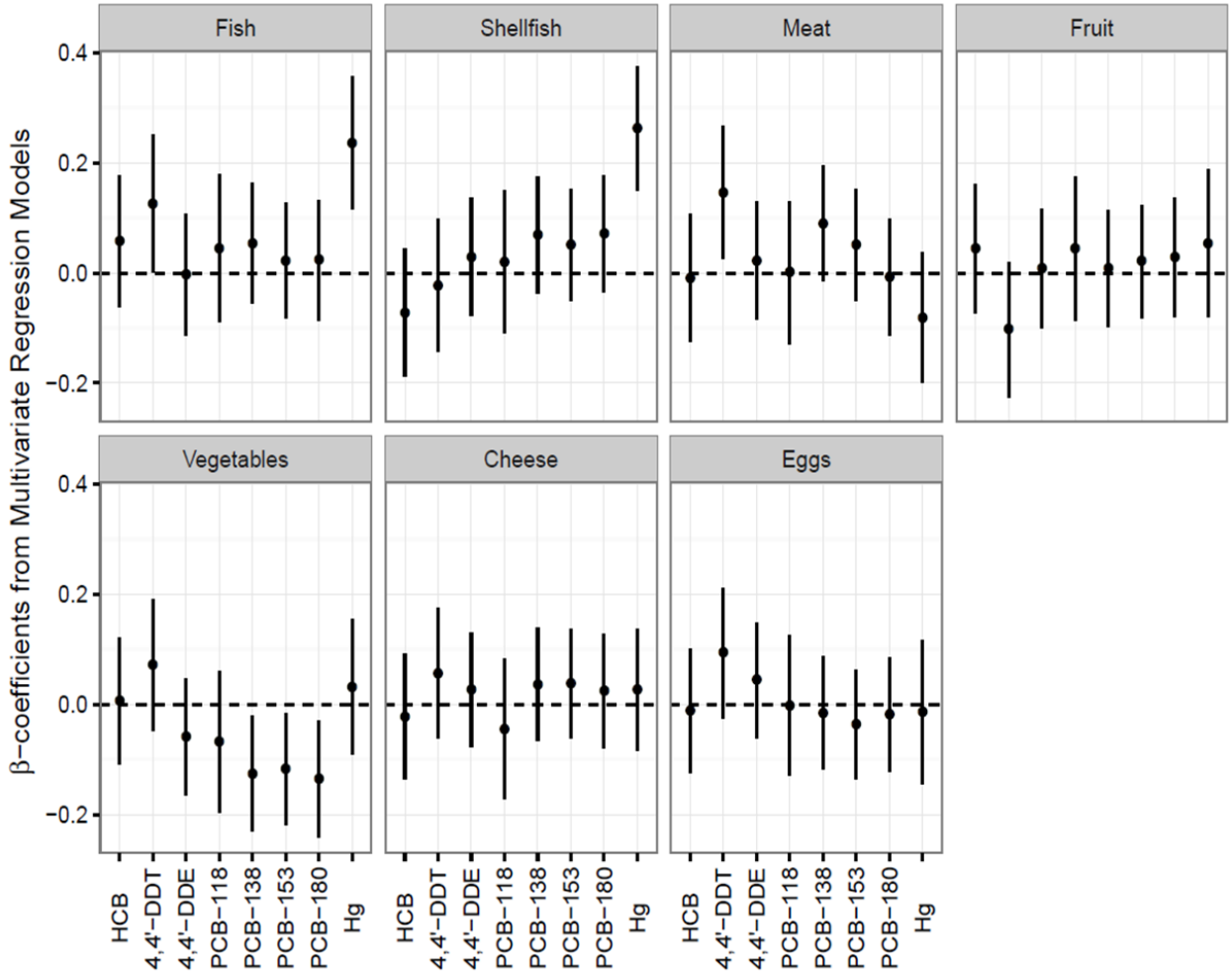

Figure 31: Associations between dietary intakes of selected items and serum OC and hair Hg concentrations of four year-old children. Bars represent the 95% confidence intervals of each beta-coefficient. The models were adjusted for child`s sex, place of birth, number of siblings, breastfeeding, maternal age, maternal and paternal occupation and educational levels.

There is also a significant dependence between the intake of 4,4`-DDT and fish and meat consumption (Figure 31). Meat intakes were also found to be associated with DDT concentrations in another Mediterranean area (Llop et al., 2010). In the analysed food items of Menorca, 87% of fish samples and 60% of meat samples presented detectable values for 4,4`-DDT. Moreover, egg intake is also related to high 4,4`-DDT levels, although without statistical significance (Figure 31). All analysed chicken eggs samples showed detectable 4,4`-DDT levels.

The significant negative relationship between higher consumption of vegetables and concentrations of PCB138, PCB153 and PCB180 in children (Figure 31) suggests that high intake of this food item could have a protective effect against PCB accumulation. However, the PCB concentrations in the vegetable samples were above the limit of detection.

Fruit consumption could also have a protective factor for the accumulation of 4,4`-DDT (Figure 31). In this respect, 4,4`-DDT was not found in any of the fruit samples analyzed. However, the results are not statistically significant.

Conclusions

In general terms, the concentrations of HCB, HCHs and DDTs in the food items produced in Menorca are low in comparison with other studies from other European regions. The concentrations of PCBs are in the range of those found in other European sites. However, the fish specimens from Menorca showed much lower PCB concentrations.

In contrast, mercury was present in all fish, shellfish and cheese samples and in 40 % of the meat samples of Menorca. However, it was not found in fruit, vegetables and eggs. Sixty-six percent of the analysed fish species had mercury concentrations above the maximum level set forth by the European Union MRL for human consumptin.

The highest OC and mercury concentrations were observed in fish, presenting the maximum values in European hake and small-spotted catshark, respectively. These two species occupy high levels in the food chain where magnification processes are strongest for hydrophobic compounds with high bioaccumulation potential.

The most important sources for the intake of OCs in four-year old children from Menorca were consumption of fish (37%) and fruit (29%). However, the EDIs of these pollutants were low in comparison to the FAO/WHO TDIs. Fish (76%) and shellfish (17%) consumption were the main mercury sources. The EWI of this metal in children from Menorca exceeded more than twice the EFSA PTWI.

A statistically significant and positive association was found between fish and shellfish consumption and hair mercury concentrations in 4 year-old children. Ninety-three percent of all dietary mercury intake originated from consumption of these food items. 4,4-DDT intake was also significantly directly associated to consumption of fish and meat. Mercury and 4,4`-DDT are the only pollutants whose concentrations in children from the cohort were significantly associated to dietary inputs. Consumption of vegetables was protective against PCB accumulation.

Proposals for countermeasures for exposure mitigation

Greece

Based on the results of the Greek cases studies and on the identification of potential sources of exposure the following recommendations for exposure mitigation can be proposed to local and national stakeholders and to the general population.

- To implement national HBM framework for the general population with specific attention to people living in most polluted areas and addressing vulnerable groups such as pregnant women, infants, children and adolescents to support the identification of hot spot areas where policy maker and health authorities need to focus.

- To avoid operation of potential dangerous industries and facilities (e.g. plastics recycling plant) close to populated areas.
- To limit the amount of material to be recycled according to the legislation.
- To increase the preparedness capacity for response to acute pollution episodes.
- To prohibit irrigation and drinking water consumption from underground wells especially in pollutes areas.
- To impose more strict regulations about effluents disposal.
- To promote the consumption of bottled water in areas for which there is evidence of potential contamination of surface and /or ground water.
- To provide financial incentives for use of natural gas for central heating and to lower taxation of oil for space heating.
- To support the adoption of newer and healthier technology such as newer pellet boilers.
- To provide financial incentives for improving thermal behavior of households.

Italy

On the basis of the results of the CROME national study and the Italian contribution to the Cross Mediterranean study some countermeasures have been identified in order to mitigate the exposure of infants, children and adolescents to metals in two areas of Italy where exposure to heavy metals might represent a risk factor. The main recommendations may be summarized as follows:

- To develop and perform a new programme of human biomonitoring of the general population with specific attention to vulnerable groups such as pregnant women, infants, children and adolescents aiming at updating reference values (RVs) for assessing time trends and to identify potential higher exposures or contaminations where policy makers and health authorities need to focus. Biomonitoring should be paralleled by evaluation of health outcomes related to growth, obesity, metabolic disorders, asthma / allergies and neurodevelopmental / neuropsychiatric disorders
- To develop and perform human biomonitoring studies addressing not only individual metals but focusing on the co-exposure to different metals; co-exposure to metals can be of more relevance in terms of association with the health status of children/adolescents;
- To empower the monitoring system of air pollution in the Lazio region and in Trieste municipality with the aim to continuously monitor air quality markers such as PM_X, NO_2, etc. but also to measure the concentration of metals directly in air, with specific attention to the areas close to industrial plants;
- To evaluate the indoor exposure of children and adolescents to metals by collecting indoor air samples, for example at selected schools in the Lazio region and in Trieste community;
- To monitor tap water quality taking samples directly from water pipes at households, in particular with regard to As concentration; perform this study in the Lazio region where As background may be high;
- To measure concentration of metals in soil, with specific attention to gardens and playgrounds where children and adolescents spend most of their time;

- To assess metals concentration in fish (for Hg), in rice (for As) and in other foods than can be indicative of local contamination to metals;
- To perform a communication strategy to reduce or avoid some lifestyles/behaviors that can be associated to the health status of children, e.g. passive smoking of the mothers and alcohol consumption and to promote the consumption of protective food especially in pregnant and lactating women;
- To set in place a system to control heavy metals' concentration in different food items collected in the local markets in municipality/areas/regions.

Slovenia

General recommendations, which should be proposed to stakeholders on a country level

Based on the HBM data and identification of potential sources of exposure the following recommendations are being proposed to stakeholders (some of them are already being taken into account in specific areas - e.g. Mežica valley):

- Dietary guidance about frequency of fish consumption and some other food items like game, offal, mushrooms.
- Consumption of protective food should be promoted.
- Behavioral recommendations on the use of general consumer products specially in the case of damaged or broken items.
- Proper disposal of waste especially if potentially containing toxic metals.
- Recommendation on land use on contaminated sites and caution when eating vegetables from contaminated land.
- Recommendations on hygiene practices i.e. regular removal of dust, washing hands before eating
- Avoid hazardous landfill.
- Use of protective clothing when renovating buildings and during farming/gardening activities
- Because omission of gardening on contaminated areas from the perspective tradition is often not possible, special attention should be paid to the education of exposed population. Workshops about the risk and also on guidelines for safer farming-gardening should be organized
- Education of general population with special attention to potentially exposed groups
- Communication material with recommendations prepared in simple language understandable to all
- Continuous biomonitoring on contaminated areas: e.g. monitoring of particulate matter in the environment should be continuously going on. Level of dust should be reduced at public and home environment. At the public level resurfacing of roads is important. Wet cleaning of public and home surfaces should be performed as often as possible.
- At current industrial areas transfer of dust from the working environment to home environment should be minimized by changing clothes.

Recommendations for fish consumption

Based on the elevated Hg levels in the coastline area of Slovenia, recommendations for fish consumption in Slovenian language (Figure 32) have been made for public that were so far presented to different target groups in Slovenia as a tool for awareness rising. The recommendations include species of fish

and how often each of them can be consumed without increasing a risk. The recommendations are posted on Slovenian webpage of the CROME-LIFE project.

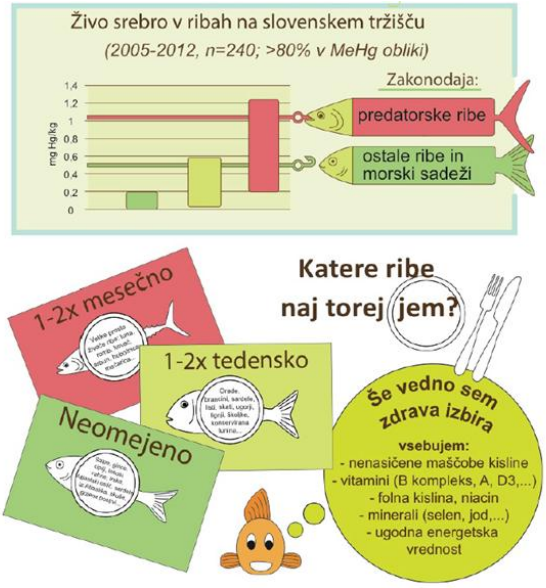

Figure 32: Outline of recommendations for fish consumption based on research on fish consumption frequency and fish availability on Slovenian market.

Specific case: former mercury mining town of Idrija

Based on the case-control study and HBM data, we recommend that pregnant women and children should not consume seasonal vegetables such as cabbage, parsley leaves and carrots from domestic gardens located in the area of the former Hg smelting plant and fish from the Idrijca river because of their higher contents of inorganic and methyl Hg. The findings of the study should be considered by the competent services and particularly by the fisheries society in the Idrija municipality in order to take appropriate action to avoid/limit Hg exposure in susceptible groups of inhabitants.

Spain

Promoting Health: Recommendations of Food Consumption as a Tool to Minimize Exposure of Pollutants in Children

As consequence of the work developed in CROME-LIFE and the results obtained in the project, a fruitful relationship was established with the General Direction of Public Health and Consumption of the Ministry of Health, Family and Social Welfare of the Government of the Balearic Islands (Palma, Mallorca), and also with the Health Area of Menorca from IB-SALUT (Mao, Menorca). It was considered to provide information and recommendations for consumption to the health care workers (HCW) of the Balearic Islands since they are opinion leaders in the field of food safety and promotion of safety life standards in the population. His task is very effective in public health. Food is an important exposure source for mercury and children are the most vulnerable age group for most of them. This high exposure to mercury is a general feature of the Spanish population in comparison to the concentrations of this pollutant in other European child cohorts, with the exception of Portugal.

With the aim to improve the knowledge about food contaminants for HCW, 275 HCW in 16 of the main hospitals from the Balearic Islands attended 2 hours of lessons in 2015. A test before and after the

lesson was given to assess the knowledge of HCW about how to prevent the exposure of children and pregnant women to food contaminants and to determine their opinion about the course. In addition, teaching materials for families was provided to the attendants.

Of those workers, 60% answered the test correctly. The average percentage of correct responses prior the training was 10%. The most valued aspects were the high profit and the opportunity to share scientific knowledge on health risks associated to food consumption between HCW and public health workers. One of their main demands was further extension of the course contents and its duration.

Accordingly, in 2016, a new online food safety course for HCW was developed. The attendees had access to more extended content. However, to access this content they had first to pass several introductory tests and to obtain a certificate of their expertise. After this new course they were requested to do a post test. Higher degree of knowledge and information was achieved with these online lessons. This personnel is then better trained for giving advice to general population and prevent better the exposure of children to contaminants. This task will be continued and the expertise developed within the Government of the Balearic Islands will be offered to other administrations at the Spanish level.

Conclusions

Recommendations to limit exposure to toxic substances and to mitigate the associated potential adverse health outcomes should be split into two main categories depending on the target group they are aiming at: policy-makers and authorities responsible for environment and health protection at local and national scale and the general population.

With regard to the first group, key recommendations include the establishment and consolidation of effective environmental monitoring system capable of tracking the status of environment (i.e. ambient air, water and soil pollution). This include also the establishment of regular monitoring of toxic substances residues in different food items collected in the local markets in the areas of study. Environmental monitoring systems should be periodically reviewed to strengthen their operation and prioritize new information so as to provide improved quality and reliability of data and information in relation to the environment which represent the essential information needed for a more effective decision-making process.

The establishment of national human biomonitoring programs for the general population with specific attention to people living in most polluted areas and to vulnerable groups is a further key recommendation for this stakeholders group. In this light human biomonitoring programs represents a powerful tool allowing to estimate population reference values, to evaluate time trends, and to identify potential higher exposures where policy makers and health authorities need to focus their efforts.

Urban planners and responsible authorities should take all the measures needed to limit/avoid the operation of potential polluters such as industries and facilities close to populated areas and to increase the preparedness capacity for response to acute pollution episodes.

Recommendations for the general population are mainly related to behavioral changes and to improved awareness and education. This in turn can be only a consequence of a more informed communication strategy leading to reduce or avoid behaviors/lifestyles having a potential negative impact on the health status. Examples includes avoiding passive smoking, limit alcohol consumption, better and more informed use of general consumer products.

Whilst is some cases there is still ignorance about the value of preventive activities in the protection of public health, education campaigns at different levels, have been proven effective in many situations. Individuals benefit from information and education that gives them an increased awareness of health risks, a greater control over their own health and their environments, and more freedom to choose a safe environment and healthier lifestyle. This supports personal and social development. When directed to communities, professionals, and policy-makers, health education for prevention of poisoning is concerned with raising awareness of problems, influencing priorities for action, and persuading about the potential effectiveness of preventive measures. Without these activities, prevention strategies such as regulation and legislation will have little impact.

Education in schools plays a key role contributing to raise awareness in the environment and health issues in the younger generations which represent also a vulnerable group. Rather than presenting a list of facts with "don`ts", a more effective approach to education is to help children to explore their attitudes, to see the possible options, then make choices and take action. To this end recommendations should also promote lifestyles which may be beneficial such as the consumption of protective food or the regular adoption of hygiene practices.

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