



Consiglio Nazionale delle Ricerche

Dipartimento Scienze del sistema Terra e tecnologie per l'ambiente



PROGETTO

*Centro Internazionale di Studi Avanzati su Ambiente,
ecosistema e Salute umana [CISAS]*

WP4. Etiological Epidemiology

To develop knowledge on the association between priority pollutants specific for each area and selected health conditions, identified on the basis of environmental and epidemiological knowledge and relevance for research and innovation

Fabrizio Bianchi, Head Unit of environmental epidemiology, IFC CNR



Background



- For the Polluted Site (PS) of **Milazzo** and **Priolo** data on mortality, incidence and hospitalization were provided (*Pirastu et al. 2012 e 2014*).
- For the PS of **Crotone** mortality data 1995-2002 were provided (*Pirastu et al 2012*), upgraded by a recent analysis on data 2006-2012, providing disaggregated data for the municipalities of Crotone and Cassano-Cerchiara (*ISTISAN 16/9*).
- In the three PSs under study several excesses of health indicators were observed.

Background



Environment characterization of target pollutants in 3 polluted sites of national interest

- **Augusta-Priolo (Sicily)**
 - Hg, Cd, Zn, Pb, PCDD, PCD, Esachlorobenzene, PAH, PCB , BTEX
- **Milazzo (Sicily)**
 - Pb, Hg, Ni, Cr, Cd, PAH, BTEX
- **Crotone (Calabria)**
 - Cd, Zn, As, Pb, Cu, Hg





AIMS of CISAS WP4

- Enhance knowledge on selected indicators through the **study of risk markers and pre-clinical signs in population groups at different levels of exposure.**
- **merging environmental, epidemiological and research criteria**

Target diseases and planned activities



- **Augusta-Priolo**
 - liver and diabetes
- **Milazzo**
 - thyroid
- **Crotone**
 - cardiovascular
 - urinary

Activities planned:

- BM surveys (exposure BM)
- cohort studies
- risk perception surveys
- linkage studies with mortality and morbidity data
- Participatory and KTE initiatives

Organization



WP4 consists of 5 tasks:

- Task 4.1** **Coordination, analysis and monitoring (Fabrizio Bianchi)**
- Task 4.2** **Risk of liver cancer in the Priolo area (Amalia Gastaldelli)**
- Task 4.3** **Risk of thyroid cancer in the Milazzo area (Letizia Pitto)**
- Task 4.4** **Risk of cardiovascular biomarkers in the Crotone area
(Alessandro Pingitore e Francesco Faita)**
- Task 4.5** **Pilot study on renal function impairment and early kidney
damage in the Crotone area (Giovanni Tripepi e Davide
Bolignano)**
- Task 4.6** **Dissemination and scientific networking (Liliana Cori)**



Task 4.1 Coordination, analysis and monitoring (Fabrizio Bianchi, IFC CNR)



Management

Project management activities, coordination with other WP, epidemiological design and statistical analysis, relationships with other local, regional, national bodies, monitoring and evaluation of actions development.

Literature review

Complete literature review on environment and health in the three study areas (Augusta, Milazzo and Crotone) as starting point of the field work (cooperation proposal to ISS)

Monitoring and evaluation

A specific list of indicators have to be defined to monitor and evaluate the task development, as a basic function of the management activity



Task 4.2 Risk of liver cancer in the Priolo area (Amalia Gastaldelli, IFC-CNR)



The Priolo PS is characterized by a diffuse environmental contamination due to toxic, persistent and bio-accumulable compounds, such as heavy metals, polychlorinated biphenyls (PCBs), dioxins and polycyclic aromatic hydrocarbons (PAHs) were detected and the past and present exposure to volatile organic compounds (VOCs) was ascertained.

The incidence of all cancers showed an excess in both genders with respect to the pool of cancer registries of central and southern Italy, in particular neoplasms in excess included liver and pancreas cancers and mesothelioma.



Task 4.2 Risk of liver cancer in the Priolo area

Amalia Gastaldelli, IFC-CNR



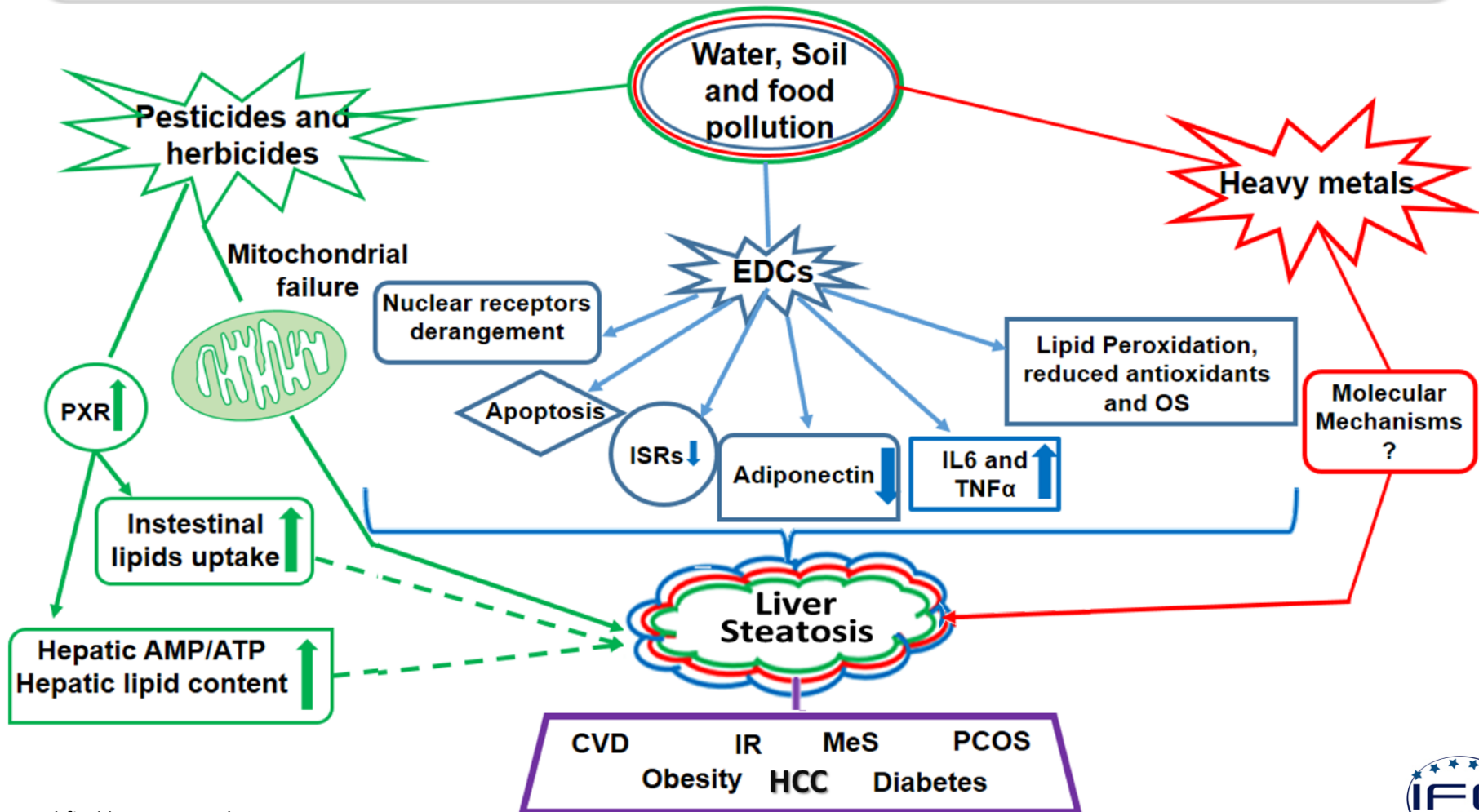
PREVENTING DISEASE THROUGH HEALTHY ENVIRONMENTS

A global assessment of the burden of disease from environmental risks

A Prüss-Ustün, J Wolf, C Corvalán, R Bos and M Neira



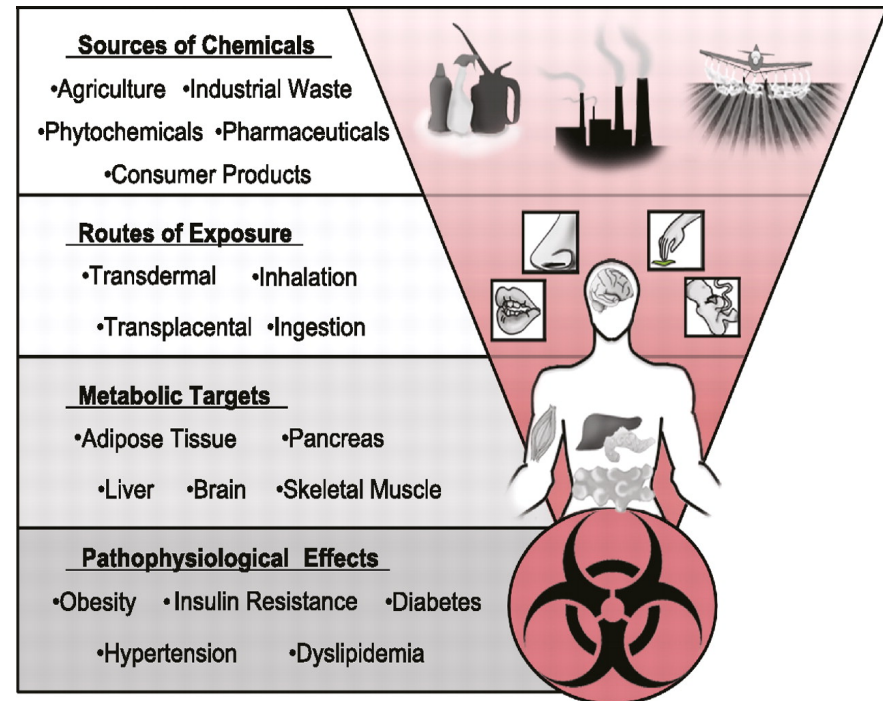
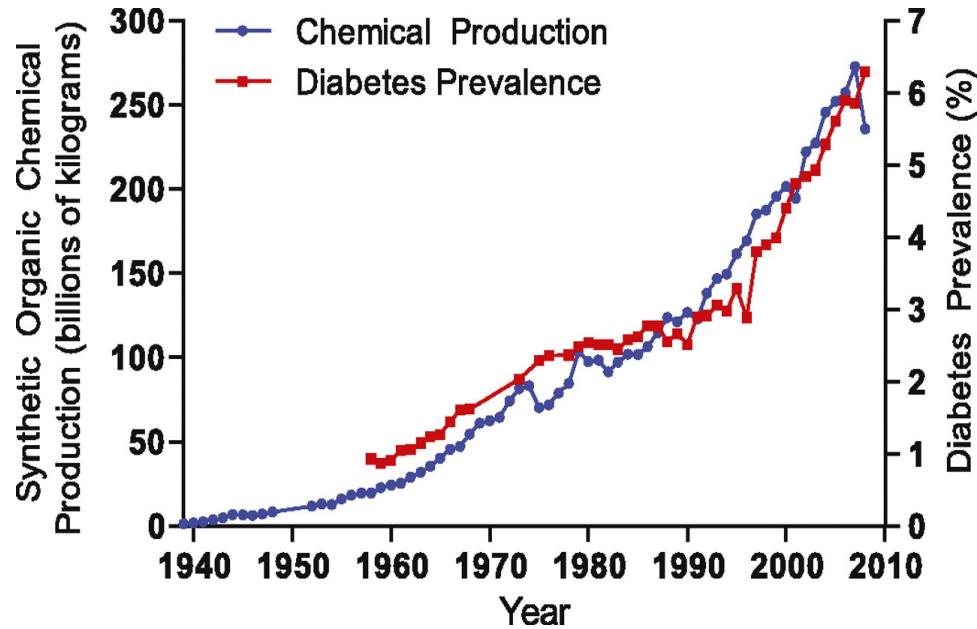
<http://www.who.int/phe/news/march2016/en/>



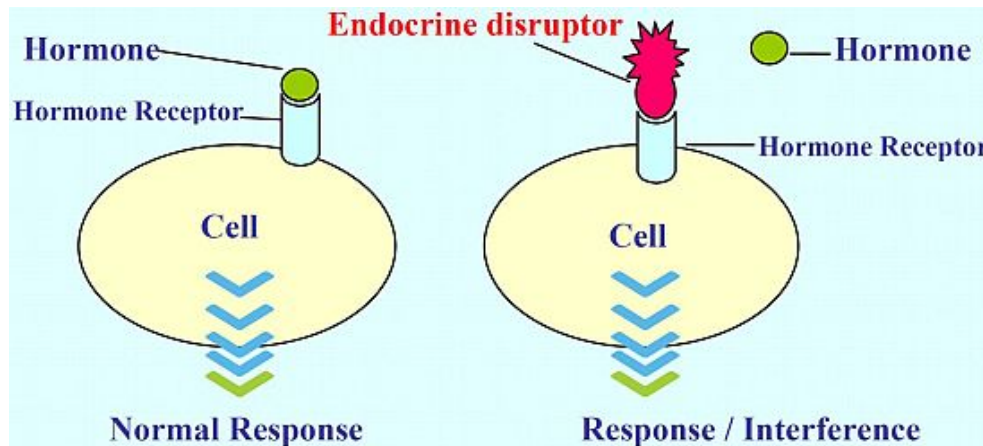
Modified by Int. J. Mol. Sci. 2013, 14, 22052-22066



Increased chemical production has been associated with increased prevalence of Diabetes, CVD, Fatty Liver, Cancer



Neel B A , and Sargis R M Diabetes 2011;60:1838-1848



Non persistent pollutants act as endocrine disruptors



Hepatocellular carcinoma (HCC) is the main type of primary liver cancer, it is the sixth most commonly occurring cancer in the world and the second largest contributor to cancer mortality.

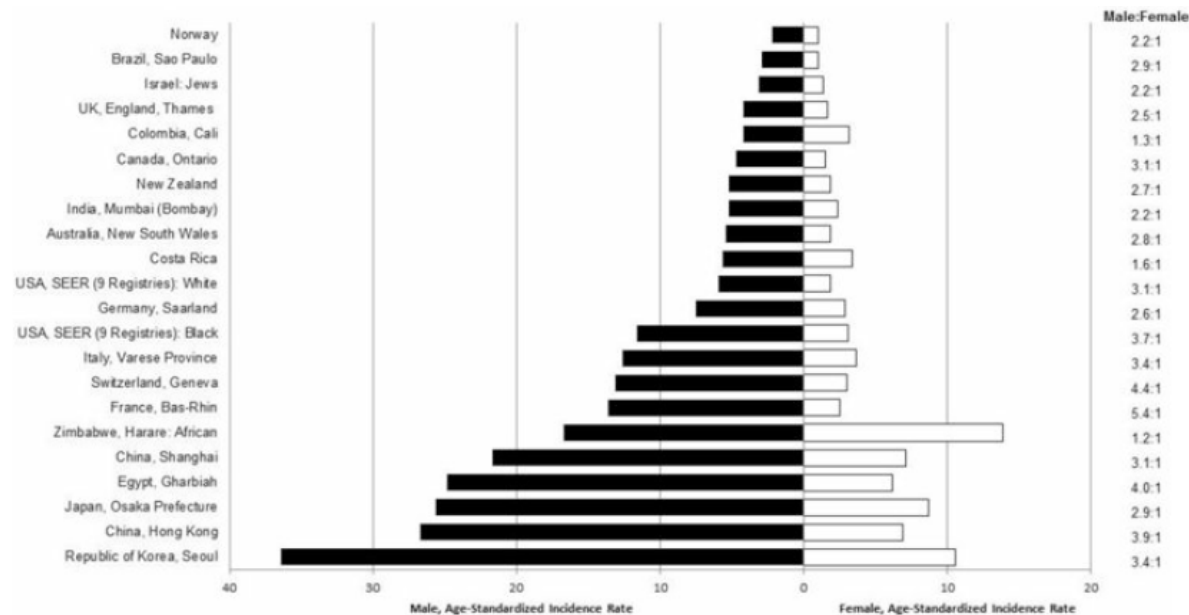


[Clin Liver Dis.](#) 2015 May;19(2):223-38. doi: 10.1016/j.cld.2015.01.001. Epub 2015 Feb 26.

Global epidemiology of hepatocellular carcinoma: an emphasis on demographic and regional variability.

McGlynn KA, Petrick JL, London WT.

The mechanisms for the development of HCC are still unknown but it might be due to alterations of lipid metabolism. In fact, the risk factors for HCC are represented not only by viral infections (HCV and HBV) and excess alcohol but also by exposure to pollutants that act as endocrine disruptors and produce metabolic alterations.



Age-adjusted incidences per 100,000 of liver cancer among men and women by region, 2003-2007. Age-adjusted to world standard.

Hypothesis

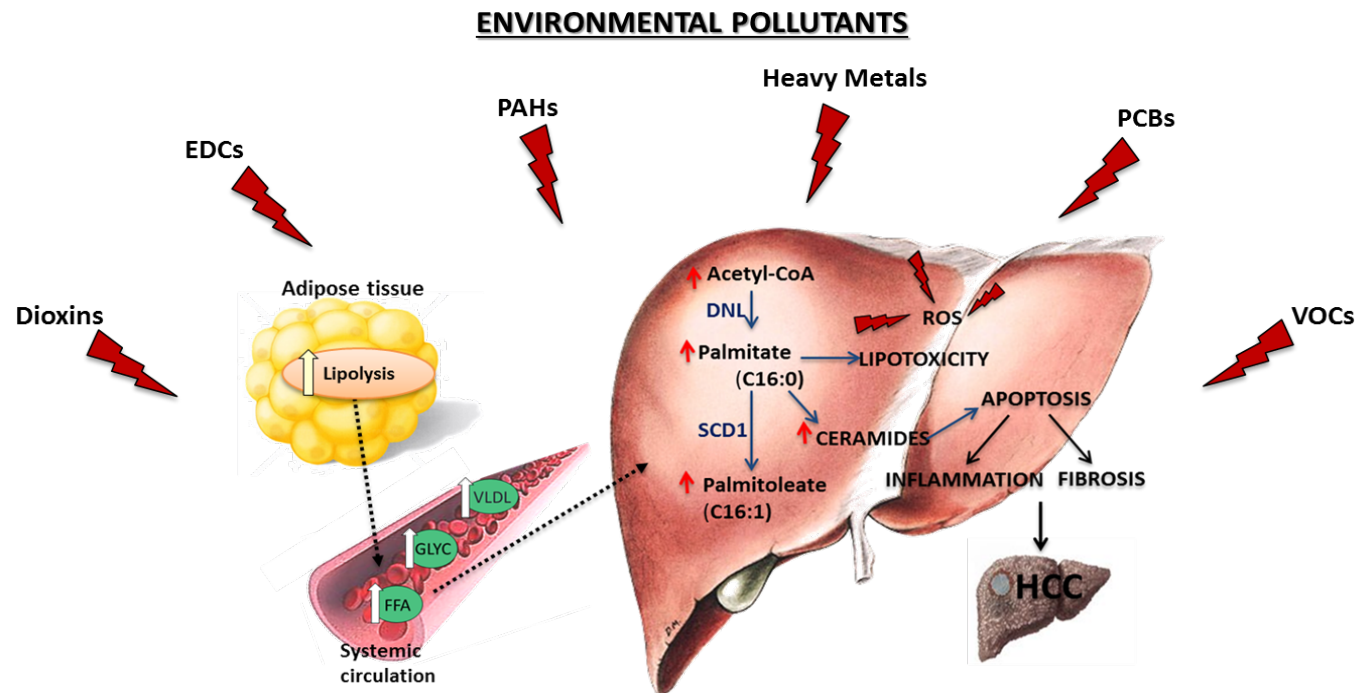


Most of the pollutants are lipid soluble and once they accumulate in adipose tissue and liver they might promote cell lipotoxicity, increase lipolysis, de novo lipogenesis (DNL) and synthesis of saturated fatty acids, in particular palmitic acid (PALM) and other classes of lipid such as ceramides, di- and tri-acylglycerols (DAG and TAG).

Fatty acids (FA) are required for energy storage, membrane proliferation, and the generation of signaling molecules and alterations in FA metabolism may be involved in the induction of the cancerogenesis event.

Cell Metab. 2013 Aug 6;18(2):153-61. doi: 10.1016/j.cmet.2013.05.017. Epub 2013 Jun 20.
Cellular fatty acid metabolism and cancer.
Currie E, Schulze A, Zechner R, Walther TC, Farese RV Jr.

Proposed mechanisms



Aim of the study



To identify if subjects living in the exposed area:

- display increased levels of established risk factors and potential biomarkers of liver disease and HCC
- display specific disease-related metabolic pathways responsible of the alteration in lipid metabolism able to promote liver disease and HCC development

Experimental design of the study



Plasma and urine samples

- **1st group (n=220):** without liver cancer living in exposed area
- **2nd group (n=220):** without liver cancer living in unexposed area
- **3rd group:** subjects with HCC will be asked to participate

- clinical and biochemical parameters
- fatty liver index (FLI)
- fibrotic liver indexes
- inflammatory markers
- adipocyte hormone leptin and the pancreatic hormone insulin

- Lipidomic profile (e.g. ceramides, di- and tri-acylglycerols) will be measured in plasma samples by liquid chromatography mass spectrometry LC-MS Q-TOF

- DNL indexes calculated as the ratio palmitic/linoleic acid (16:0/18:2)
- Free fatty acid (FFA) concentration and composition



These parameters will measure in plasma, by gas chromatography mass spectrometry (GC/MS)



Expected Results



- To identify classes of toxic lipids that, due to the high concentrations of environmental pollutants in the Priolo area, can promote and stimulate alterations of lipid and fatty acid metabolism that may be involved in the development of hepatocellular carcinoma.
- To identify potential biomarkers involved in specific metabolic pathways that could be used as target to prevent/treat hepatocellular carcinoma.

Task 4.3. Risk of thyroid cancer in the Milazzo area (Fabrizio Bianchi e Letizia Pitto)



In the Milazzo PS, including the municipality of Milazzo, Pace del Mela e San Filippo del Mela, the incidence of thyroid cancer (TC) in 2003-2010 has been reported in excess (not statistically significant) among men and women (24% and 40% increases respectively), and the hospitalization for TC in 2005-2010 showed statistically significant excess of 55% among men and 24% among women. (*Pirastu et al. 2014*)

Task 4.3. Risk of thyroid cancer in the Milazzo area (Fabrizio Bianchi e Letizia Pitto)



Excesses of mortality and morbidity were observed in several PSs in addition to Milazzo (Brescia-Caffaro, Mantua Lakes, Sassuolo-Scandiano, Taranto), in both genders (*Pirastu et al. 2014*)

Since long time is well known the critical role of the thyroid gland in the development/cellular differentiation, and fetal tissue, in the control metabolic homeostasis and function of the majority of organs and systems (cardiovascular and central nervous system first of all), including the remaining endocrine organs and the reproductive system.



Task 4.3. Risk of thyroid cancer in the Milazzo area (Fabrizio Bianchi e Letizia Pitto)



This role is emphasized by the relief, clinical and experimental, that even **minimal change in thyroid function** with circulating levels of hormones while still resulting in the reference range of normality '(so-called **hyper or hypothyroidism preclinical**) are able to generate negative consequences, particularly at fetal and neurological level.

Hormone-like molecules mimetic PCB, Bisphenol and others is well recognized, while the role of heavy metals, such as mercury, lead, cadmium, is less known.



Task 4.3. Risk of thyroid cancer in the Milazzo area (Fabrizio Bianchi e Letizia Pitto)



Subtask 3.1 Epidemiological investigation

To examine potential mechanisms of toxic action on thyroid function of mercury, lead, cadmium, and other heavy metals with an integrated approach that includes the use of an animal model widely used and validated for toxicological which zebrafish.

The primary aim is to identify if subjects living in the exposed area display increased levels of established risk factors and biomarkers of function/thyroid carcinogenesis.



Task 4.3. Risk of thyroid cancer in the Milazzo area (Fabrizio Bianchi e Letizia Pitto)



Subtask 3.1 Epidemiological investigation

The secondary aim is to discover if subjects living in the exposed area display alteration in metabolism that can promote thyroid cancer.

Subjects: we will study subjects without known thyroid cancer, aged 18-44 years, recruited in the Milazzo area and in another area without contamination.



Task 4.3. Risk of thyroid cancer in the Milazzo area (Fabrizio Bianchi e Letizia Pitto)



Subtask 3.1 Epidemiological investigation

In all subjects we will collect plasma and urine samples, and the following parameters will be measured: TSH, FT3, FT4, urinary or blood levels of various heavy metals of interest, adjusted for creatininuria.

A thyroid sonographic measurements (glandular volume) will be done in each subject.



Subtask 3.2 Experimental model



Zebrafish an in-vivo model to investigate the effects of environmental pollutants on Thyroid function and on occurrence of thyroid carcinoma

Zebrafish are widely used in toxicological research because of its small size, easy culture, high reproductive performance, rapid organogenesis and sensitivity to the harmful chemicals.



84% of genes known to be associated with human disease have zebrafish counterparts



Recently, zebrafish studies have led to **important insights into thyroid development**, and have been used to model endocrine cancer.

The **thyroid system in zebrafish is similar to mammalian**, which can provide a valuable reference for human beings.

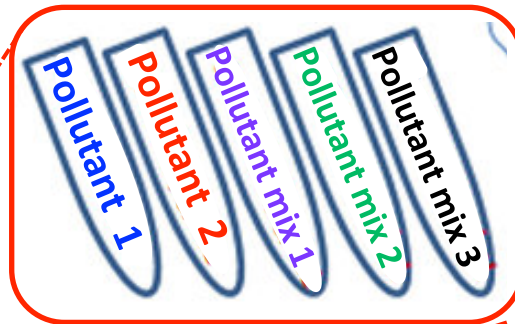
In the present study, zebrafish will be used for elucidating the effects of several environmental pollutants identified in the area of interest of Milazzo, on disruption of thyroid function and expression and on insurgence of thyroid carcinoma.

Investigation on the impact of pollutants on thyroid metabolism



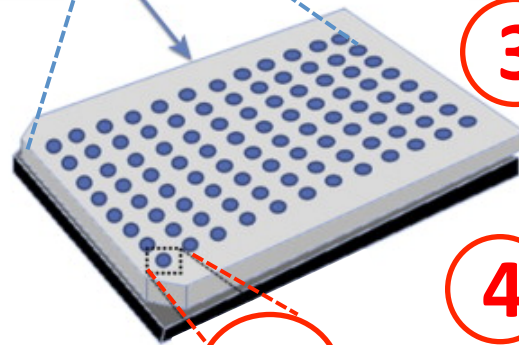
1

Fish crossing and eggs fertilization



Arrayed embryos

2



3

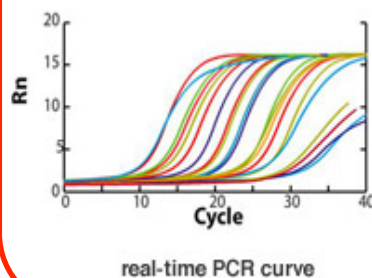
xenobiotic addition

4

Embryo development in the presence of xenobiotics

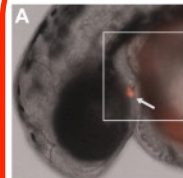


Quantitation analysis of thyroid regulating genes by Quantitative Real Time PCR.

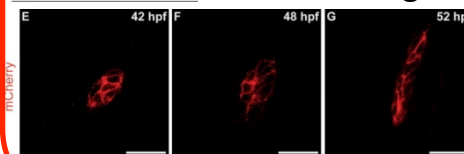


Analysis of **Dio1** and **Dio2**, **TR- α** and **TR- β** , **tsh- β** gene, the iodine transporter **slc5a5** gene, the **thyroglobulin** and **transthyretin**.

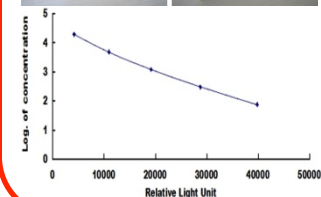
Morphological studies using of the transgenic line *tg(tg:mCherry)*



This line allows **live imaging** of thyroid development in embryos from budding stage throughout formation of functional **thyroid follicles**.



T3, T4 quantification by Elisa Kit

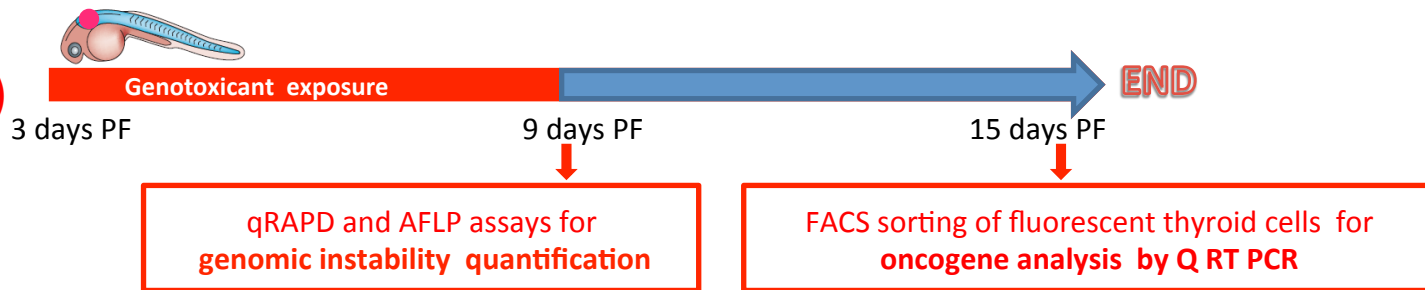


Investigation on the impact of pollutants on thyroid neoplasia

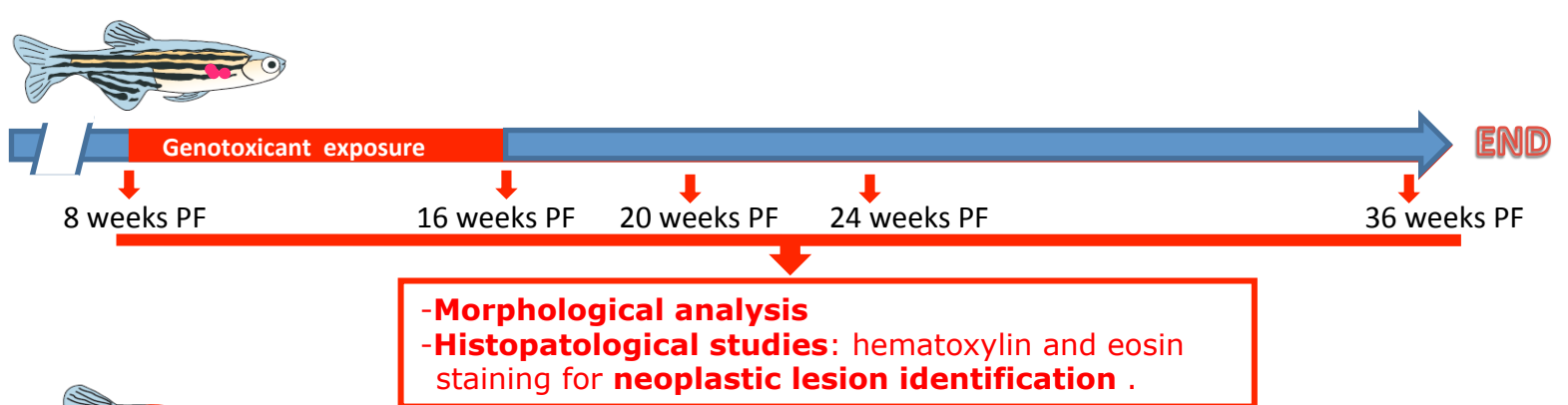


To evaluate the genotoxic effect of the pollutants under studies, different exposure protocols will be utilized on the **transgenic *tg(tg:mCherry)* line** and on the **mutant *p53*^{-/-} line** which lacking of the tumor suppressor p53 gene will result in an increase of the neoplasia frequencies making easier the generation of tumor free curves.

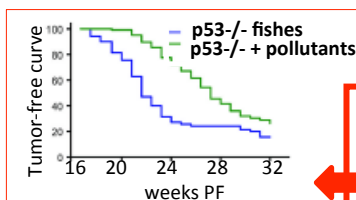
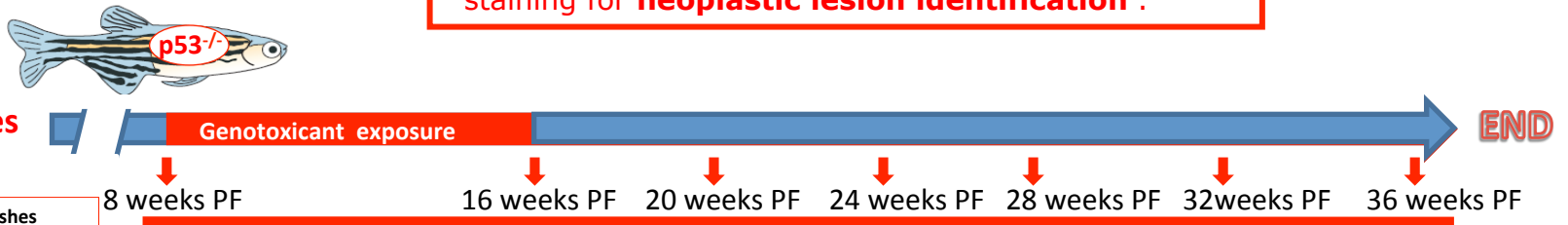
1 Treatment of *tg(tg:mCherry)* embryos



2 Treatment of *tg(tg:mCherry)* juvenile fishes



3 Treatment of *p53*^{-/-} juvenile fishes



Generation of tumor free curves.



Task 4.4. Risk of cardiovascular biomarkers in the Crotona area



Background

In the Crotona area high concentration of heavy metals was reported (*Troisi et al 2002*).

The Study SENTIERI on 1995-2002 mortality data, showed an excess for circulatory diseases among males (+13%) (*Pirastu 2012*)

Mortality for **kidney tumors** was observed in excess among males and females (+ 105% and + 37% respectively), while an excess of **urinary tract and nephrosis kidney** failure emerged among females (+43%). Hospital admission due to **ischemic heart disease, acute ischemic diseases, nephritis, nephrotic syndrome and nephrosis** showed excesses for both genders (+42-68%), for **kidney cancers** among males (+49%).(*ISTISAN 16/9*)



Task 4.4. Risk of cardiovascular biomarkers in Crotona



Background

Heavy metals and CardioVascular Diseases: a true relationship

"...4 metals having no role in the human body (xenobiotic), mercury, lead, cadmium, and arsenic, have epidemiological and mechanistic links to atherosclerosis and CVD."

Solenkova NV et al. Am Heart J 2014 (Review)

"Blood lead and calcium, at levels well below current safety standards, were associated with an increased prevalence of peripheral arterial disease..."

Navas-Acien A et al. Circulation 2004

"Urine cadmium ... was associated with increased cardiovascular mortality..."

Tellez-Plaza et al. Epidemiology 2013

"Long-term exposure to low to moderate arsenic levels was associated with cardiovascular disease incidence..."

Moon KA et al. Ann Intern Med 2013

Task 4.4. Risk of cardiovascular biomarkers in Crotona



Oxidative stress and inflammation

“The overall vascular effect of mercury include increased oxidative stress and inflammation, reduced oxidative defense, thrombosis, vascular smooth muscle dysfunction, endothelial dysfunction, dyslipidemia, and immune and mitochondrial dysfunction.”

Houston MC J Clin Hypertens 2011

“The potential association between chronic heavy metal exposure, like arsenic, lead, cadmium, mercury, and CVD has been less well defined. The mechanism through which heavy metals actmay still remains unknown, although impaired antioxidants metabolism and oxidative stress may play a role.”

Alissa EM et al. J Toxicol 2011

Task 4.4. Risk of cardiovascular biomarkers in Crotona



Effects on cv biomarkers

*“...**mercury** is associated with increasing BP and pulse pressure ... after considering the effect of fish nutrients ... and other confounders.”*

Valera B et al. Hypertension 2009

*“The results of this study suggest a deleterious impact of **mercury** on BP and HRV in adults.”*

Valera B et al. Environ Health 2008

*“**Mercury** exposure was significantly associated with increased BP and IMT”*

Choi AI et al. Environ Health Perspect 2009

*“An association has been shown between exposure to high levels of **arsenic** and cardiovascular risk factors such as ... subclinical carotid atherosclerosis.”*

Stea F et al. Environ Sci Pollut Res Int 2014

“... the increase of IMT with age was higher than that observed in the healthy population ...”

Stea F et al. Environ Sci Pollut Res Int 2016

*“... THU ameliorates **cadmium**-induced hypertension, vascular dysfunction , and arterial stiffness ...”*

Sangartit W et al. PLoS One 2014

*“Acute exposure to **diesel exhaust** is associated with an immediate and transient increase in arterial stiffness.”*

Lundback M et al. Part Fibre Toxicol 2009

Task 4.4. Risk of cardiovascular biomarkers in the Crotone area

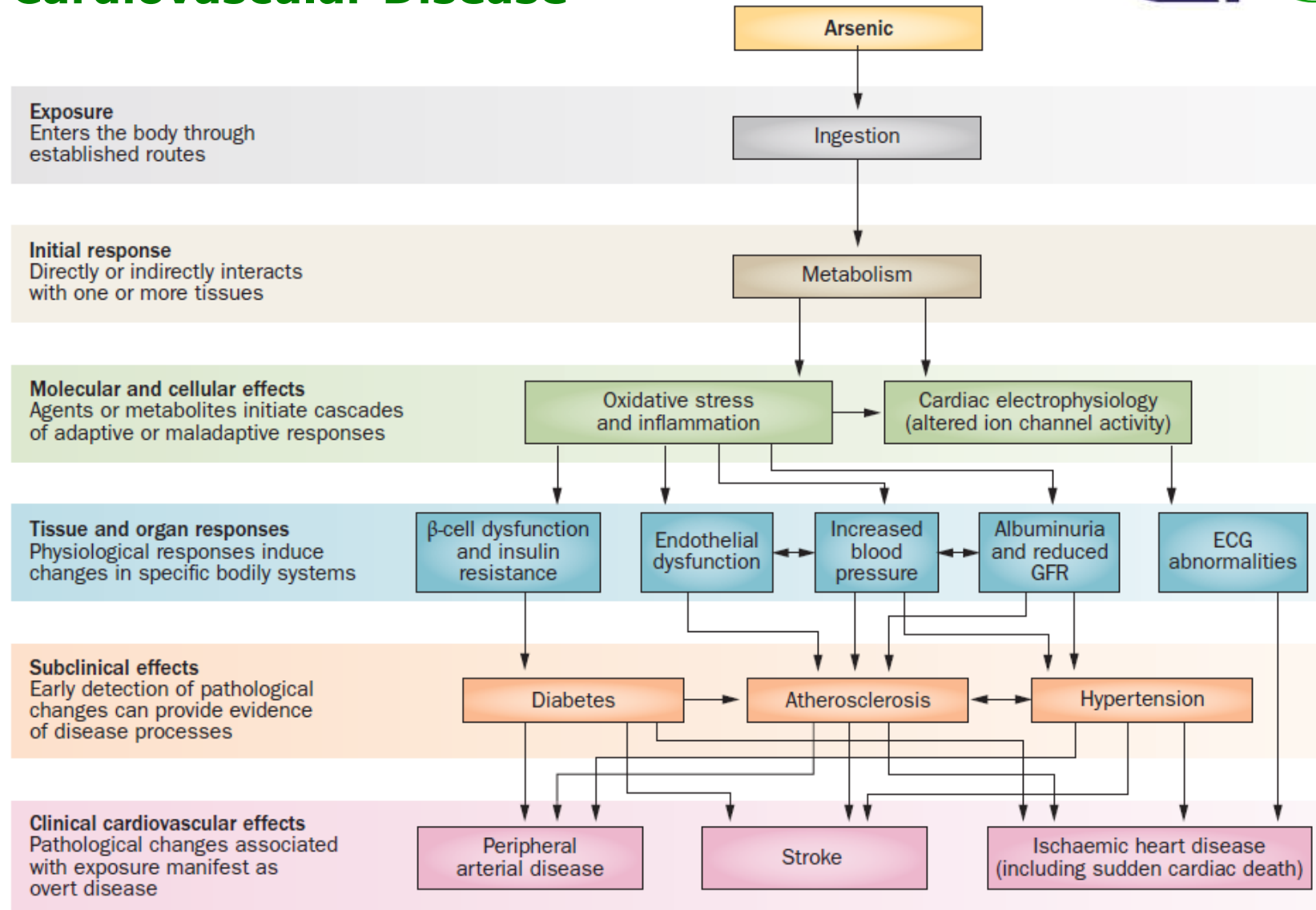


Study design and Subject

- 300 subjects aged 40-70 years without previous cardiovascular diseases and with or without known conventional CV risk factors. Selected according to the residence at low middle and high level of environmental pollution
- CV risk score, Framingham or Euro Score, will be assessed for each subject
- A platform will be used providing an integrated and multipurpose computer-based program for CV risk assessment
- Questionnaires on life-style habits and psychosocial parameters will be conducted



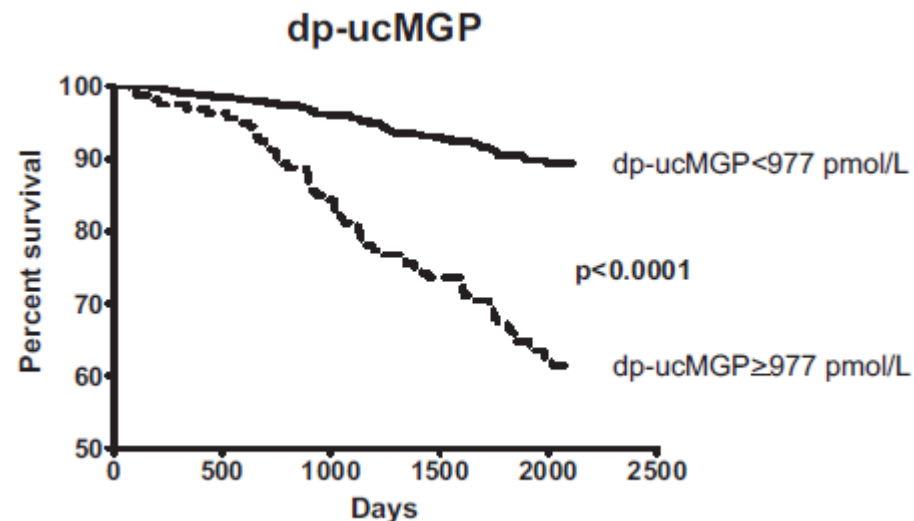
The Multiple Arsenic Pathways to Cardiovascular Disease



Cosselman K et al. Nat Rev Cardiol 2015

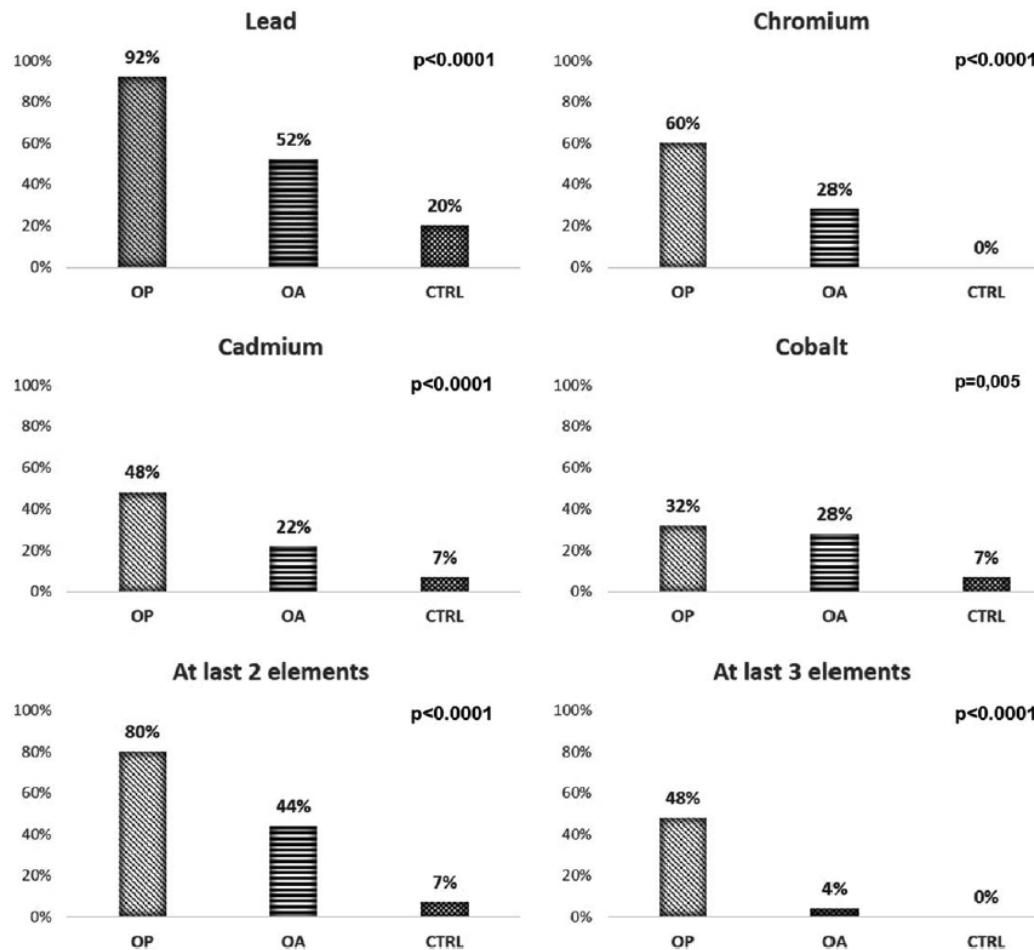


Vitamin K is the essential co-factor for activation of matrix Gla-protein (MGP), the natural inhibitor of tissue calcification. Biologically inactive, desphospho-uncarboxylated MGP (dp-ucMGP) is a marker of vascular vitamin K status



The key finding of our study was that elevated dp-ucMGP concentrations were associated with higher cardiovascular and total mortality in stable patients with manifest vascular disease.

Heavy Metals Accumulation Affects Bone Microarchitecture



the presence of heavy metals into bone shed new light on the comprehension of the pathogenesis of osteoporosis since these elements could play a non redundant role in the development of osteoporosis at cellular/molecular and epigenetic level

OA = pts with osteoarthritis
OP = pts with osteoporosis and femoral fracture

Scimeca M et al. Environ Toxicol 2016

Task 4.4. Risk of cardiovascular biomarkers in the Crotona area



Cardiovascular analysis

Carotid femoral Pulse Wave Velocity and Central Blood Pressure:

- PulsePen (Diatecne, Italy) device
- Arteriograph (Tensiomed, Hungary) device
- PWVcf, P_c, Alx, PWVao

Local carotid assesement:

- Carotid echocardiography
- Carotid Studio (Quipu, Italy) device
- PulsePen (Diatecne, Italy) device
- IMT, CS

Task 4.4. Risk of cardiovascular biomarkers in the Crotona area



Characterization of carotid atherosclerotic plaques:

- Carotid echocardiography with Doppler
- Custom designed texture analysis software

Endothelial function:

- Brachial echocardiography with Doppler
- FMD Studio (Quipu, Italy) device
- EndoPat (Itamar, Israel) device
- FMD, PVA, RHI

Task 4.4. Risk of cardiovascular biomarkers in the Crotona area



Cardiovascular analysis

Electrocardiogram (ECG) analysis:

- Custom designed device for ECG acquisition
- Custom designed software for ECG analysis
- HR, QT, HRV

Biohumoral tests:

- Inflammatory markers
- Oxidative stress markers
- NT-proBNP, tPa, CD40
- Bone health markers

Task 4.5. Pilot study on renal function impairment and early kidney damage in the Crotone area (Giovanni Tripepi e Davide Bolignano)



- The incidence and prevalence of chronic kidney disease (CKD) have risen constantly over the last 3 decades, becoming now a growing public health problem.
- There is now considerable evidence pointing at the role of environmental pollution as a prominent aetiological factor of CKD of multifactorial or unknown origin (CKDmfo/CKDu)
- Several mechanistic and experimental studies have defined the role of ions, heavy metals and fluorides as key determinants of early glomerular and tubular damage, which usually anticipate the frank impairment of renal function, leading to End-Stage Kidney Disease (ESKD)



Task 4.5. Pilot study on renal function impairment and early kidney damage in the Crotone area (Giovanni Tripepi e Davide Bolignano)



- To assess the entity of renal function impairment and early tubular damage in a sample of subjects geographically exposed to a different gradient of environmental pollution by heavy metals
- This will be achieved by:
 - 1) identifying the overall prevalence of CKDmfo/CKDu in this population
 - 2) quantifying the entity of early renal damage by measuring tubular biomarkers in individuals with apparently normal renal function.

We expect to find a significantly increased proportion of subjects presenting with CKDmfo/CKDu or altered (increased) levels of tubular biomarkers of early renal damage according to a geographical gradient of environmental pollution.



Task 4.5. Pilot study on renal function impairment and early kidney damage in the Crotone area (Giovanni Tripepi e Davide Bolignano)



- **100 adults with no history of renal disease** will be enrolled. These subjects will be shared according to the place at low middle and high level of environmental pollution.
- **Renal function** will be estimated as glomerular filtration rate (eGFR) by using the Cockcroft-Gault and the MDRD formula by assessing individual serum creatinine levels. **Serum cystatin-C** will also be analyzed and implemented in cystatin-C based formulas for improving estimation precision of renal function
- **Biomarkers of tubular damage**, including NAG, β 2-MG, KIM-1, NGAL and L-FABP, will be measured in urine samples using ELISA commercial available kits and indexed by urinary creatinine excretion levels.



Task 4.6. Dissemination & scientific networking

Liliana Cori (IFC CNR)



The task will be developed in the framework of the WP4 activities, in coordination with the general communication plan (WP1).

Three different studies involving human subjects are planned in areas defined at risk for environmental pollution.

In each study → questionnaire to investigate past and current risks, information received concerning environment and health, risk perception.

Task 4.6. Dissemination and scientific networking

Liliana Cori (IFC CNR)



The main actors directly involved

- Participating subjects (donors)
- Local General Practitioners (LGP)
- Local Health Authorities (LHA) and Ethical Committees
- Public Administrator in Health and Environment sectors

The main actors indirectly involved

- Scientific community
- Local media people
- Local stakeholders

Task 4.6. Dissemination and scientific networking

Liliana Cori (IFC CNR)



Dissemination and scientific networking activities

- A. Internal circulation of information*
- B. Preparatory activities*
- C. Legally binding activities*
- D. Training*
- E. Circulation of results within the Scientific Community*
- F. Dissemination of results*



ERA-ENVHEALTH

NETWORK

www.era-envhealth.eu

**General Assembly meeting
Bilthoven, October 27, 2016
Hosted by RIVM**



Acronym	Name	Country
ADEME	French Environment and Energy Management Agency	France
ANSES	French agency for food, environmental and occupational health & safety	France
Centre Léon Bérard	University Lyon 1	France
CNR	Italian National Research Council	Italy
EPA	Environmental Protection Agency	Ireland
FPS	Federal Public Service Health, Food Chain Safety and Environment	Belgium
MEDDE	Ministry of Ecology, Sustainable Development and Energy	France
Folkhälsomyndigheten	Public Health Agency of Sweden	Sweden
RIVM	National Institute for Public Health and the Environment	Netherlands
SPW	Wallonic public service	Belgium
Swedish EPA	Swedish Environmental Protection Agency	Sweden
UA	University of Aveiro	Portugal
UBA	Federal Environment Agency	Germany
UoWM	University of Western Macedonia	Greece





ERA-ENVHEALTH

NETWORK



www.era-envhealth.eu

La rete ERA-ENVHEALTH ha finanziato progetti di ricerca su ambiente e salute:

marzo **2008** sono stati finanziati due progetti:

- **Risk assesment of the impact of climate change on Human health**
 - **ENHanCE**: Wellbeing e Environmental change and rising doctrends: implications for public health
- gennaio **2012** progetto **ACCEPTED**: Assessment of changing conditions, environmental policies, time-activities, exposure and disease.





ERA-ENVHEALTH

NETWORK



www.era-envhealth.eu

Dal 2013 la rete prosegue a titolo volontario (Roma, Stoccolma, Bruxelles, Bilthoven)

Tra le attività principali del progetto:

- RETE DI ESPERTI
- BRINGING THE GAP BETWEEN SCIENCE & POLICY





ERA-ENVHEALTH

NETWORK



www.era-envhealth.eu

Joint activities promotion

COST project – Twinning and other proposal

Newsflash ← 2 volte l'anno

GA annual meeting 2017 → ITALY

Sixth WHO Europe Ministerial Conference on
Environment and Health: 13-15 June 2017 in
Ostrava, Czech Republic

