NEUROSOME

Exploring The Neurological Exposome

UNICEF Multiple Indicator Cluster Survey (MICS)

BIOMONITORING OF LEAD IN A CHILDREN'S COHORT

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Outline

H2020-MSCA-ITN-2017 GA - 766251

Biomonitoring Of Lead In A Children's Cohort

A collaborative project by

UNICEF Georgia, Tbilisi, Georgia

Istituto Superiore di Sanità, Rome, Italy

Georgian National Center for Disease Control and Public Health, Tbilisi, Georgia

Coordinated by Alessandro Alimonti

Outline

Georgias overview

Lead as permanent heatlh issue

Methodology

Results, finding and perspectives

Further reading

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Socio-economic Status

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Georgia

Index	Value	Italian values
Population	3,717,100	60,551,416
GDP (MUS$)	15.1	1,934.8
Life expectancy at birth (y)	73.26	82.5
Gross National Income (MUS$)	9,186	41,154
Poverty headcount ratio at national poverty lines (%)	21.9	<0.6
HDI	0.780	0.880

Source: World Bank, IARC. 2019

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Demographic Information

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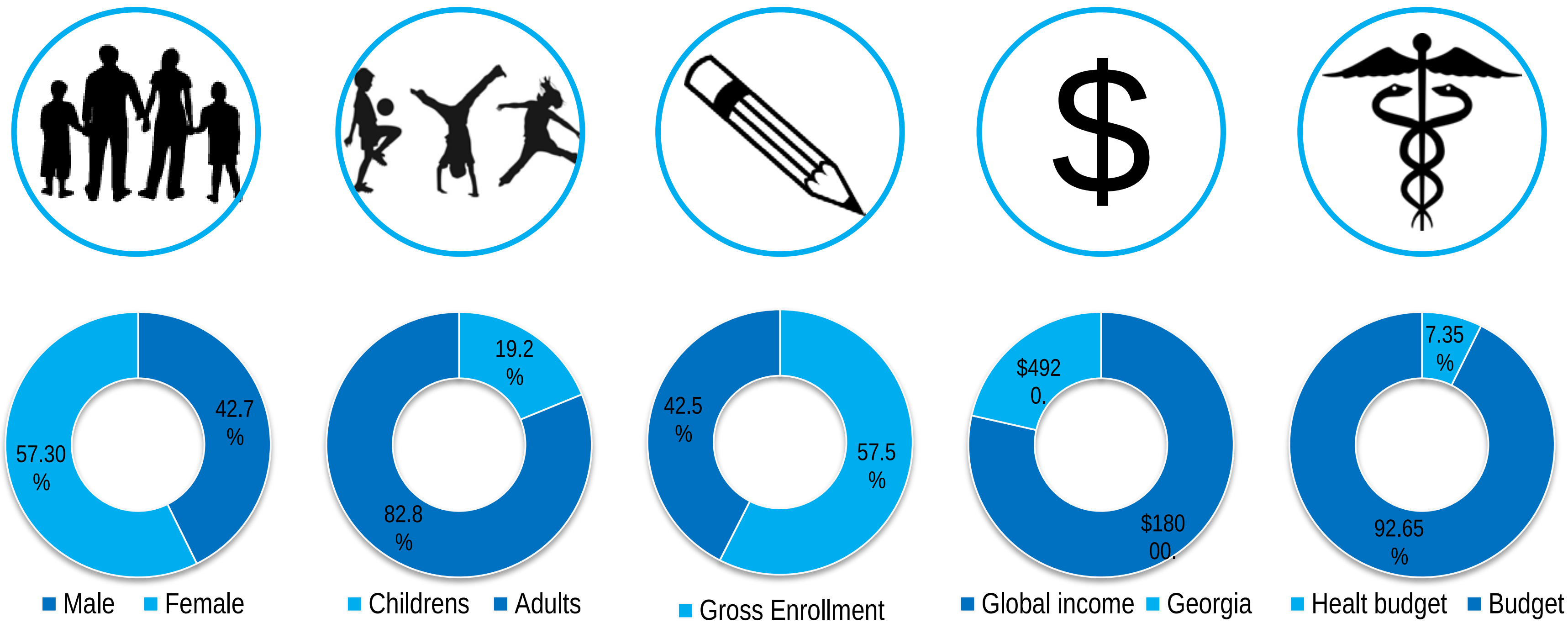

Source: World Bank, WHO, UNESCO. 2019

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Child Health Statistics

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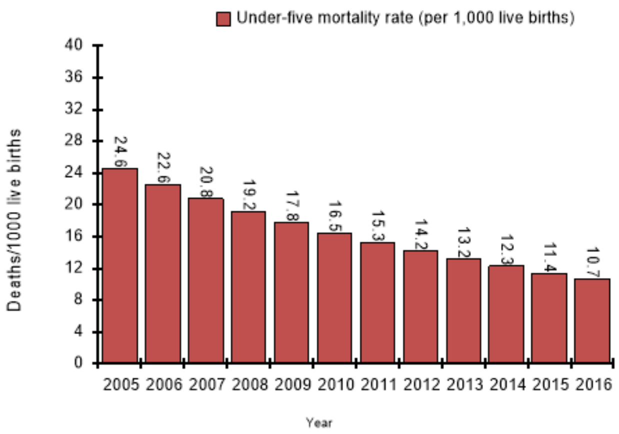
Indicator	Geogia		Italy	
	Year	Value	Year	Value
Births attended by skilled health personnel (%)	2014	99.9	2013	99.9
Population using improved sanitation facility (%)	2015	86.3	2015	99.5
Children aged 1 year immunized against measles (%)	2016	93.0	2016	85.0
Probability of dying under five (per 1 000 live births)	2017	11.0	**2017**	**3.4**
Population using improved drinking-water sources (%)	2015	100.0	2015	100.0



Source: World Bank, WHO. 2019

Lead, permanent health Issue

Lead (Pb)

Metallic element bluish-gray, with a presence of 0.0013% on the earth crust.

Rarely found in the elementary form, most frequently in mineral form as sulfide, sulfate, carbonate.

In the environmental is spread principal by industrial activities mining, fuels, waste treatment, among other.

Lead is a non-essential metal for biological activities on humans. The International Agency for Research on Cancer (IARC) has classified as A2 (probably carcinogenic to humans).

Source: IARC. 2019

Source of exposure

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Source: WHO, FAO (2019)

Routes of absorption	Percentage
Breathing	40
Ingestion	15
Dermal and other	25

Maximal lead levels in drinking water (WHO)	0.010 mg/L
Maximum daily diet burden children (EFSA)	1.32 -2.54 mg/ kg b.w. per day

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Exposure

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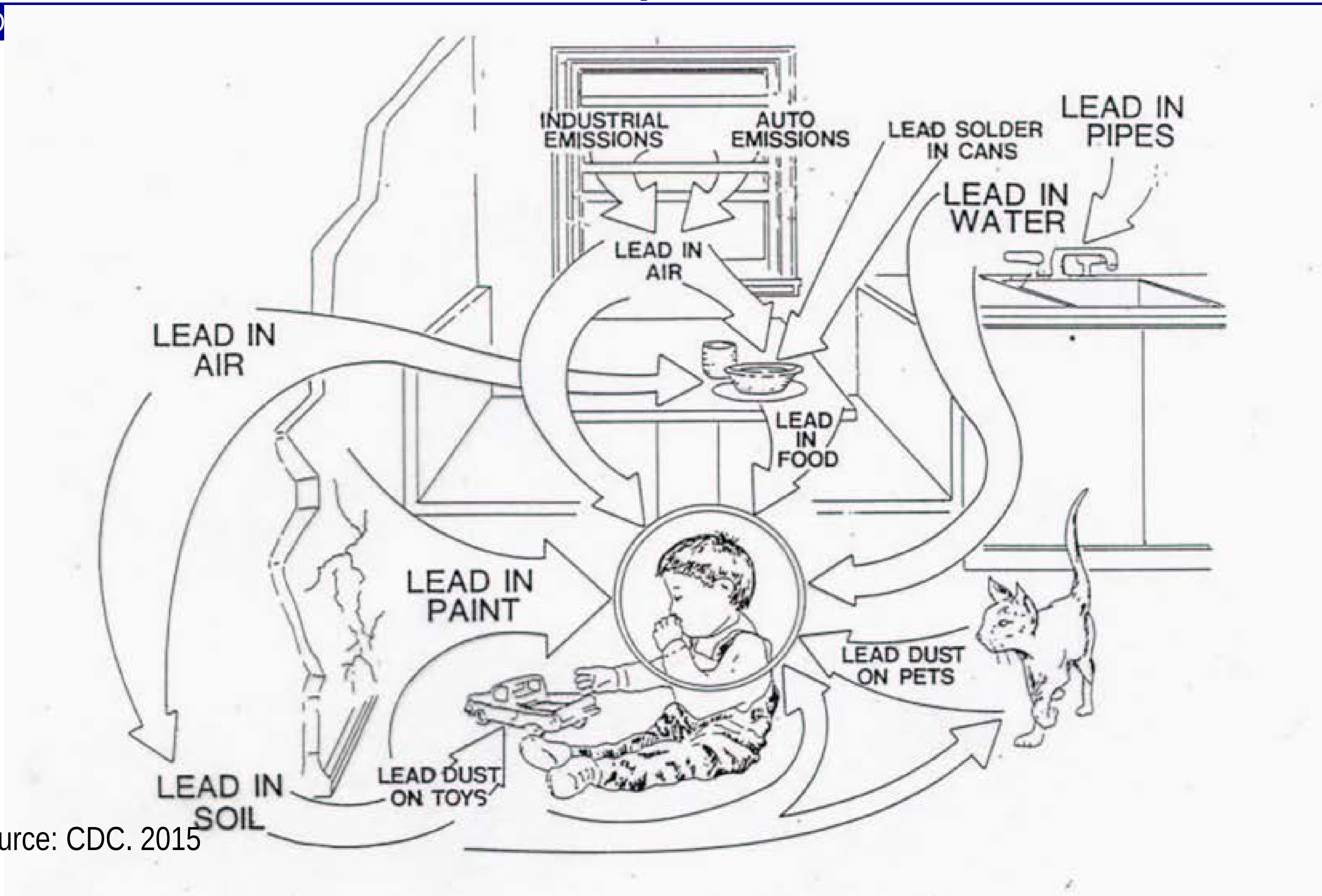


Source: CDC. 2015

Exposure can be measured by biomarkers

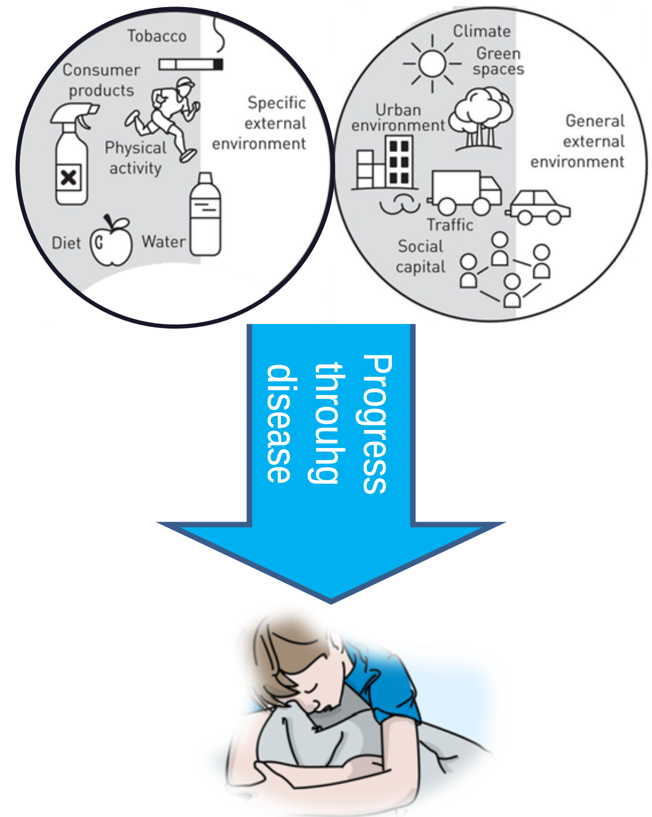
Biomarkers of Exposure

Biomarkers of Susceptibility

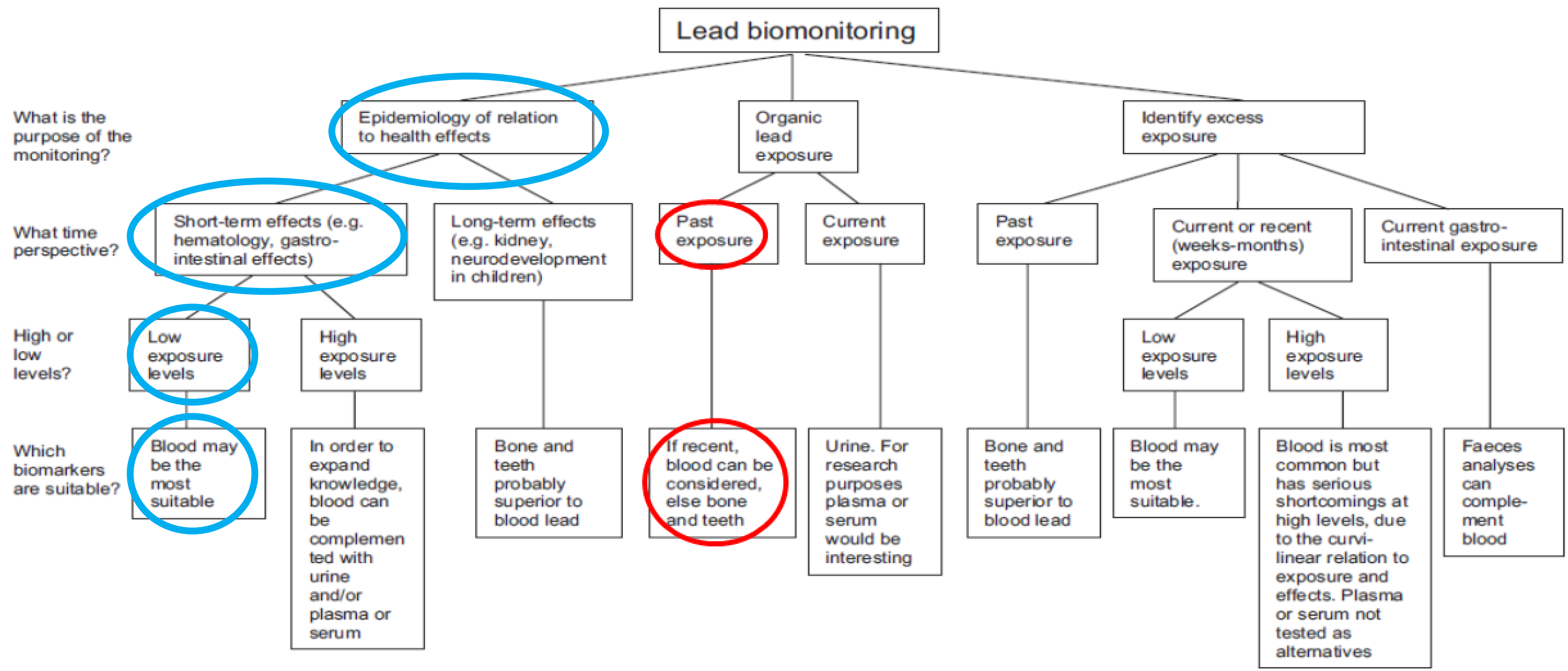
Biomarkers of effects

Marker of individual susceptibility

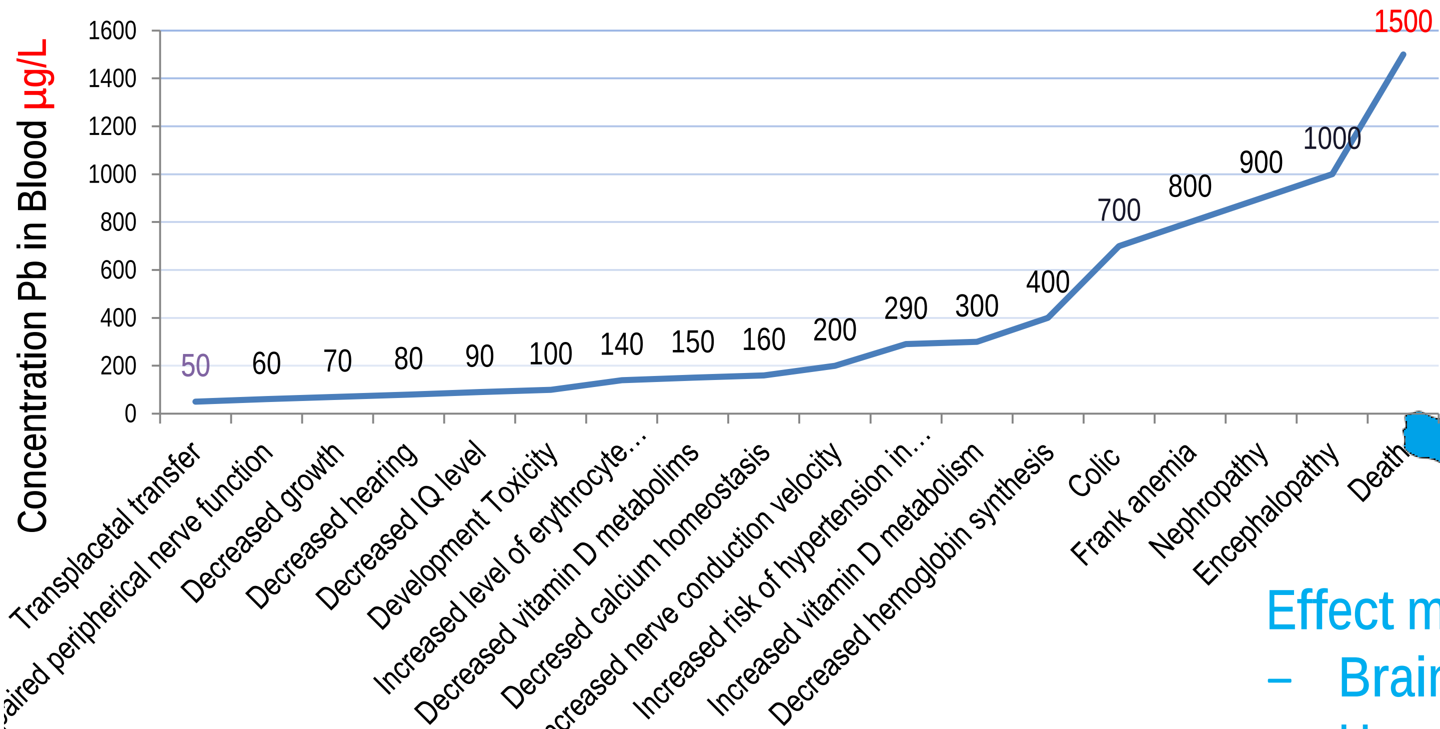
- Exposure
- Internal dose
- Effect bio-dose
- Alteration of function struture
- Clinical manifestation



Choice of biomarker exposure



Pediatric health effects of lead

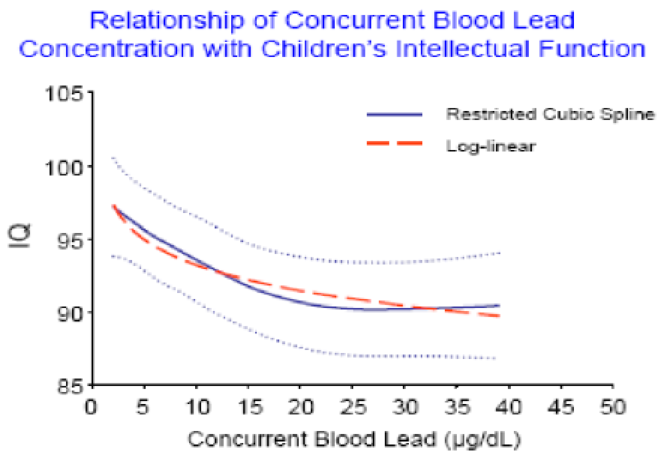


Source: Bellinger & Bellinger. (2006)

Effect mainly on:

- Brain
- Hemopoietic system
- Kidney

Neurotoxic effect of lead



Effects on intra and intercellular signaling, cell adhesion, protein folding, ionic transport, enzyme regulation, neurotransmitter release, etc.

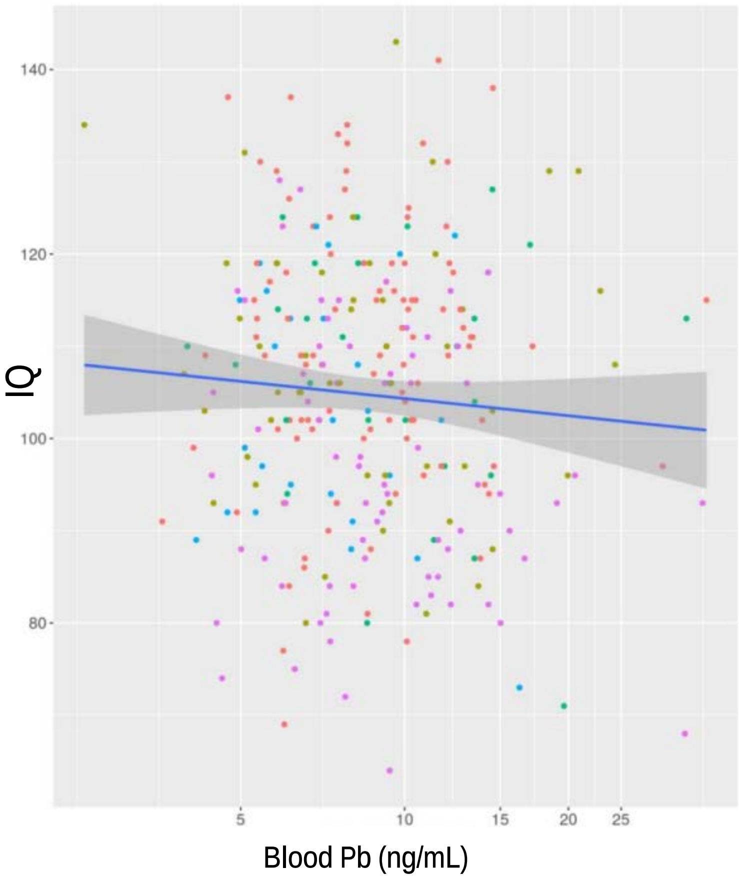
Action against brain learning and memory processes.

Ability to also replace sodium (Na) by altering the generation of action potentials in excitatory tissues.

Exposures even at low concentrations can cause a decrease in attention / hyperactivity (ADHD) in children.

Source: Lanphear et. al. 2005

IQ total in function of blood Pb (Taranto Study)



"… Note that the lead poisoning, although not in a statistically significant way, is constantly e negatively associated with IQ in all 5 models and the magnitude of the effect is comparable with that observed in other Italian territories (Lucchini et al., 2012a)…"

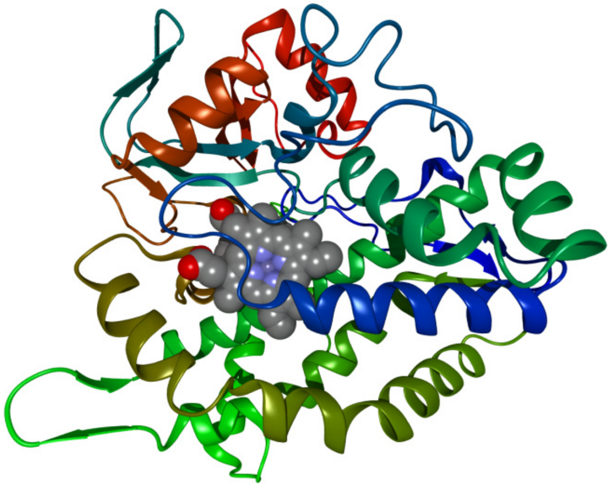
(Alimonti et. al., 2016)

Hemopoietic system effects

Lead inhibits three important **enzymesdelta aminolevulinic acid dehydratase, delta aminolevulinic acid synthase,** and **ferrochelatase**.

The critical target, however, seems to be the enzyme's heme synthesis, essential for the insertion of iron into the precursor, **protoporphyrin IX** . Derivate in are reduction of circulating levels of hemoglobin and the inhibition of **cytochrome P 450-dependent**

Inhibition of normal hemoprotein function, which results in basophilic stippling of erythrocytes related to clustering of ribosomes and microcytosis.



Cytochrome P 450

Source: Papanikolaou et. al. 2005

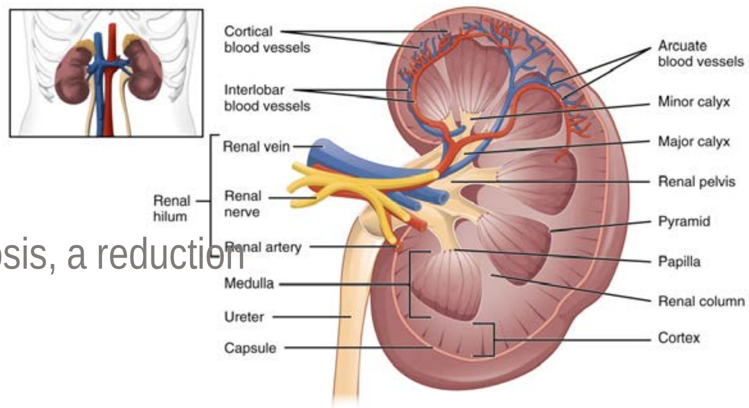
1

Kidney effects

Involves inhibition in the proximal tubular lining cells and renal insufficiency.

Early or acute nephropathy induce Fanconi's syndrome:

- Aminoaciduria
- Glycosuria
- phosphaturia with hypophosphatemia, and
- increased sodium and
- decreased uric acid excretion



Chronic lead nephropathy generates progressive interstitial fibrosis, a reduction in the **glomerular filtration rate**, and **azothemia**.

The acute form of nephropathy is most frequently in children. Studies show that Fanconi's syndrome can persist up to **13 years after lead poisoning**.

Source: Papanikolaou et. al. 2005

Economic Perspectives

Direct cost

Medical treatment estimates in US expenses US$ 43 billion annually.

Indirect cost

Educational services, institutionalization or even incarceration of people who suffered lead.

Benefits

For every US$ 1 spent to reduce lead hazards, there would be a benefit of US$17–220.

Studies calculates increase children's intelligence, can traduce in benefit of between US$ 110billion and US$ 319 billion in each birth cohort in the United States

Source: Landrigan et al., (2002), Grosse et al., (2002), Gould, (2009)

Legislations and guidelines related to Pb (Since 1986)

Year	Guidelines/Act	Relative to	Limit
1986	86/278/EEC	Soil quality	50 – 300 mg/Kg
1994	98/24/EC	Occupational exposure	0.15 mg/m³
1994	Directive 94/62/EC	Packaging	100 ppm by weight
1995	General standard for contaminants and toxins in food and feed	Food production	0.1 – 0.2 mg/Kg
1998	98/83/EC	Drinking water	10 µg/L
2000	Air Quality Guidelines for Europe	Air quality	0.15 µg/L
2001	Water quality: Guidelines, standards and health	Water quality	0.010 mg/L
2004	1935/2004/EC	Contact with Food	Limit do not defined
2006	Regulation (EC) No 2006/1881	Food production	Do not defined
2008	2008/50/EC	Air quality	0,5 µg/m³
2009	Mortality and burden of disease attributable to selected major risks	General health	Limit do not defined
2009	1223/2009	Cosmetic	Forbidden
2010	Partnership for Clean Fuels and Vehicles	Air quality	Do not applies
2013	2013/39/EU	Surface water	1.2 µg/L
2011	2011/65/EU	Electrical items	0.1% by weight
2016	Integrated Review Plan for the National Ambient Air Quality Standards for Particulate Matter	Air quality	0.15 µg/m³
2017	EU 2017/738	Toys production	3.4 mg/Kg

Source: WHO, EC, EPA, Canadian Council of Ministers of the Environment 2019

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Reference Values (RVs)

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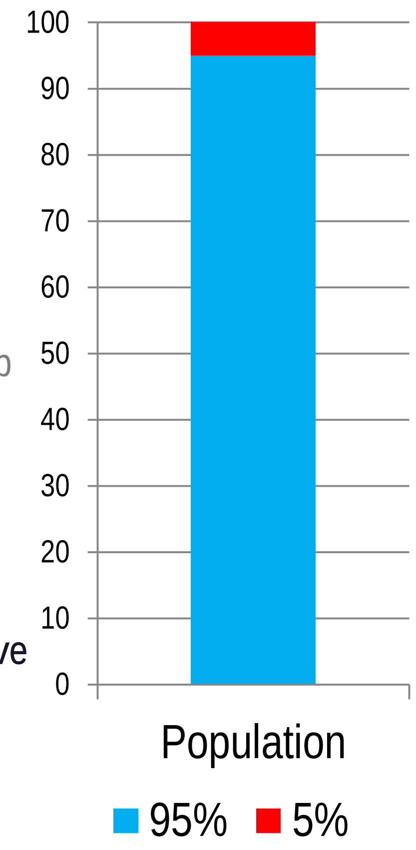
ACGIH provides a BEI of 30 µg/100 mL for Pb in blood (occupational setting)

NHANES reports 1.12 µg/dL for Pb in blood and 0.46 µg/L in urine of the general adult US population.

GerES reports 70 µg/L for females, 90 µg /L for males and 33.8 µg/L for children for Pb in blood.

PROBE study reports a 21.6 µg /L for Pb in blood for the Italian for adolescents population.

The HBM´s values, HBM I -alert value- HBM II -action value- for Pb in blood **to date have been suspended.**



Source: ACGIH, (2002). Il NHANES, (2009-10). Bundesgesundheitsbl., (2009).

Human BioMonitoring studies that include Pb

Year(s)	Name of the Study or Acronym	Country/ Region	Population
2003-2015	**NHANES**	**USA**	**General population**
2005,2008	KorSEP	Korea	**Adults**
2006-2007	ENNS	France	**General population**
2007–2019	CHMS	Canada	General population
2008	Cz-HBM	Czech Republic	General population
2009-2010	BIOAMBIENT.ES	Spain	**Adults**
2005-2009	LIFE-study	EEUU	**Adult (couples)**
1990-1992 **2003-2006**	**GerES**	**Germany**	**Adults** **Children**
2008-2010	PROBE	Italy	**Adult and adolescents**
1991-1992	ALSPAC	England	**Mother-child pair**
1997-1999 2002-2008	INMA	Spain	**Children**
1996	JECS	Japan	**Mother-child pair**

Source: Lou et.al. (2014), Trierr et.al. (2017) Vogel, 2019.

Human BioMonitoring studies that include Pb

Year(s)	Name of the Study or Acronym	Country/ Region	Population
1999-2008	Norwegian Mother and Child Cohort MoBa	Norway	**Mother-child pair**
2002-2006	Danish National Birth Cohort	Denmark	**Mother-child pair**
2002-2006	EDEN	France	**Mother-child pair**
2002-2006	The Generation R study	Netherlands	**Mother-child pair**
2002-2003	FLEH	Belgium	**Mother-child pair**
2003-2009	HELIX	EU	**Mother-child pair**
2007-2008	Rhea	Greece	**Mother-child pair**
2008-2011	MIREC	Canada	**Pregnant Woman**
2009-2012	IRNPQ	Canada	**Mother-child pair**
2006-2007	ENFAMS	France	**Children**
2007-2009	KANC	Lithuania	**Children**
2016	TARANTO	Italy	**Children**

Source: Lou et.al. (2014), Trierr et.al. (2017) Vogel, 2019.

Methodology

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Methodology of quantification

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Factor Dilution 1:3

Boold Sample (BS)

1) 1000 µL BS

2) 2000 µL HNO_3

Digestion

3) 3h - 83 ±3°C

4) 1000 µL digest BS

5) 3500 µL H_2O

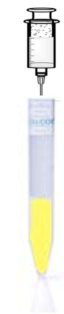
Dilution 1:5

6) 50 µL IS

In [100ppb]

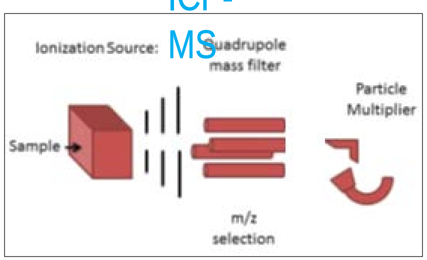
Internal standard addition

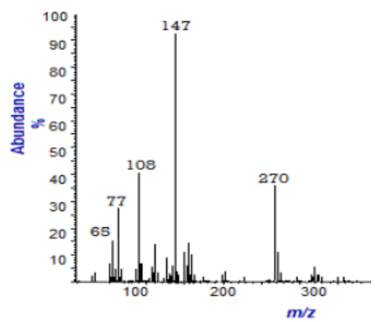
Final dilution factor 1:15

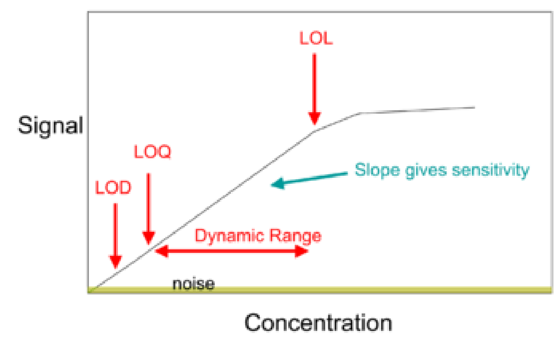


Preparated Samples

ICP-MS







Development and accreditation of the method

The analytical procedure validation process

First validation level

Evaluate method performances:
- specificity
- LoD and LoQ
- linearity
- trueness
- recovery
- intermediate precision

Develop Internal Quality Control (IQC) procedure:
- procedural blank
- calibration curve linearity
- recovery on IQC samples
- repeatability limit on IQC samples
- use of control chart (CC)

Calculate the expanded uncertainty
- uncentainty (U) associated through profile

Second validation level

Participate to External Quality Assessment Schemes (EQUAS):
- identify a suitable Proficiency Testing (PT)
- participate to PT regularly
- verify the outcome and apply remedial action when necessary

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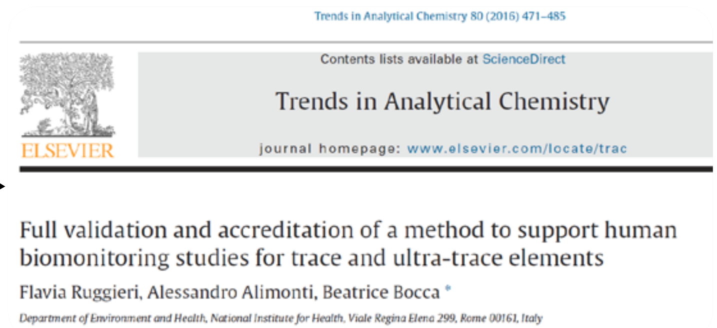
Trends in Analytical Chemistry 80 (2016) 471–485

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

ELSEVIER

Full validation and accreditation of a method to support human biomonitoring studies for trace and ultra-trace elements

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Department of Environment and Health, National Institute for Health, Viale Regina Elena 299, Rome 00161, Italy

Source: Ruggieri, Alimonti, Bocca. 2016

Method used for Pb quantification

- Biological matrix: blood
- ^{208}Pb
- Reference cetificed material used: ClinCheck® Level I (59.1 µg/L) and II (228 µg/L)
- Interval of concentration (sensitivity): [ng/L - µg/L]

linearity: 0.50 – 500 µg/L

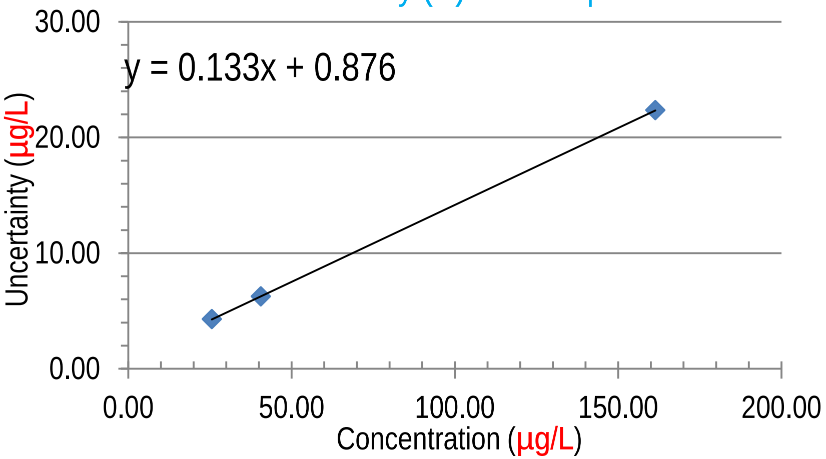
LoD: 0.45 µg/L

LoQ: 1.48 µg/L

CV% recovery 80-120% (~106%)

Uncentainty (*U*) associated through profile:

Profile of uncertainty (*U*) for Pb quantification



Result, finding and perspectives

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Summary of Results

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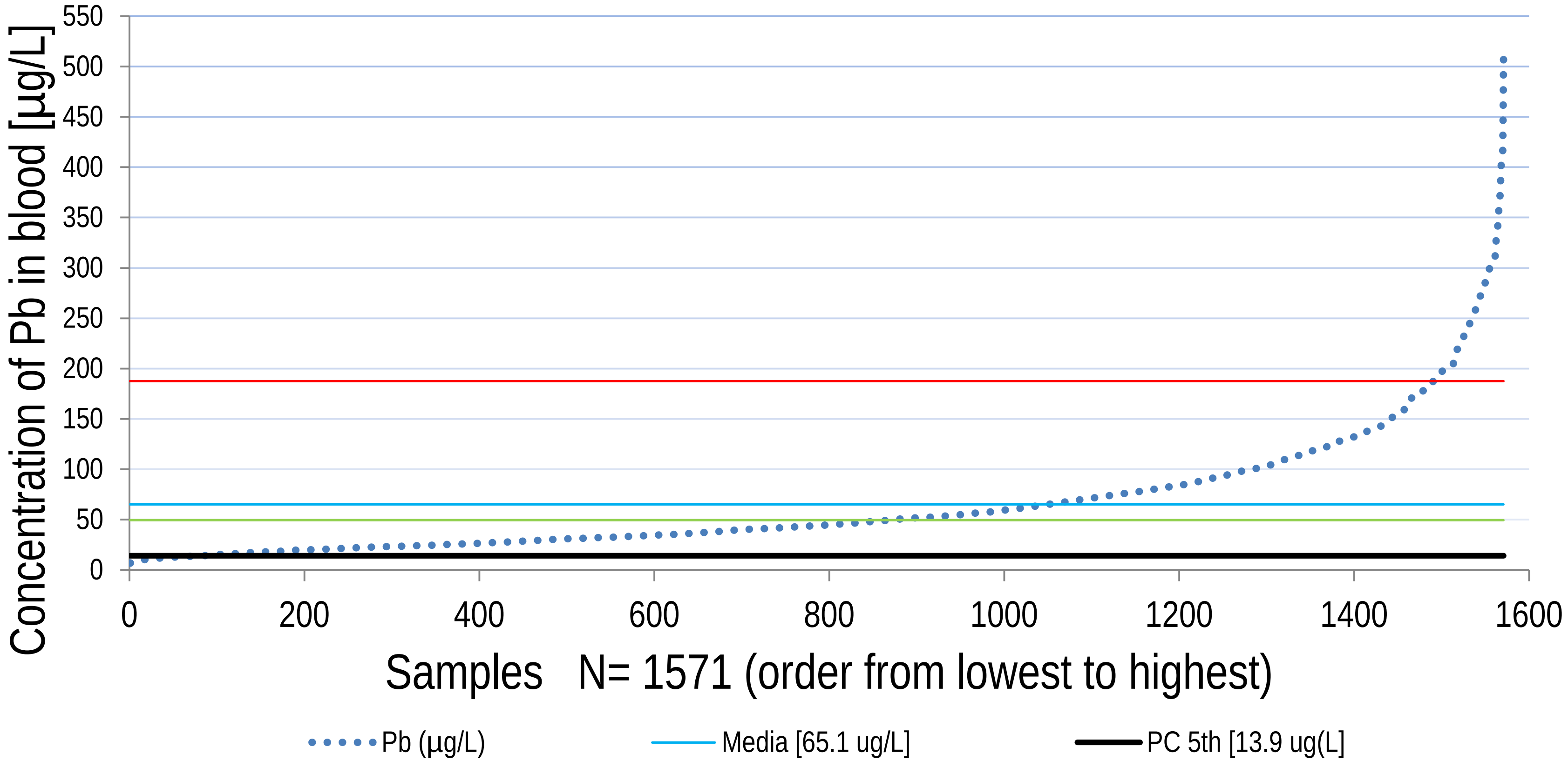
	Mn (µg/L)	As (µg/L)	Cd (µg/L)	Hg (µg/L)	Pb (µg/L)
LoDs µg/L	0.47	0.27	0.24	0.48	0.45

	Mn (µg/L)	As (µg/L)	Cd (µg/L)	Hg (µg/L)	Pb (µg/L)
Samples N	1,571	1,571	1,571	1,571	1,571
media	12.9	1.66	1.05	0.86	65.1
min	3.00	<LoD	<LoD	<LoD	6.78
max	94.9	43.1	11.5	11.9	518.5
mediana 50th	12.0	0.45	0.48	0.60	49.49
5th	7.10	0.28	0.25	0.49	13.94
95th	21.3	8.35	3.25	1.91	187.4

Lead (Pb)

Value of Pb in blood samples (µg/L) order from lowest to highest



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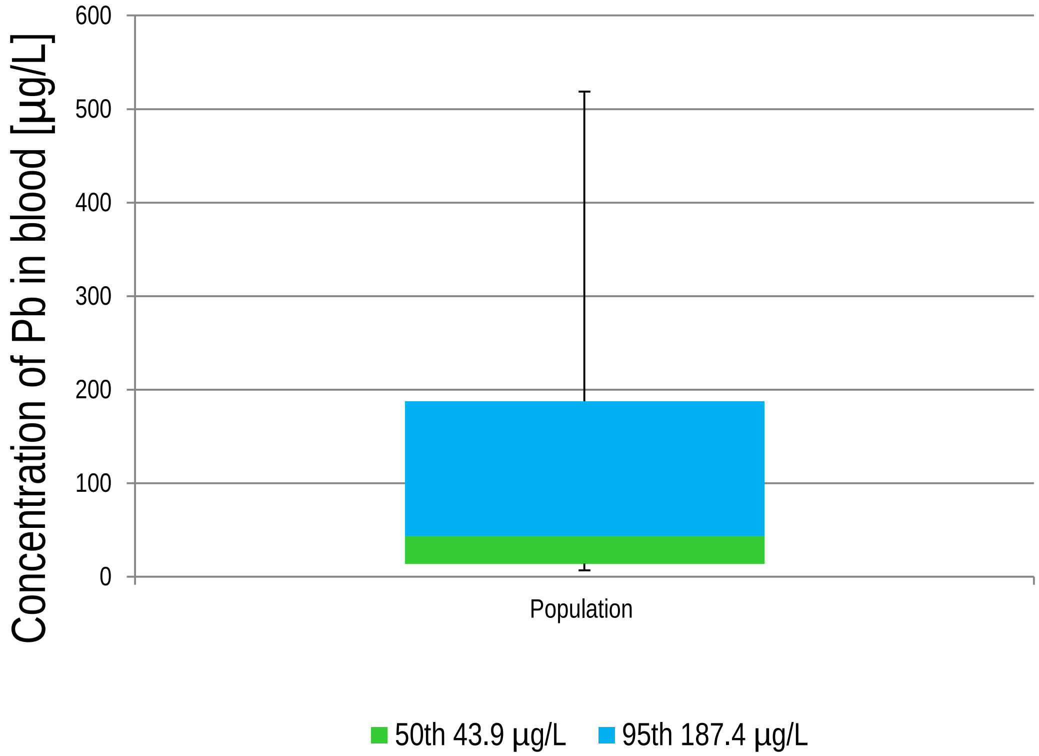
Georgia's children study 2019



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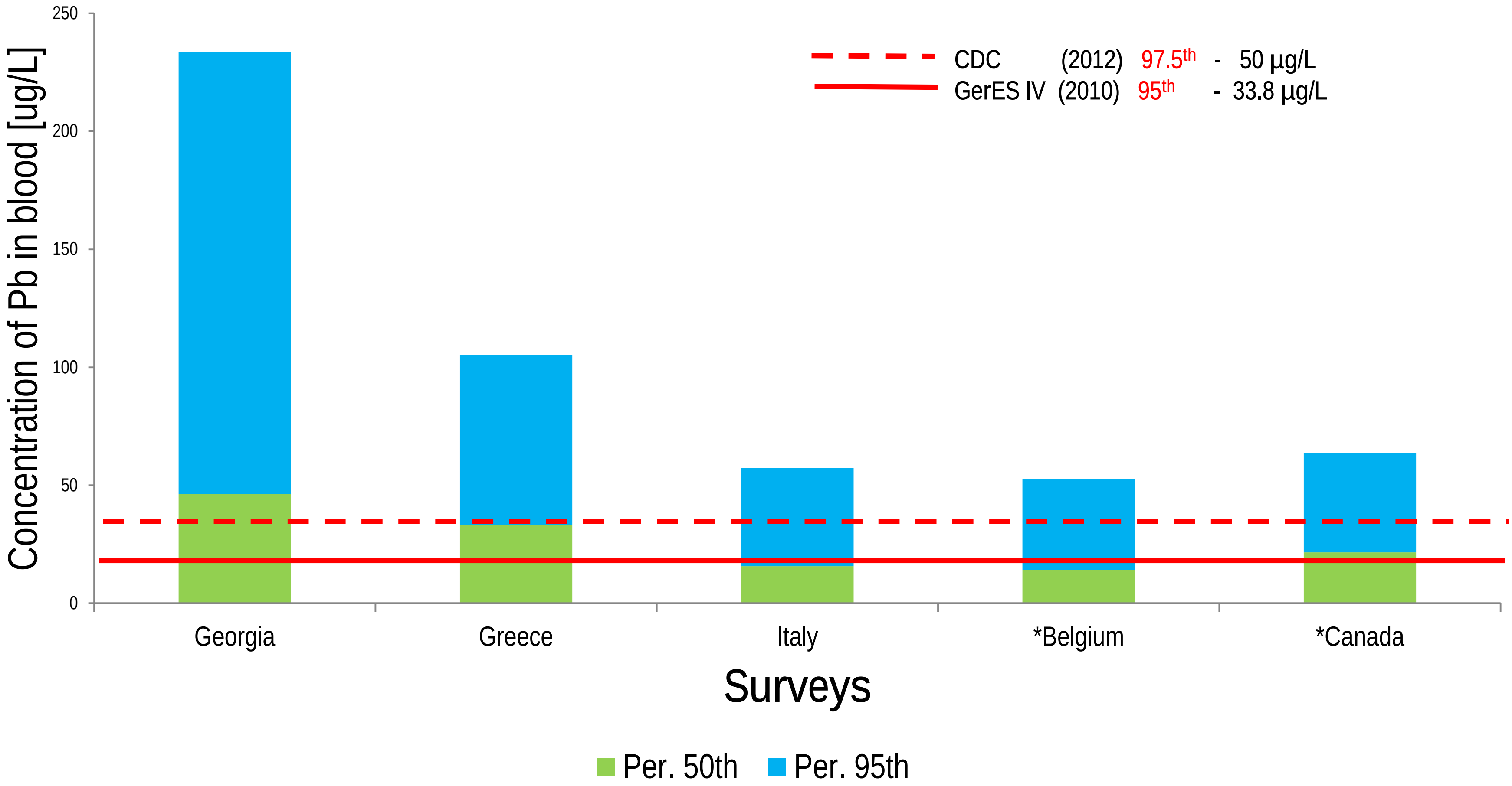

Finding and perspectives

The current **world goal** is to reduce exposure at the lowest level possible.

Georgian context a great challenge will be to evaluate the potential lead sources, both indoor and outdoor, as first attempt to reduce their contribution.

The generation and implementation guidelines-police in order the regulated the spread of lead in their environment.

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Further reading

H2020-MSCA-ITN-2017 GA - 766251

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Nowadays still matters

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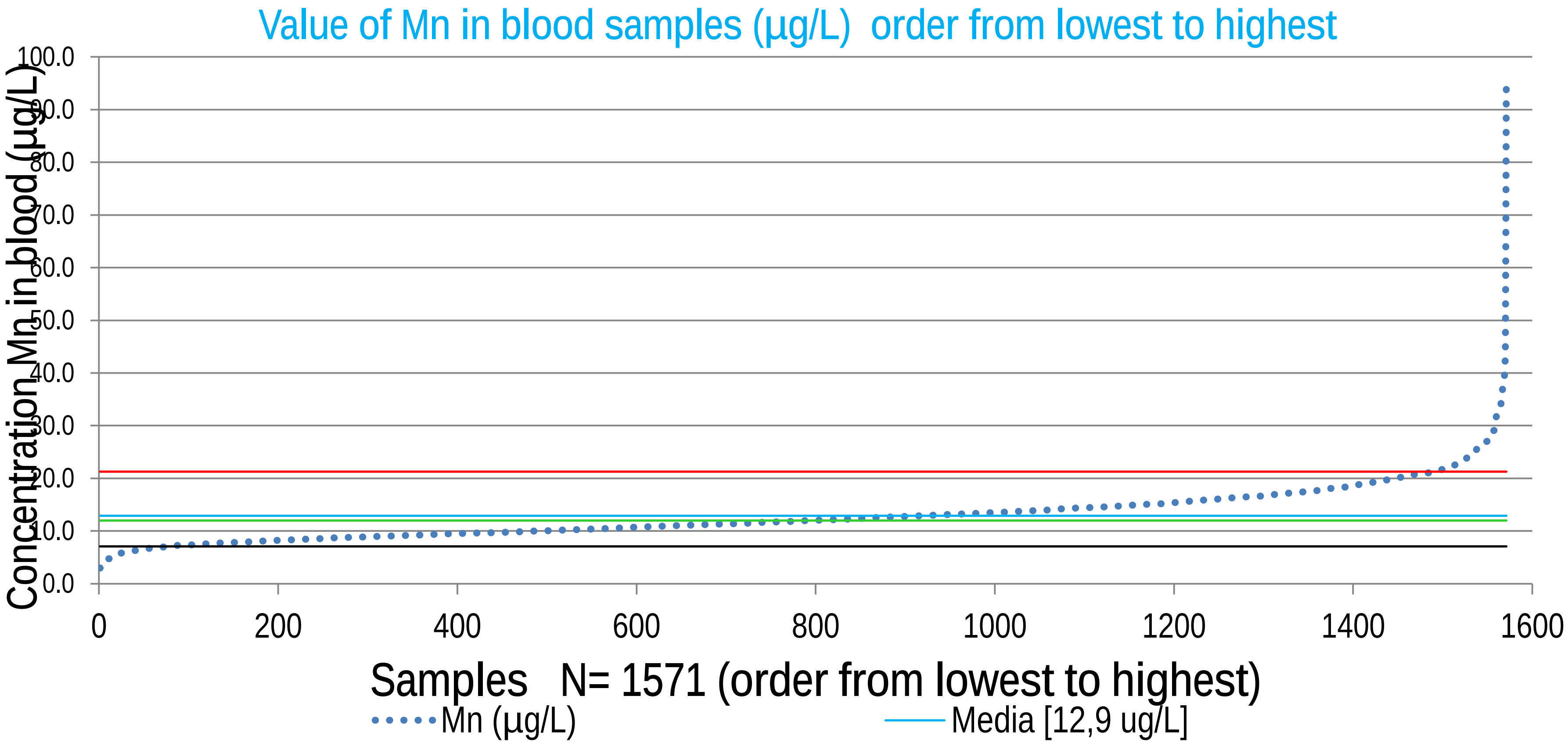
Source: WHO (2019)

Manganese (Mn)


Value of Mn in blood samples (µg/L) order from lowest to highest
Concentration Mn in blood (µg/L)
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
200
400
600
800
1000
1200
1400
1600
Samples N= 1571 (order from lowest to highest)
Mn (µg/L)
Media [12,9 ug/L]

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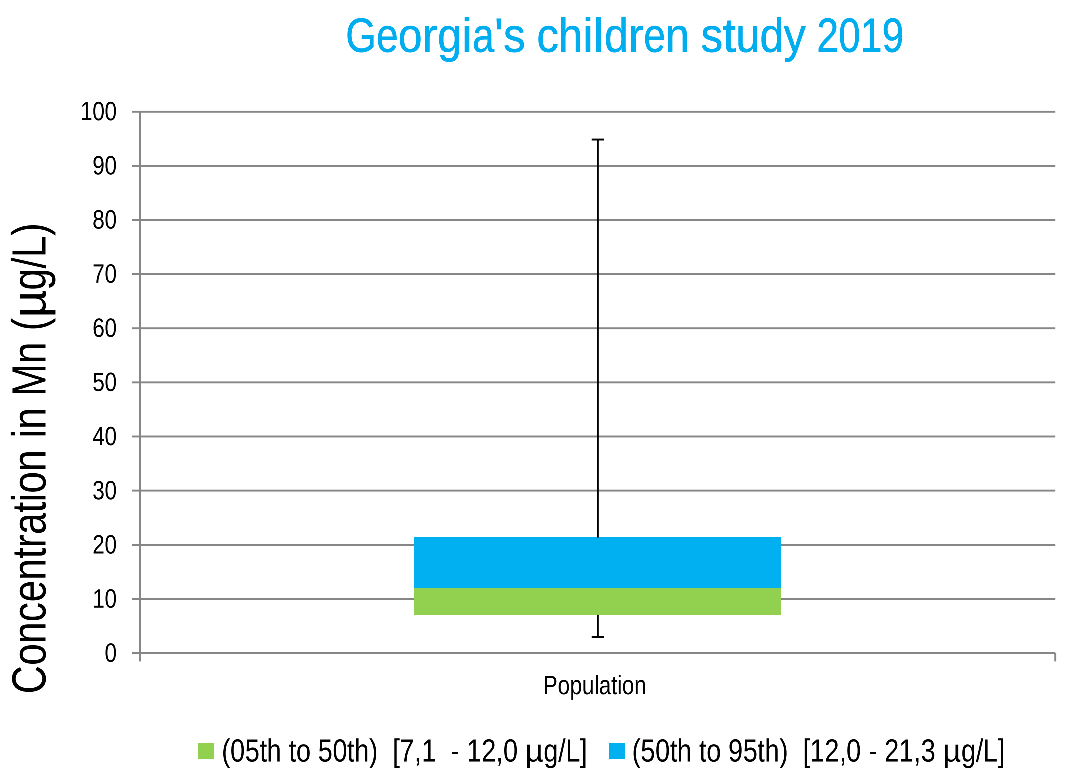

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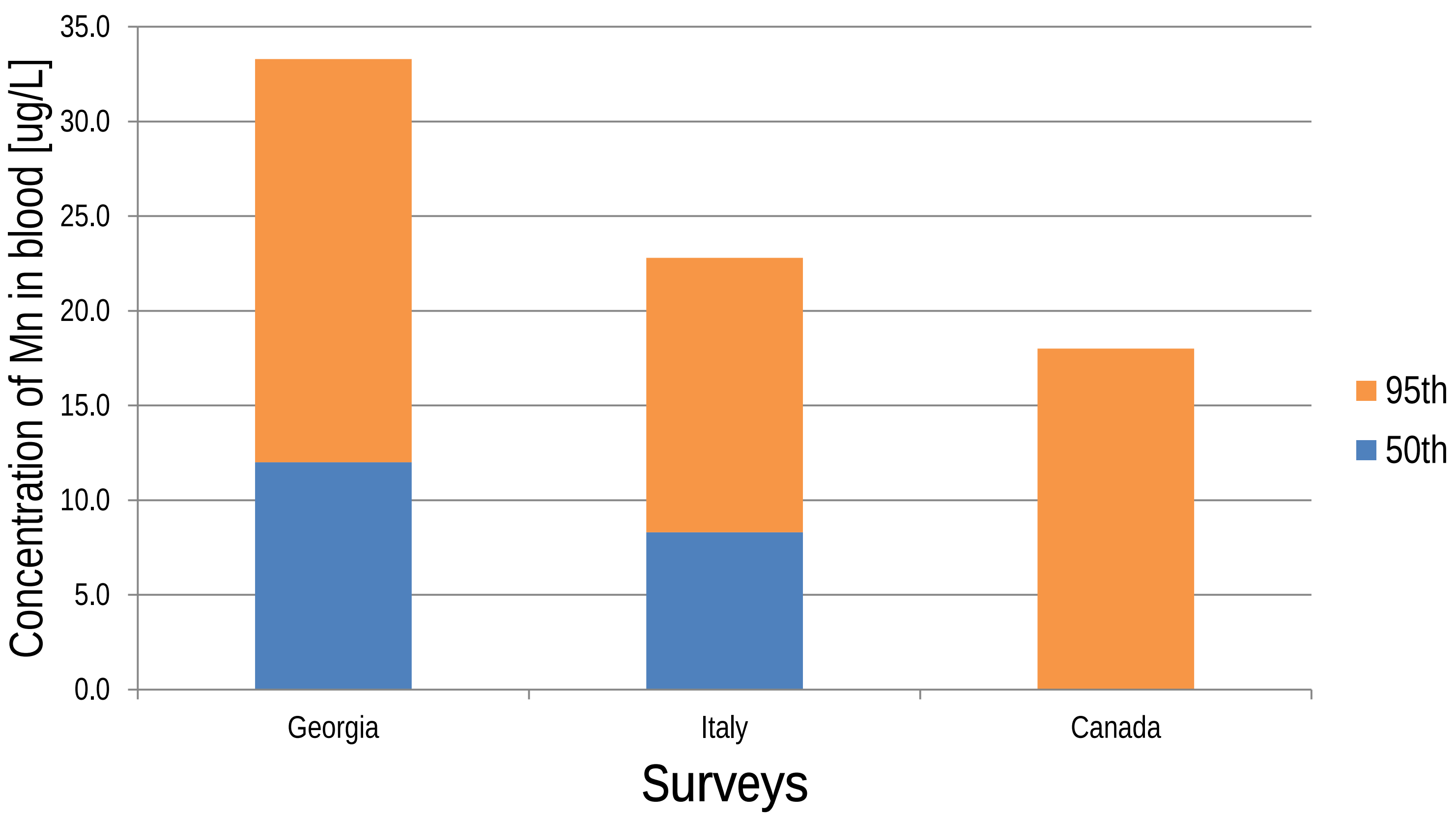
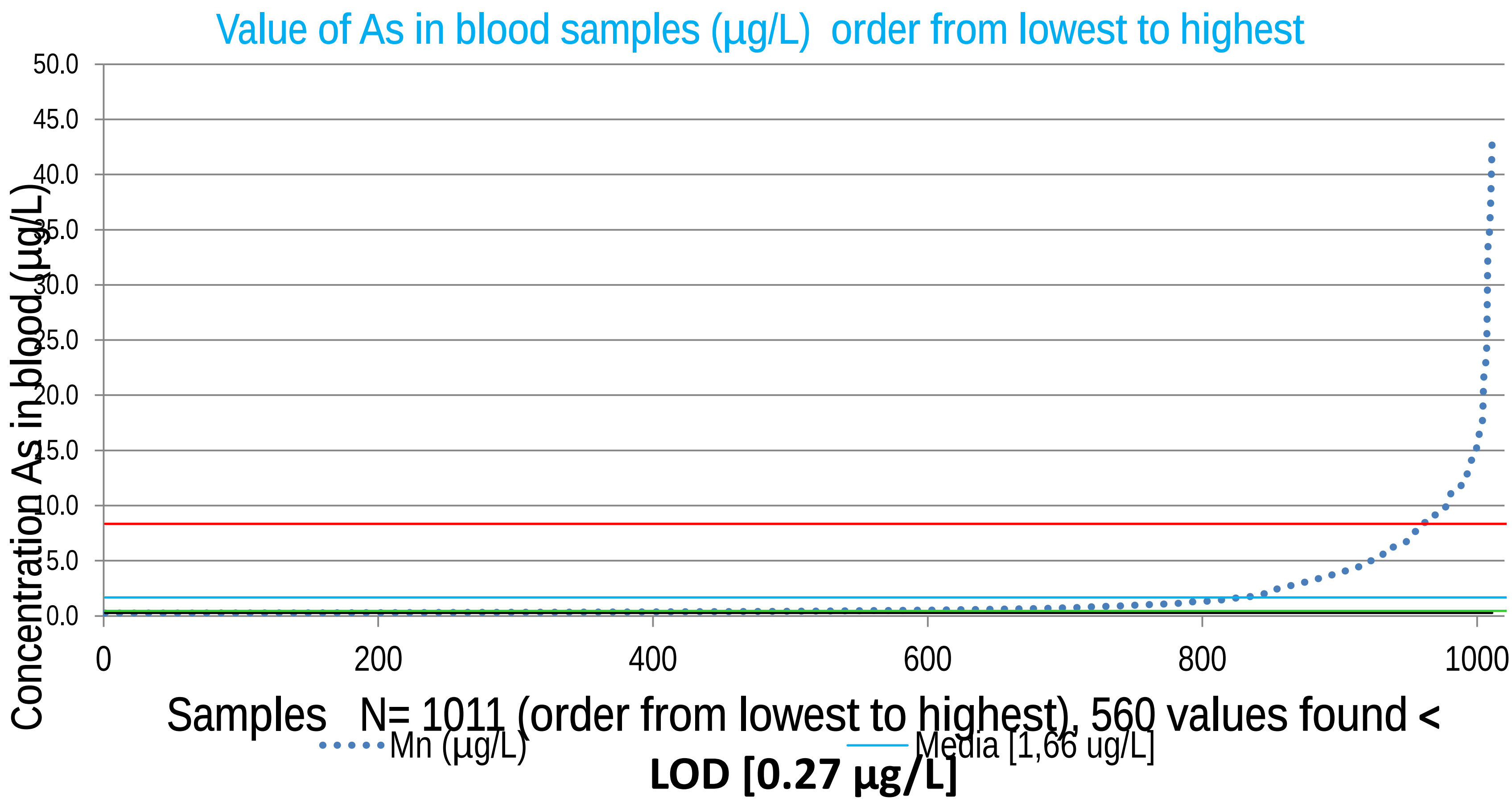


Arsenic (As)


Value of As in blood samples (µg/L) order from lowest to highest
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0
200
400
600
800
1000
Concentration As in blood (µg/L)
Samples N= 1011 (order from lowest to highest), 560 values found <
LOD [0.27 µg/L]
Mn (µg/L)
Media [1,66 ug/L]

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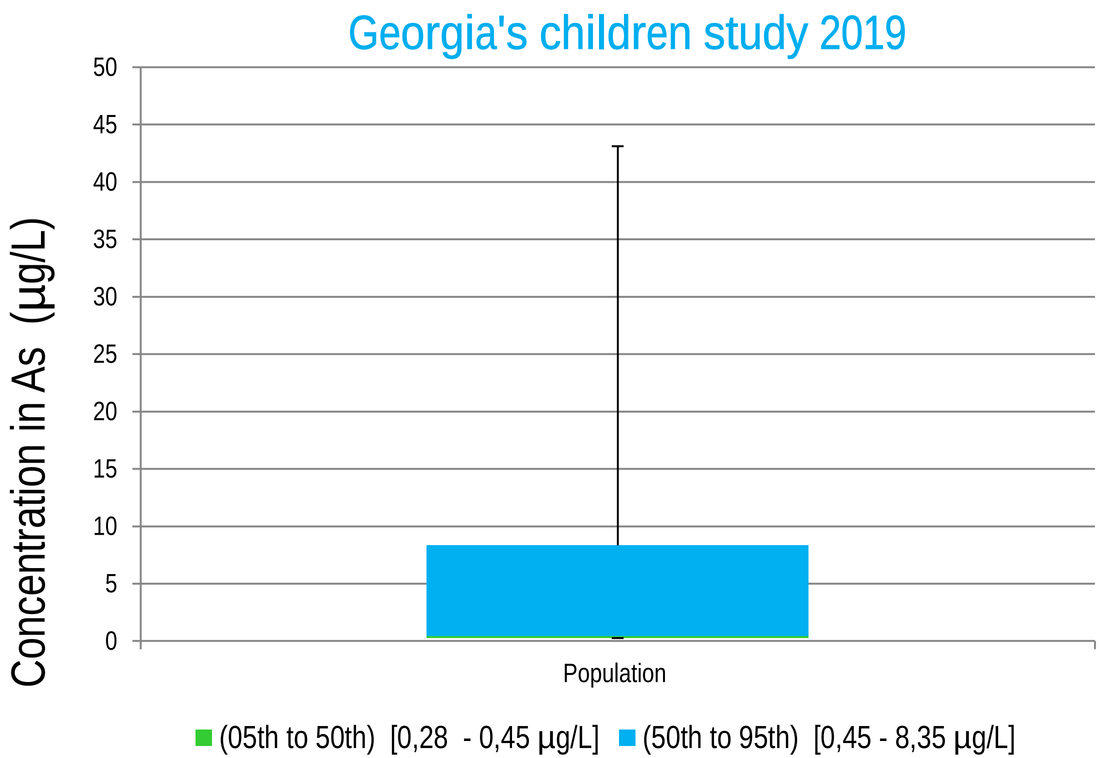

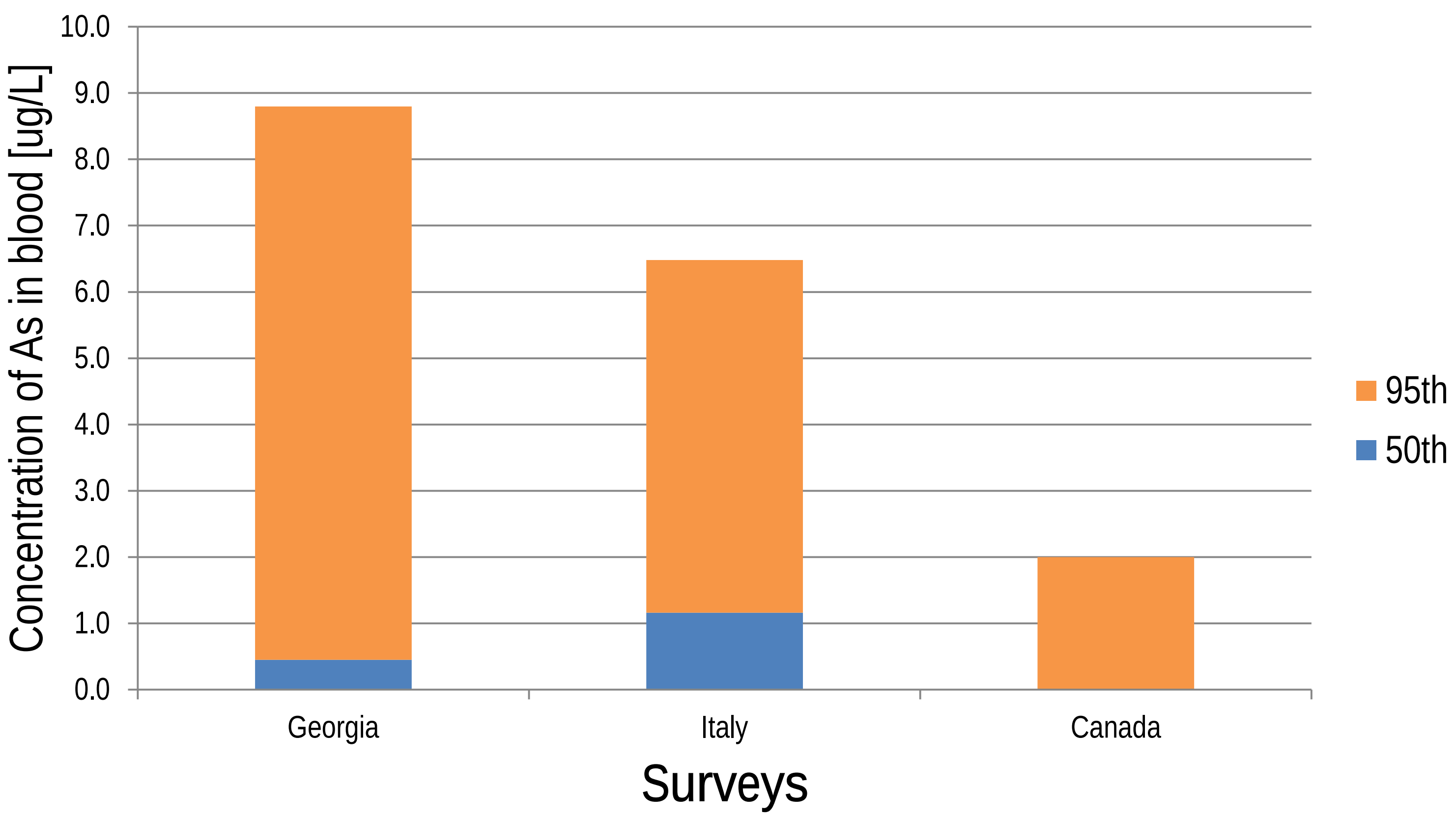
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Concentration of As in blood [ug/L]
Georgia
Italy
Canada
Surveys
95th
50th

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Cadmium (As)

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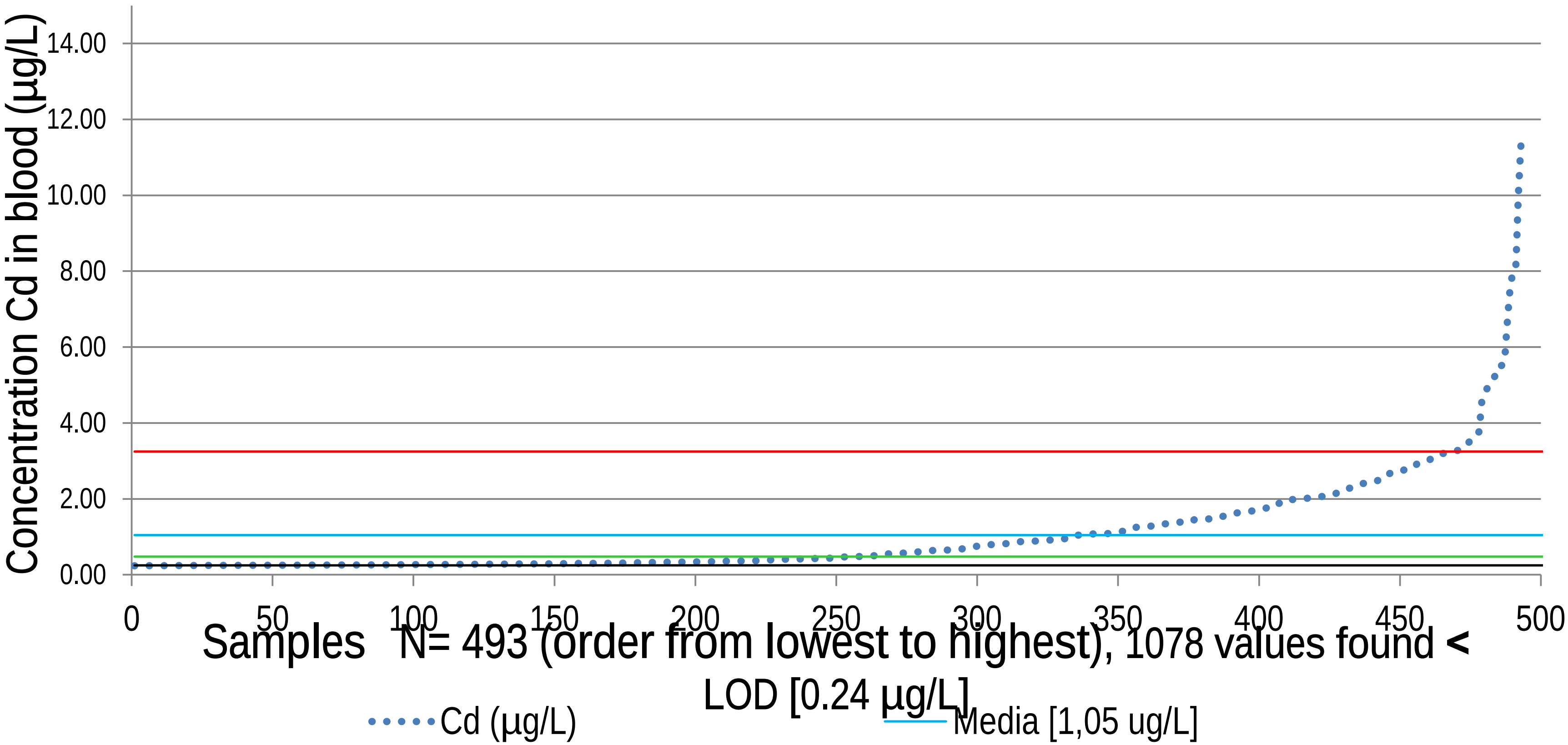
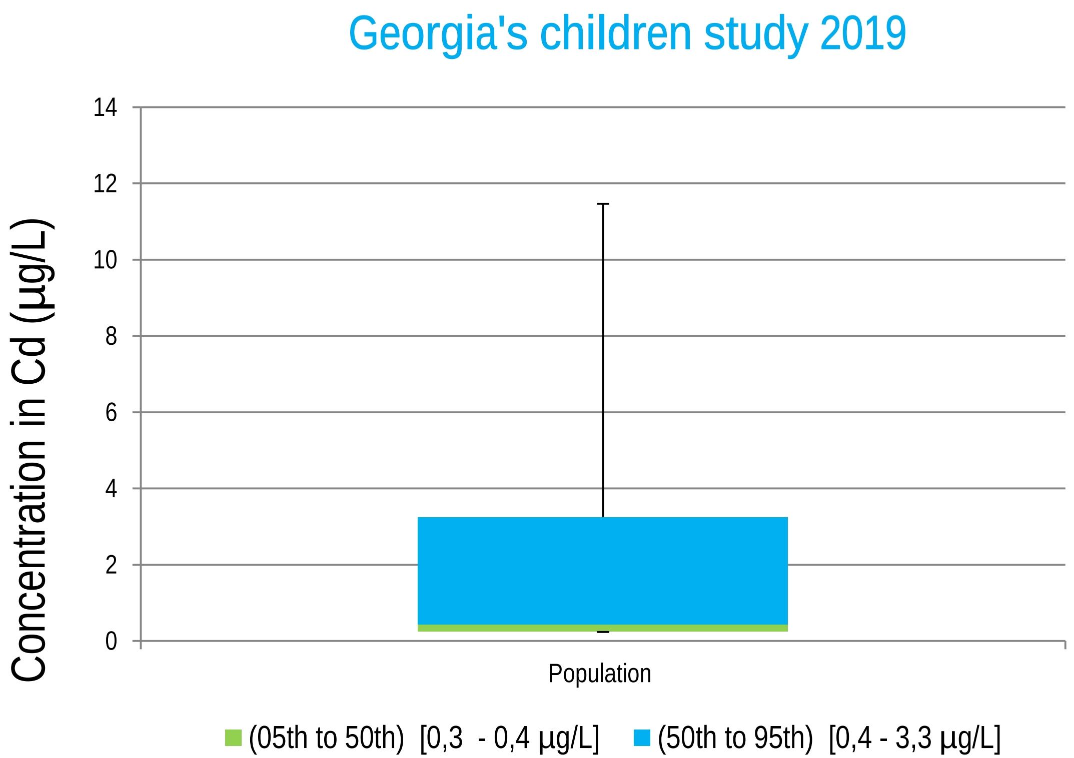
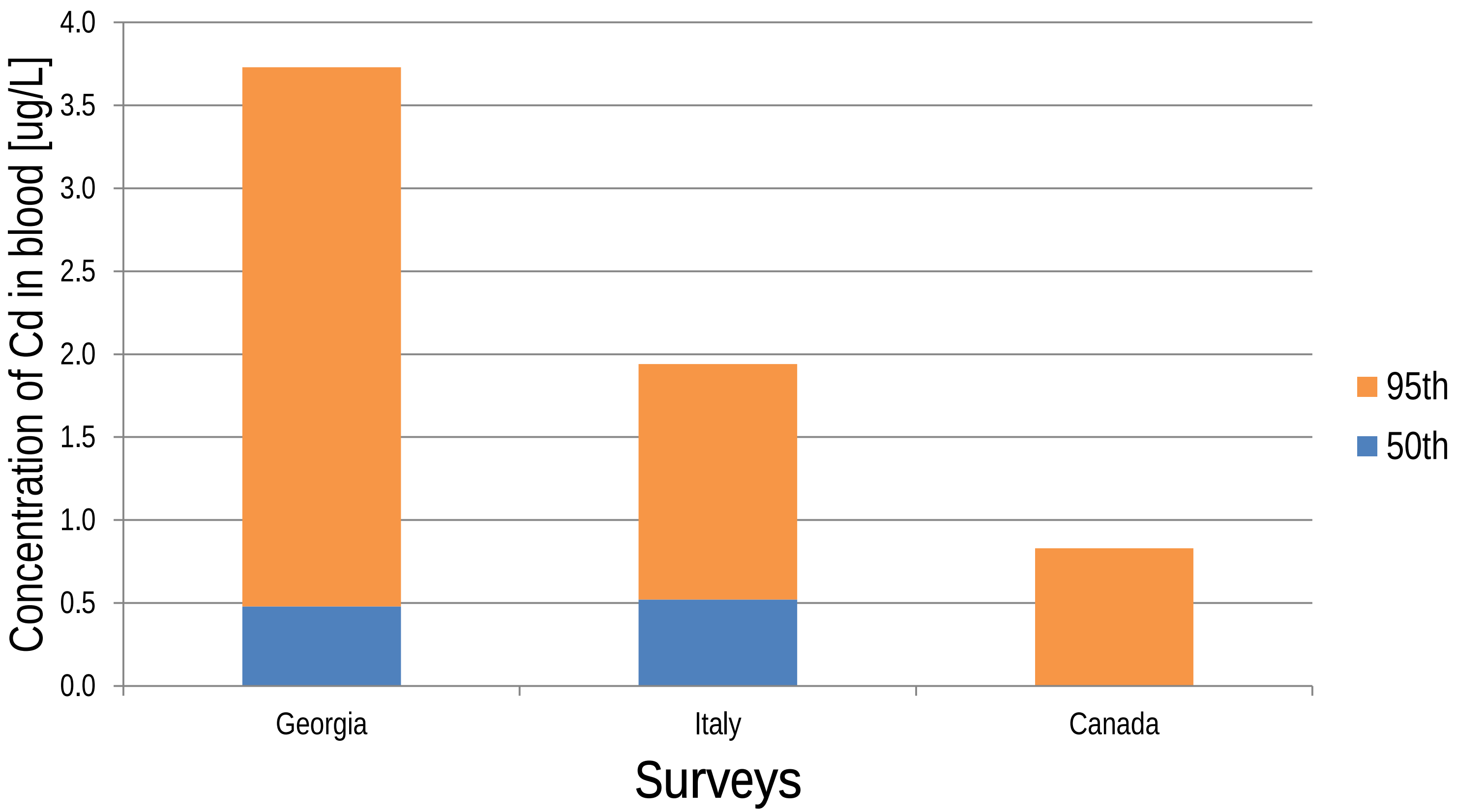
H2020-MSCA-ITN-2017 GA - 766251
NEUROSOME: First training event
Heraklion, Crete, May 2019

Value of Cd in blood samples (µg/L) order from lowest to highest

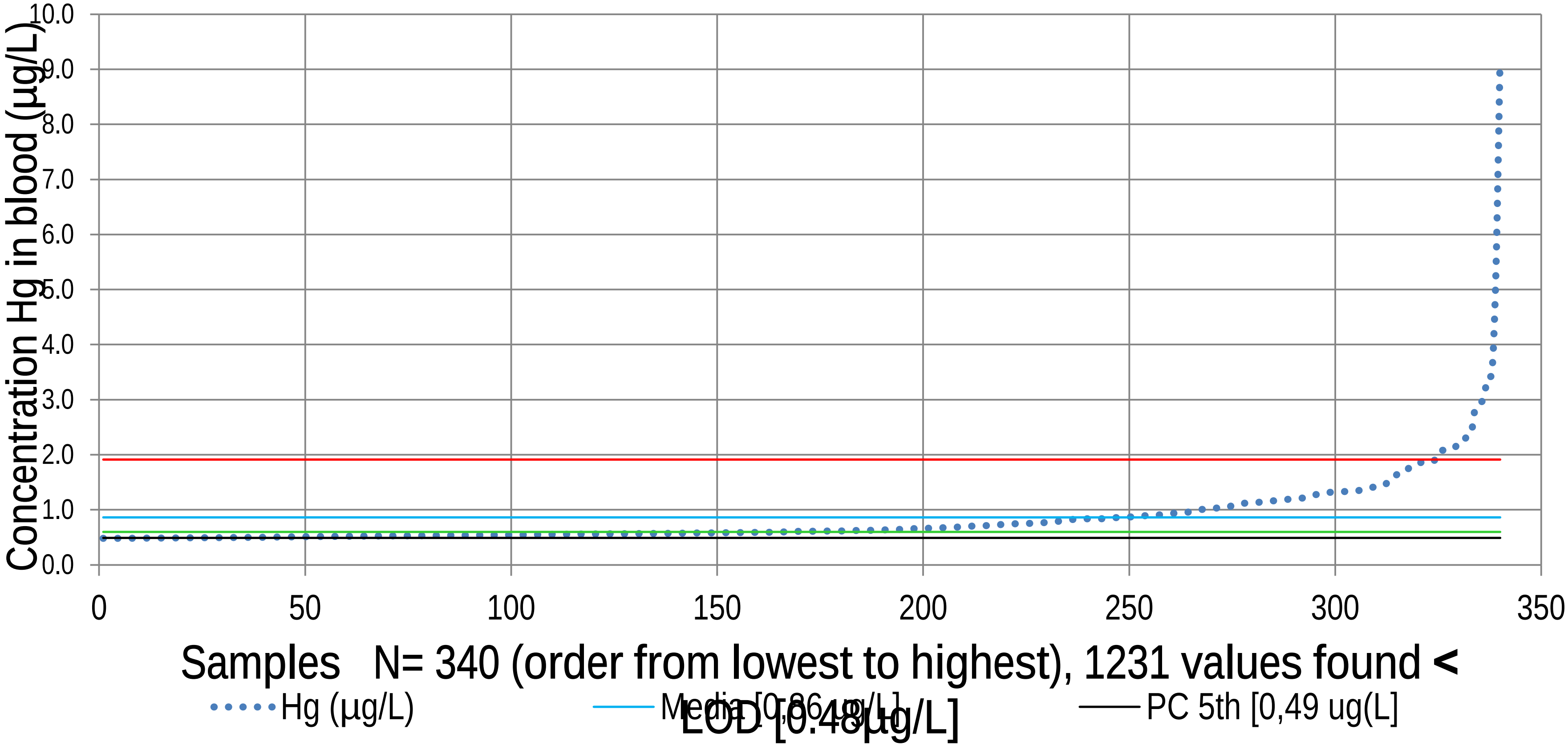


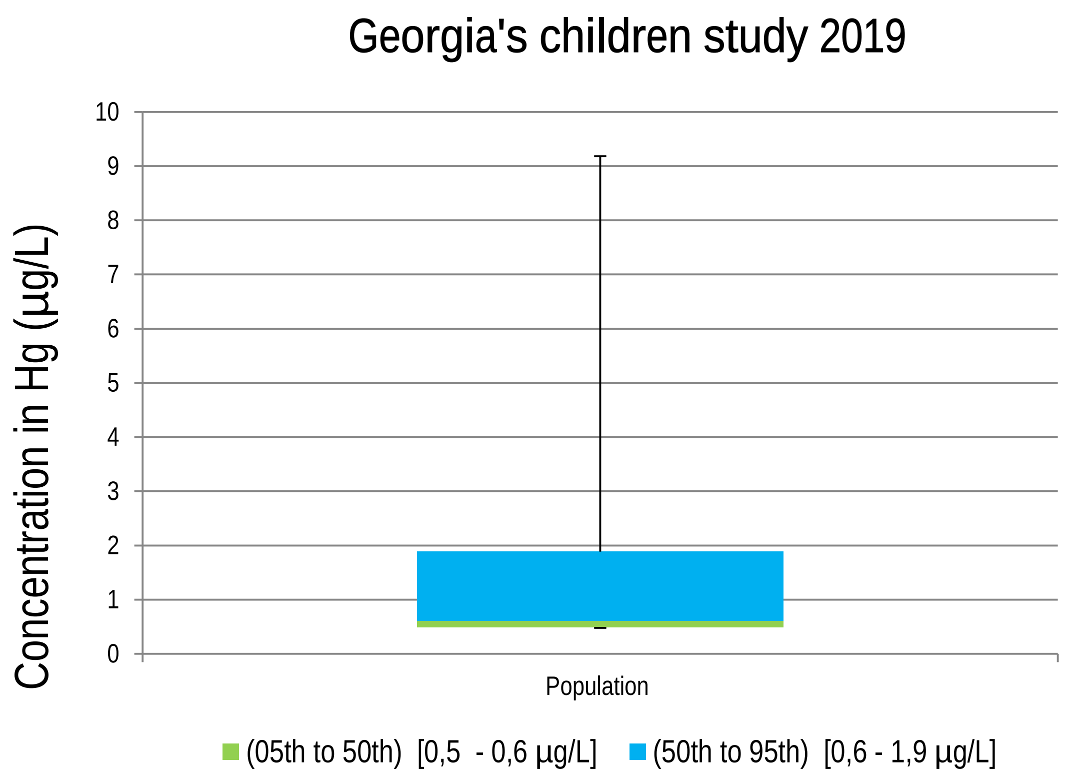
NEUROSOME
H2020-MSCA-ITN-2017 GA - 766251
NEUROSOME: First training event
Heraklion, Crete, May 2019

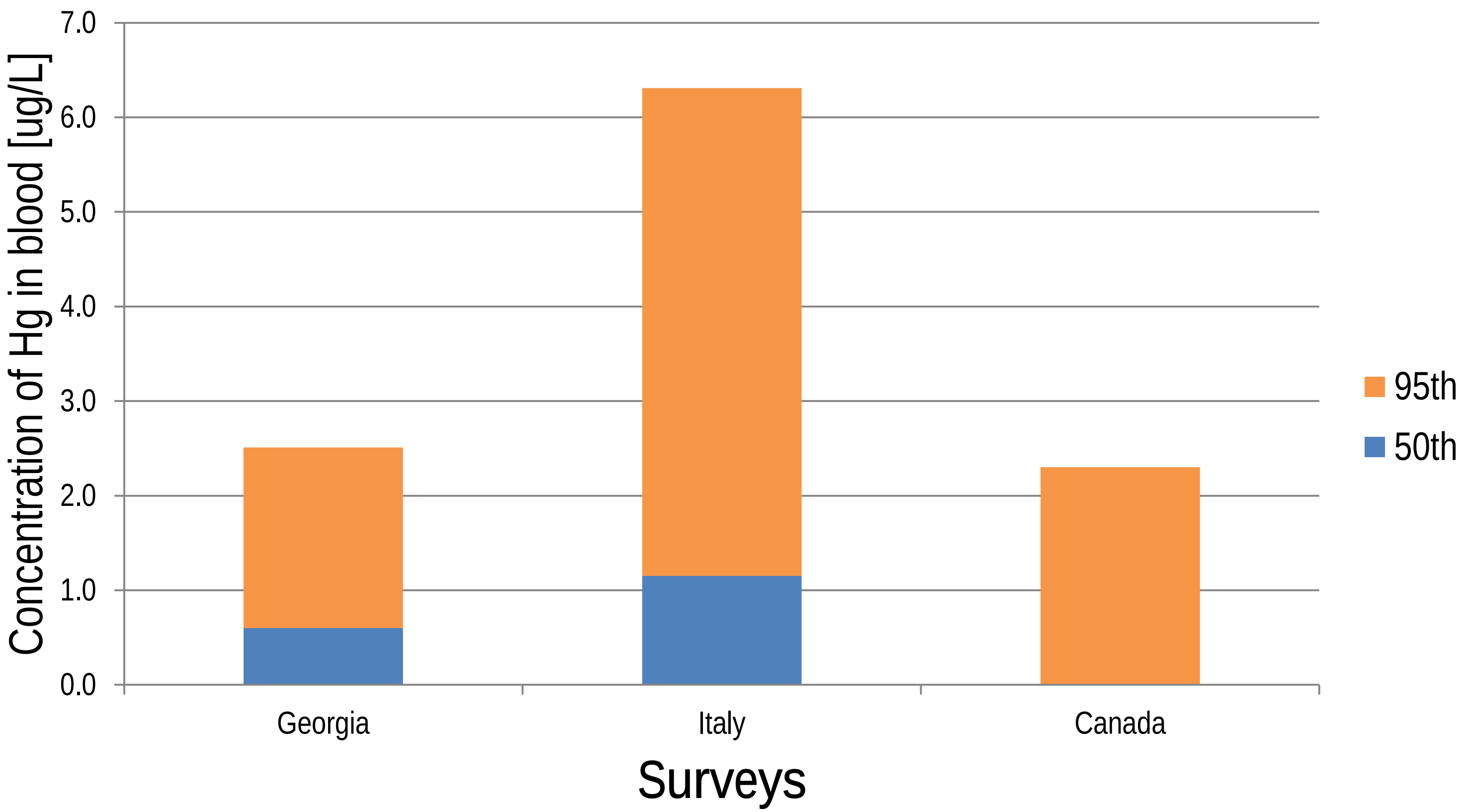


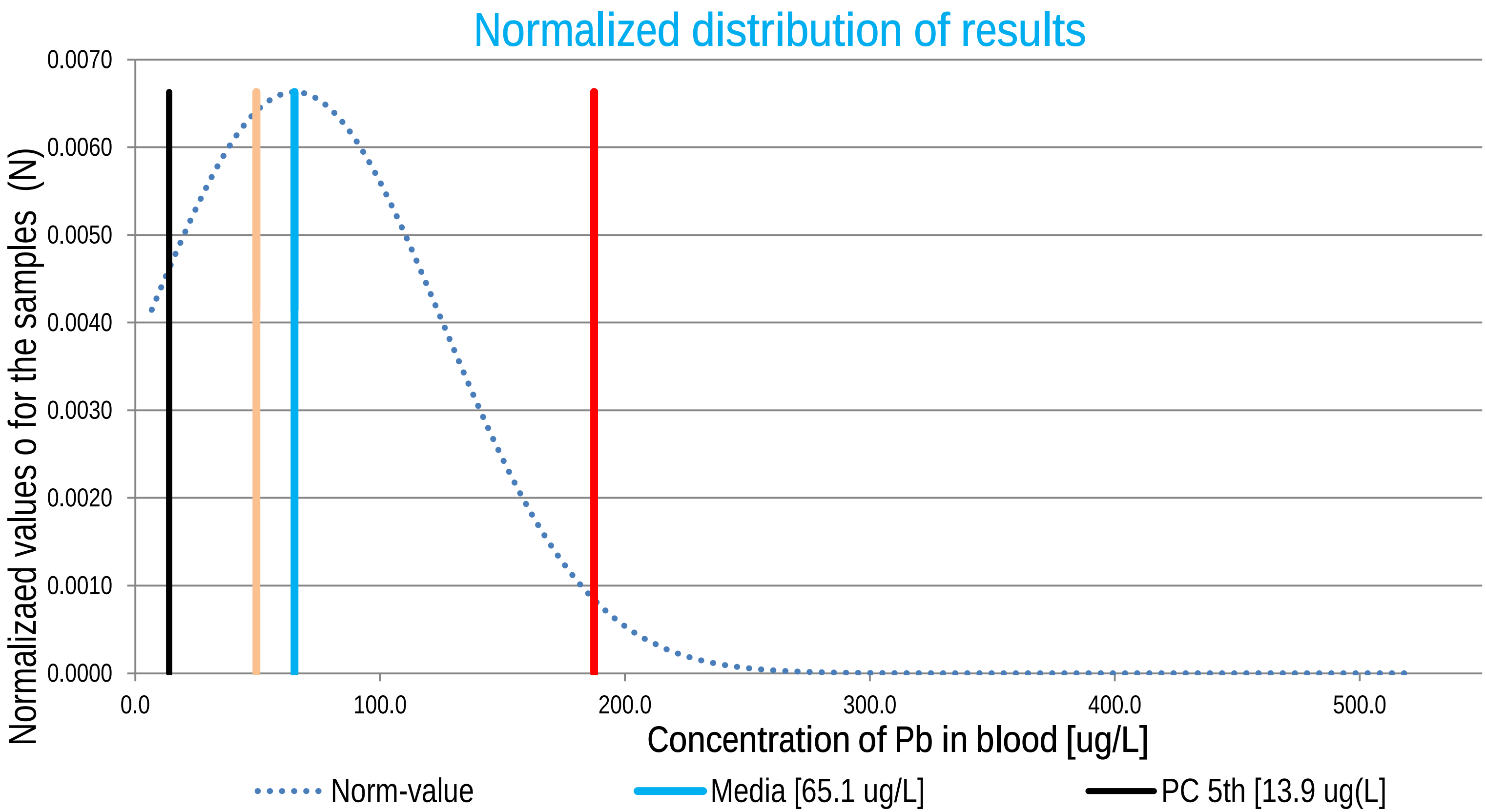

4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Concentration of Cd in blood [ug/L]
Georgia
Italy
Canada
Surveys
95th
50th

Mercury (Hg)


Concentration Hg in blood (µg/L)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
50
100
150
200
250
300
350
Samples N= 340 (order from lowest to highest), 1231 values found < LOD [0.48µg/L]
Hg (µg/L)
Media [0,86 µg/L]
PC 5th [0,49 ug(L]


Georgia's children study 2019
Concentration in Hg (µg/L)
10
9
8
7
6
5
4
3
2
1
0
Population
(05th to 50th) [0,5 - 0,6 µg/L]
(50th to 95th) [0,6 - 1,9 µg/L]


Concentration of Hg in blood [ug/L]
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Georgia
Italy
Canada
Surveys
95th
50th



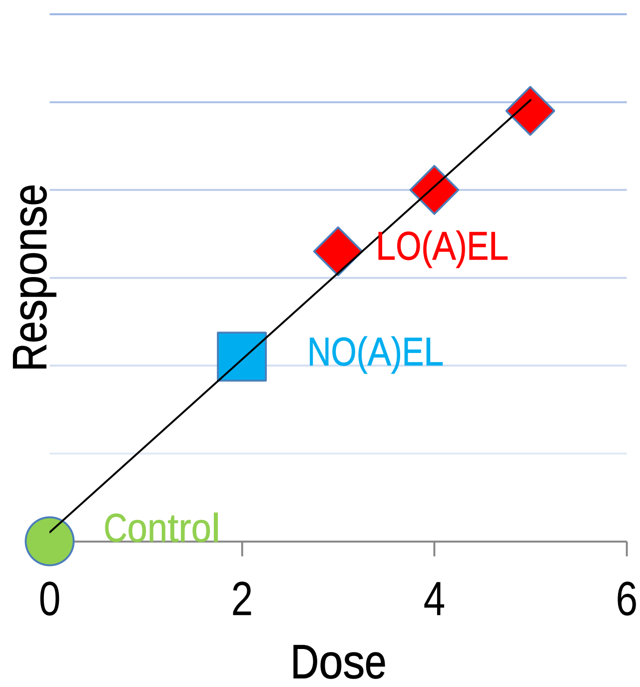
Data from ISS - Esposizione umana a contaminanti ambientali (DAMSA) - 2019

NEUROSOME

Internal dose

H2020-MSCA-ITN-2017 GA - 766251

Endocrinological					
75 men	Occupational	50–98 µg/dL (PbB range)	Decreased serum T_3 and T_4	No significant correlation for FT_4 and TSH in this PbB range. TSH, T3, FT4, and T4 increased in the range 8–50 µg/dL.	López et al. 2000
58 males, mean age 31.7 years	Occupational	51.9 µg/dL (mean PbB)	TSH significantly higher than in controls (mean PbB 9.5 µg/dL in controls)	Cross-sectional study. The association between PbB and TSH was independent of employment length. T3 was lower in a subgroup of 17 workers employed for 17.5 years than in those employed for 2.4 years.	Singh et al. 2000a
68 children, 11 months–7 years old	General population	2–77 µg/dL (PbB range) 25 µg/dL (mean PbB)	No effect on serum T_4 or FT_4	Covariates: sex, race, SES, and hemoglobin; 56% of the children had PbB <24 µg/dL.	Siegel et al. 1989
30 children, 1–5 years old	General population	33–120 µg/dL (PbB range)	Decreased serum Vitamin D levels	15 children with mean PbB of 18 µg/dL served as a comparison group.	Rosen et al. 1980
Immunological					
38 children, 3–6 years old	General population	PbB >10 µg/dL	Increased IgE and decreased IgG and IgM in females	35 children with PbB <10 µg/dL served as controls. No such effect was seen in males or in the combined analysis of males and females.	Sun et al. 2003
279 children, 9 months–6 years old	General population	1–45 µg/dL (PbB range)	Increased serum IgE	No other parameter of cellular or humoral immunity showed a significant association with PbB. Covariates: age, race, sex, nutrition, and SES.	Lutz et al. 1999



NEUROSOME

Quantification techniques for lead

H2020-MSCA-ITN-2017 GA - 766251

Technique	Acronym	Limit of detection	Observations
Flame Atomic Absorption Spectrometry	FAAS		
Graphite Furnace Atomic Absorption Spectrometric	GFAAS		
Inductively Coupled Plasma Mass Spectrometry	ICP-MS		
Electrothermal Atomic Absorption Spectrometry	ETAAS		
Inductively coupled plasma optical emission spectrometry	ICP OES		
X-ray Fluorescence Spectrometry	XRF		
Neutron activation analysis	NAA		
inductively coupled plasma-optical emission spectrometry	ICP-OES		
Cold Vaporatomic Fluorescence Spectrometry	CVAFS		
Spectroscopy Extended X-Ray Absorption Fine Structure	EXAFS		
Sensings	---		Mainly for *in situ* work

Source: Sitko et.al., (2012), Losec et. al., (2015) Istituto Superiore di Sanità

Piombo

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