DEMOCOPHES

NATIONAL REPORT ON HUMAN BIOMONITORING IN MOTHERS AND CHILDREN

BELGIUM – FPS n°1 – D17 – A52

The Belgian Steering Committee on HBM

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DEMOCOPHES - BELGIUM

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2. Abstract

Background: A harmonized European human biomonitoring pilot study was set up within the frame of the EU projects DEMOCOPHES and COPHES. In 17 European countries environmental pollutants were measured in order to gain experience among these countries and to obtain European-wide comparison values on some biomarkers. The study also serves as a demonstration project for its use in supporting environment and health policy in Europe.

Methods: In Belgium 129 school children (6-11y) and their mothers (≤ 45y) were selected, and morning urine and hair samples were collected. Field work was performed in the period of October 2011 until February 2012. Participants were recruited in the rural areas of Brakel, Ellezelles, Frasnes-lez-Anvaing and in the urban area of the capital Brussels. In urine the heavy metal cadmium, , the nicotine metabolite cotinine, several phthalate metabolites (MEHP, 5oxo-MEHP, 5OH-MEHP, MBzP, MiBP, MnBP) as well as bisphenol A (BPA) and triclosan were measured. Hair samples were analysed for mercury. By means of multiple linear regression analysis, the influence of life style, diet and home environment – information which was gathered via personal interview with the mothers – was analyzed for their influence on the urinary and hair exposure markers.

Results: 14.1% of the children/mothers who received an invitation for the study wished to participate. This number was most probably influenced by the fact that the inclusion criteria were indicated on the invitation letters distributed. Due to stringent selection on living area, age sex, and the exclusion of two or more children of the same mother, only 5.3% of all contacted people could take part in the study.

Metals: cadmium and mercury

Mercury is a naturally occurring element polluting the environment amongst others from the earth crust and is also released into the environment through man-made sources such as the burning of fossil fuels, waste incineration, plastic production plants and (forest) fires. Cadmium is a metal present in small quantities in air, water and soil. It is a by-product in the extraction of zinc, lead and copper. It is also used in batteries and in paint pigments. The levels of mercury in hair and cadmium in urine were higher in mothers than in children, with a geometric mean (GM) of respectively 0.383 vs. 0.204 µg/g for mercury and 0.21 vs 0.04 µg/L for cadmium. Urinary cadmium reflects indeed accumulation in the kidney during the years. The cadmium levels were higher in the urban area in both mothers and their children, and boys had increased levels compared to girls. Fish consumption and also amalgam fillings in mothers were determinants of hair mercury levels. Compared to the European average measured in the 17 participating countries, Belgian mothers and children had higher significant values for mercury and children had significant lower levels of cadmium in urine.

Cotinine

Cotinine is formed from nicotine after it enters the human body. It is an excellent biomarker to measure exposure to tobacco smoke, and is detectable for several days after inhalation. In the

Belgian study group, 9.3% of the mothers and none of the children were smokers. Nevertheless, cotinine was detectable in the urine of 30.4% of the children and 34.4% of the mothers. The average levels were higher in smoking mothers and in children who have reported to be exposed to environmental tobacco smoke. The urinary concentrations of the children and mothers were good correlated, indicating that smoking behavior of the mother had its influence on exposing the child passively to smoke or possibly was associated with a different attitude in protecting the child from smoky environments (Spearman rank r= 0.60). In the 17 European countries the average cotinine levels in children and mothers were significantly higher than the concentrations measured in the Belgian participants.

Phthalates

Phthalates are a group of compounds widely used in the manufacture of plastics, to make them soft and flexible. They are found in daily life products such as soaps, cosmetics, soft plastic toys, bottles, raincoats, shoes and food packaging. Most of the measured phthalate metabolites were higher in children compared to the mothers: MEHP+5oxo-MEHP+5OH-MEHP (GM 21.28 vs 36.72 µg/L), MBzP (GM 6.47 vs 8.78 µg/L), MiBP (GM 38.08 vs. 58.16 µg/L) and MnBP (GM 30.86 vs 38.97 µg/L). These metabolites originate respectively from the phthalates DEHP (di-2-ethylhexyl phthalate), BBzP (butylbenzyl phthalate), DiBP (di-isobutyl phthalate) and DnBP (di-n-butyl phthalate). The higher levels of children compared to their mothers, might be explained by a higher food consumption relative to their body weight, longer time since they urinated at the time of first morning void (in average 11 vs. 8h for mothers), and more exposure via mouth-hand contact. MiBP and MnBP were the most apparent phthalates present in urine of the Belgian children and their mothers. In the mothers, additionally MEP was present to the same extent as MiBP and MnBP. The urinary phthalate metabolite MEP originating from diethyl phthalate (DEP), was the only phthalate higher in the mothers compared to the children (GM 36.30 vs 26.18 µg/L). It is present in personal care products, which were more (often) used by mothers compared to the children.

DEHP is mainly taken up via food (Wittasek et al., 2011). Food items contain various levels of phthalates dependent on packaging and processing. It was not possible to pinpoint this exposure route, based on a general questionnaire as used in this study. In the mothers (N=129) and children (N=129) DEHP metabolite levels (MEHP, 5OH-MEHP and 5oxo-MEHP) were correlated to MiBP, MnBP and MBzP (Spearman rank r=0.30-0.55, p=0.0001). MiBP, MnBP and MBzP were often even better correlated (r= 0.48-0.60, p=0.0001). This possibly suggests similar exposure sources for DnBP, DiBP and BBP. For those phthalates indoor air and personal care products are known to have similar or sometimes higher importance than exposure via food. In this study group the levels of the urinary components MBzP, MiBP and MnBP, were determined by the presence of PVC flouring or wall paper in the participants' homes. The metabolites MiBP and MnBP were also associated with the use of personal care products in children.

MEP is the metabolite of DEP, a phthalate widely used in consumer products. In the current study, this compound was clearly to a lesser extent correlated to the other phthalate metabolites (r= 0.13-0.50, p=0.14-0.0001). The levels of urinary MEP were higher in children using plastic toys, in participants having PVC flouring/wall paper or having recently done renovation activities in house. MEP was also linked to consumption of canteen food (mothers) and chewing gum (children).

In general the variability in phthalate levels among the different European countries was relatively low. Belgian participants had lower levels of DEHP metabolites and MEP. Furthermore MBzP, MiBP and MnBP (in mothers only), were higher in Belgium compared to the European average.

Bisphenol A

Bisphenol-A (BPA) is used in coatings on the inside of cans, in plastics manufacture, in paints, varnishes and glues, and in the thermal paper used, for example, in cash machines in supermarkets.. The urinary levels were comparable for mothers and children: GM 2.55 vs 2.35 µg/L. Bisphenol A was correlated with all phthalate metabolites in both age groups (with MEP, however to a lesser extend). The BPA urinary concentration was higher if the mothers reported to consume canned food several times per week vs. once per week or less. In children, this was not observed. Bisphenol A was measured in only 6 out of the 17 European countries. The concentration ranges among those countries differed only to a minor extend, as well as among age groups and among individuals of one country.

Triclosan

Triclosan is a bactericide which is commonly used as disinfectant and conservation agent, mainly in cosmetics. For that purpose, it can also be added to materials, such as textiles, leather, plastics and rubber. Urinary triclosan levels were higher in the mothers compared to the children (GM 2.72 vs. 1.23 µg/L). In children, triclosan correlated well with the phthalate metabolites. Triclosan levels were higher if they had a moderate (vs low) use of personal care products (like make-up, shampoo, eye make-up, hair styling products, body lotion, crèmes, fragrances, deodorants, nail polish, or massage oil) and sun screens. This was not observed in mothers, possibly because of the general use of these products among all mothers.

Conclusion: The levels of the contaminants measured in the Belgian study population given in the table 0 below were nearly all below used health based guidance values, as far as they were available. Belgian participants had levels above the European average for mercury in hair and some phthalate metabolites in urine (MiBP, MBzP and MnBP). On the other hand, the urinary levels of cotinine, cadmium and the phthalate metabolites of DEHP and DEP were significantly lower than the European average.

The concentrations of all measured pollutants correlated good till rather good between mothers and their children. This means that the home environment and food consumption, which is (rather) similar among family members, determined a considerable part of the exposure. This study shows that several environmental contaminants in children and their mothers could be explained by information asked about life style, nutrition and home environment. Human urine and hair appeared to be good matrices to assess contaminants (chronically) present in daily life.

The results and conclusions were presented in a national symposium which took place on November 28th. This symposium was followed by a discussion on policy implications in a closed meeting and conclusions, gaps and propositions for action will be presented to the Joined Ministerial Conference on Environment and Health for political approval.

Table 0: Summary table of the biomarkers values for Belgian mothers and children recruited from October 2011 till February 2012

Biomarker	Population	Unit	N	%>LOQ	GM	low CI	up CI	P_{50}	P_{90}	P_{95}	N > health guidance	Observed associations with questionnaire data
heavy metals												
Mercury	mother	(µg/g)	129	95.30%	0.38	0.33	0.44	0.44	0.997	1.24	0	↑regular fish consumption ↑number of amalgam dental fillings
	child	(µg/g)	127	80.30%	0.20	0.17	0.24	0.24	0.58	0.82	0	↑regular fish consumption urban > rural
Cadmium	mother	µg/L	125	99.20%	0.21	0.18	0.24	0.22	0.51	0.65	2>HBM-I	urban > rural
		µg/g creat.	125	99.20%	0.18	0.16	0.21	0.20	0.37	0.46		
	child	µg/L	125	86.40%	0.04	0.04	0.05	0.05	0.14	0.18	0	urban > rural ↑education level of parents ↑age, boys > girl
		µg/g creat.	125	86.40%	0.04	0.03	0.05	0.05	0.11	0.14		
Smoking												
Cotinine	mother	µg/L	125	30.40%	-	-	-	0.35	74.1	905.1	n.a.	↑ smoker or last 24h exposed to ETS
		µg/g creat.	125	30.40%	-	-	-	0.35	109	776.5		

	child	µg/L	125	34.40%	-	-	-	0.35	2.9	6	n.a.	↑last 24h exposed to ETS
		µg/g creat.	125	34.40%	-	-	-	0.35	3.3	5.6		
Phthalate metabolites of DEHP												
MEHP	mother	µg/L	125	92.80%	2.16	1.81	2.59	2.3	7.2	9.1	n.a.	n.t.
		µg/g creat.	125	92.80%	1.93	1.65	2.26	1.94	4.91	6.56		
	child	µg/L	125	95.20%	2.32	1.97	2.74	2.2	8.7	13	n.a.	n.t.
		µg/g creat.	125	95.20%	2.08	1.77	2.44	1.94	7.08	10.13		
5OH-MEHP + 5oxo-MEHP	mother	µg/L	125	100.00%	18.72	16.22	21.6	18.5	57	86	0	none
		µg/g creat.	125	100.00%	16.7	14.89	18.73	15.9	40.11	53.18		
	child	µg/L	125	100.00%	34.09	29.14	39.89	30	111	184	1> HBM-I	none
		µg/g creat.	125	100.00%	30.46	26.12	35.51	27.3	96.47	157.41		
Phthalate metabolite of BBP												
MBzP	mother	µg/L	125	100.00%	6.47	5.55	7.54	6.4	17	23	n.a.	↑ PVC in floors/walls
		µg/g creat.	125	100.00%	5.77	5.05	6.59	5.52	15.53	19.82		
	child	µg/L	125	100.00%	8.78	7.44	10.36	8.6	27	35	n.a.	↑ PVC in floors/walls
		µg/g creat.	125	100.00%	7.84	6.68	9.21	8.01	23.34	31.94		
Phthalate metabolite of DiBP												
MiBP	mother	µg/L	125	100.00%	38.08	32.48	44.65	33	115	175	n.a.	↑ PVC in floors/walls
		µg/g creat.	125	100.00%	33.97	29.69	38.87	29.5	101.14	142.68		

	child	µg/L	125	100.00%	58.16	49.29	68.63	54	187	362	n.a.	↑ PVC in floors/walls ↑ use personal care products ↑age
		µg/g creat.	125	100.00%	51.96	44.35	60.88	46.42	135.94	278.89		
Phthalate metabolite of DBP												
MnBP	mother	µg/L	125	100.00%	30.86	26.88	35.43	31	89	119	n.a.	none
		µg/g creat.	125	100.00%	27.53	24.57	30.85	25.34	57.37	79.31		
	child	µg/L	125	100.00%	38.97	34.46	44.08	40	98	122	n.a.	↑ PVC in floors/walls ↑ use personal care products
		µg/g creat.	125	100.00%	34.82	30.96	39.15	33.68	84.68	99.35		
Phthalate metabolite of DEP												
MEP	mother	µg/L	125	100.00%	36.3	29.35	44.9	34	168	240	n.a.	↑ renovation in home last 2y ↑PVC in floors/walls ↑ regular use of chewing gum ↑ regular use of toys
		µg/g creat.	125	100.00%	32.39	26.38	39.76	32.69	156.45	221.62		
	child	µg/L	125	100.00%	26.18	21.56	31.8	23	103	169	n.a.	↑ renovation in home last 2y ↑PVC in floors/walls ↑ regular use of personal care products ↑ regular use of canteen food
		µg/g creat.	125	100.00%	23.39	19.41	28.19	20.88	93.48	114.18		
Triclosan												
	mother	µg/L	125	100.00%	2.72	1.87	3.96	1.56	122.39	347	n.a.	urban > rural

		µg/g creat.	125	100.00%	2.42	1.67	3.53	1.23	103.37	222.44		
	child	µg/L	125	97.60%	1.23	0.89	1.7	0.83	7.88	121.9	n.a.	↑regular use of personal care products ↑use of sunscreens ↑age ↑last 24h exposed to ETS
		µg/g creat.	125	97.60%	1.1	0.8	1.52	0.85	7.71	85.13		
Bisphenol A (BPA)												
	mother	µg/L	125	100.00%	2.55	2.16	3.02	2.3	7.47	11.63	0>HBM-I	↑regular use of canned food
		µg/g creat.	125	100.00%	2.28	1.94	2.67	2.11	5.72	9.44		
	child	µg/L	125	96.80%	2.35	1.94	2.84	2.27	8.15	13.44	0>HBM-I	None
		µg/g creat.	125	96.80%	2.1	1.73	2.54	2.03	6.68	11.42		

3. Objectives

The pilot project DEMOCOPHES aims to measure pollutants in humans on a European scale and to perform this a in coherent and harmonized way throughout Europe by means of common protocols, strategies and scientific tools. Therefore the EU project COPHES provided the 17 participating individual European countries with protocols and supported to organize the national field work activities within DEMOCOPHES. More specifically the DEMOCOPHES project wants to develop/ evaluate the following skills:

- To gain practical knowledge of access to study populations, recruitment procedures and response rates in the different European Countries.
- To test the developed guidelines, protocols and technical procedures for field work, questionnaires, chemical analyses, data handling and processing in the different countries.
  To test ethical guidelines and gain experience on ethical rules, within the frame of social and legal aspects of the different participating countries.
  To receive practical information on, evaluate and improve overall performance of national units and national laboratories involved via an inter-laboratory comparison.
  To collect comparable HBM data from different European countries.
  To obtain basic data (values) on selected biomarkers (mercury in hair and cadmium, cotinine, bisphenol A , triclosan and phthalates in urine) from all participating countries.
  To obtain basic data on the distribution of specific biomarkers among defined/selected study population strata/sub-groups of the general population.
  To test out the linkeage of HBM values with environment and health indicators.
  To assess the costs of the applied HBM program, preferably including a concept to improve time and cost efficiency.
  To develop practices and guidelines for effective communication and raise awareness for the wider public and policy makers.
- To establish protocols for the translation of HBM results into policy recommendations

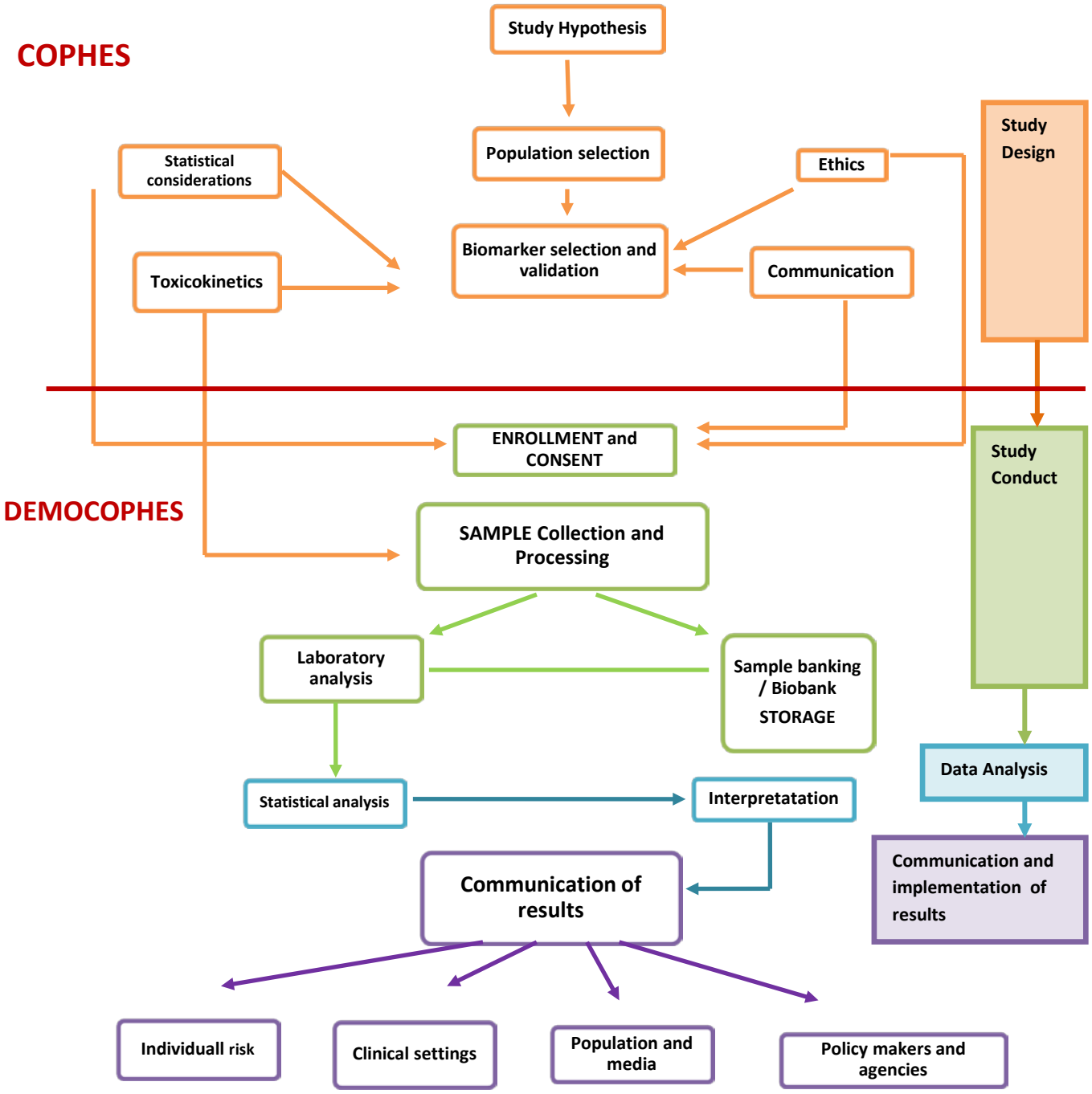


Figure 1: Schematic overview of tasks in the COPHES and DEMOCOPHES projects: the study design and protocols were worked out within the COPHES group. They needed to be implemented in each country

4. Management & organization

4.1 Financing and steering committee

DEMOCOPHES was funded by the European Union with a LIFE+ 'Policy and governance', Grant Agreement LIFE09/ENV/BE/000410. The Belgian project was co-financed by the Ministers of Environment and Health. It was coordinated by the Federal public service for health, food chain safety and environment (FOD) and followed up by the Belgian Cell Environment and Health including all federal, Flemish, Walloon and Brussels governmental entities working on Health and Environment.

The steering committee consisted of representatives of those offices, contractors performing practical work/reporting and other representatives of different environment and health related administrations:

Dominique Aerts (FPS, DG5, project leader DEMOCOPHES), Pierre Biot (FPS, DG5, project leader DEMOCOPHES), Karen Van Campenhout and Caroline Teughels (Flemish Environment Nature and Energy Department, unit Environment and Health, LNE), Priscilla Declerck (Brussels Environmental Institute, BIM-IBGE), Christine Vinkx (FPS, DG4), Sophie Lokietek and Laurence Nick (Walloon Public Service, SPW-DSE), Philippe Collard (Walloon Public Service, SPW-CPES), Yseult Navez (FPS, Diensten van de voorzitter), Hana Chovanova (Agentschap voor Zorg en Gezondheid), Safia Korati (FPS, DG5, MRB-Reach), Ludwine Casteleyn (KULeuven), Gudrun Koppen and Tine Caeyers (VITO, contractor coordination, interpretation & communication), Marie-Christine Dewolf (HPH-HVS, contractor fieldwork for the French speaking part), Els Van de Mieroop (PIH, contractor fieldwork for the Flemish speaking part), Catherine Pirard (CHU Liège, contractor analysis of mercury in hair), Koen De Cremer (WIV-ISP, contractor analysis of cadmium, cotinine & creatinine in urine), Adrian Covaci (UA, contractor analysis of BPA & triclosan in urine), Guido Vanermen (VITO, contractor analysis of phthalates in urine).

To avoid language problems, all members of the steering committee agreed to organize their 1.5 to 2 monthly meetings in English. Most e-mail traffic and especially all official e-mail traffic was done in English.

4.2 Institutes and their tasks

The project ran in close cooperation with various Belgian institutes and universities. The Belgian Cell Environment and Health launched in May 2011 tenders for the three main tasks in the Belgian human biomonitoring work: (i) technical coordination, communication and statistics work, (ii) field work and recruitment and (iii) sample analysis. These tasks were taken up by respectively one, two and four contractors.

Technical coordination

In Belgium field work was done by two separate field work centers operative in the two main language regions of the country. Therefore there was a need for a technical coordination center, which took care of the communication and cooperation between the institutes responsible for field work, and for centralized follow-up of the recruitment. Besides coordinating the activities, the technical coordinator was responsible for the preparation of the 'Belgian fieldwork manual', organization of field work meetings, reporting on field work activities and laboratory analyses, shipping samples to the respective laboratories compilation of all collected data into a data file, cleaning of data and data management, and (temporarily) storage of the samples. Before the start of the recruitment, in August and September 2011, two field work meetings were organized by the technical coordination center, where field work procedures were discussed upon. The Flemish Institute of Technological Research (VITO, Mol) was technical coordinator of the project.

Fieldwork centers

Two field work centers were responsible for: submitting the research protocol to the Ethics Committee and Privacy Commission, selection of the schools, contacting and communication with the management of the schools, recruitment/contacting participants, arranging and performing urine and hair collection at school (or at home), interviewing the mothers and keeping listings of the progression of the fieldwork. The field work centers, were PIH (Provinciaal Instituut voor Hygiëne, Antwerp) for recruitment of the Dutch-speaking participants and HPH – HVS (ASBL Hygiène Publique en Hainaut – Hainaut Vigilance Sanitaire) for the French-speaking participants. The centers were located 115 km apart from each other in each of the major two language regions of Belgium. Each staff consisted of a field work coordinator and minimal two field workers who were in charge of this project (Figure 2).

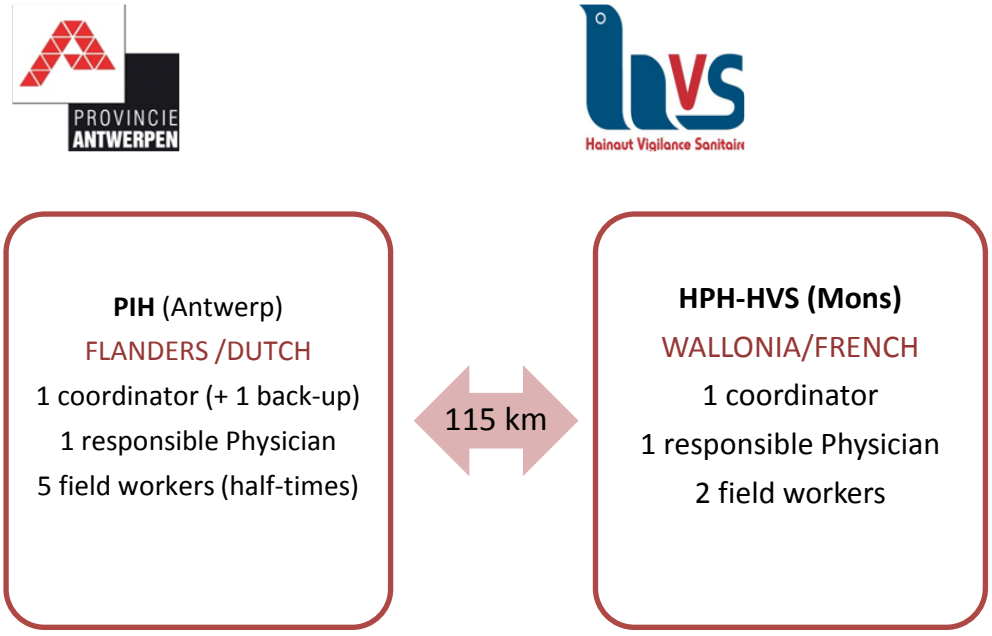


Figure 2: The Belgian field work centers and personnel in charge of the recruitment within DEMOCOPHES

Chemical analyses labs

Four labs were in charge of the chemical analysis of the hair and urine samples: (i) mercury in hair: University Hospital Liège, Service de Toxicologie clinique, Médico-légale, de l'environnement et en entreprise (CHULiège) ; Cadmium, Cotinine and creatinin in urine: Scientific Institute of Public Health (WIV-ISP); Phthalates in urine: Flemish Institute of Technological Research, Environmental analysis techniques (VITO); Bisphenol A & Triclosan in urine: Center for Toxicology, University Antwerp (UA).

The participating laboratories, had to: pass at least one ICI[1] and two EQUAS[2] inter-laboratory rounds, which were organized by COPHES/DEMOCOPHES, needed to have written and validated SOPs for the biomarkers in question, had a an established system of internal and external quality control (QC), preferably had a reviewable record of previous analyses, and had an accreditation according to DIN EN 45001, ISO/IEC 17025 or equivalent was required from commercial laboratories and/or certification according to the rules of good laboratory practice (GLP).

[1] Interlaboratory Comparison Investigation (ICI)
[2] External Quality Assessment Scheme (EQUAS)

Table 1 List of the Belgian study management and contractors' coordinates

Lab/ institute	name	responsability	telephone	e-mail
FOD	**Pierre Biot**	coordination	02 5249616	pierre.biot @environnement.belgique.be
FOD	**Dominique Aerts**	coordination	02 5249697 0477790827	Dominique.Aerts @health.fgov.be
VITO	**Gudrun Koppen**	technical coordination	014 335165 0493100999	gudrun.koppen@vito.be
VITO	**Tine Caeyers**	Reporting, data management	014335116	Tine.caeyers@vito.be
VITO	**Eva Govarts**	Data cleaning, statistics	014335166	eva.govarts@vito.be
VITO	**Roel Smolders**	CAPI, interpretation, link COPHES	015335159	roel.smolders@vito.be
VITO	**Elly Den Hond**	COPHES link	014335161	elly.denhond@vito.be
VITO	**Ab Borburgh**	biobank	014335247	ab.borburgh@vito.be
VITO	**Daniëlla Ooms**	Sample reception/aliquoting	014335210	Daniella.ooms@vito.be
VITO	**Hilde Leppens**	Sample reception/aliquoting	014335210	Hilde.leppens@vito.be
PIH	**Els Van de Mieroop**	Field work leader	032591261	els.vandemieroop@pih.provant.be
PIH	**Vera Nelen**	Study Medical Doctor	032591290	Vera.nelen@pih.provant.be
PIH	**Bhoudane Hanane + Katrijn**	Field work – sam-pling/database	032591276	Hanane.BOUHADAN@pih.provant.be]
PIH	**Ghis Meysen**	Field worker-participants database & contact Flanders	032591265	Ghis.MEYSEN@pih.provant.be
PIH	**Liliane Thijs**	Field worker-participants database & contact Flanders	032591276	liliane.thijs@pih.provant.be
PIH	**Daniëlle Stappers**	Field worker-questionnaires	032591272	danielle.stappers @pih.provant.be
PIH	**Mia Verwimp**	Field worker	032591267	mia.verwimp@pih.provant.be
PIH	**Guy Thys**	database	032591273	guy.thys@pih.provant.be
HPH	**Marie-Christine Dewolf**	Field work leader	065403681 0473410894	marie_christine.dewolf @hainaut.be
HPH	**Etienne Noël**	Study Medical Doctor		etienne.noel@hainaut.be
HPH	**François Charlet**	Field worker	065403616	francois.charlet@hainaut.be
HPH	**Marie-Agnès Monnoye**	Field worker	065403632	Marie_Agnes.Monnoye @hainaut.be
VITO	**Guy Vanermen**	Tubes, analysis urine: phthalates	014335018	guido.vanermen@vito.be
VITO	**Jo Lievens**	Tubes, analysis urine: phthalates	014335016	Jo.Lievens@vito.be
VITO	**Ludwig Goetelen**	Tubes, analysis urine: phthalates	01433 5037	ludwig.goetelen@vito.be
WIV/ISP	**Koen De Cremer**	Analysis urine: Cd, creatinin, cotinine	026425184	Koen.DeCremer@wiv-isp.be
WIV/ISP	**Ilse Van Over-meire**	Analysis urine: Cd, creatinin, cotinine	026425169	ilse.vanovermeire@wiv-isp.be
WIV/ISP	**Joris Van Loco**	Analysis urine: Cd, creatinin, cotinine	026425353	Joris.VanLoco@wiv-isp.be
CHULiège	**Raphael Denooz**	Analysis Hg in hair	043668816 043667683	raphael.denooz @chu.ulg.ac.be
CHULiège	**Catherine Pirard**	Analysis Hg in hair	043668816 043667683	c.pirard@chu.ulg.ac.be
UA	**Adrian Covaci**	Analysis urine: bisphenol A + triclosan	032652498	adrian.covaci@ua.ac.be

4.3 Timing

The COPHES preparatory materials were ready by the end of March 2011. The tendering processes were then initiated and finalized in June 2011.

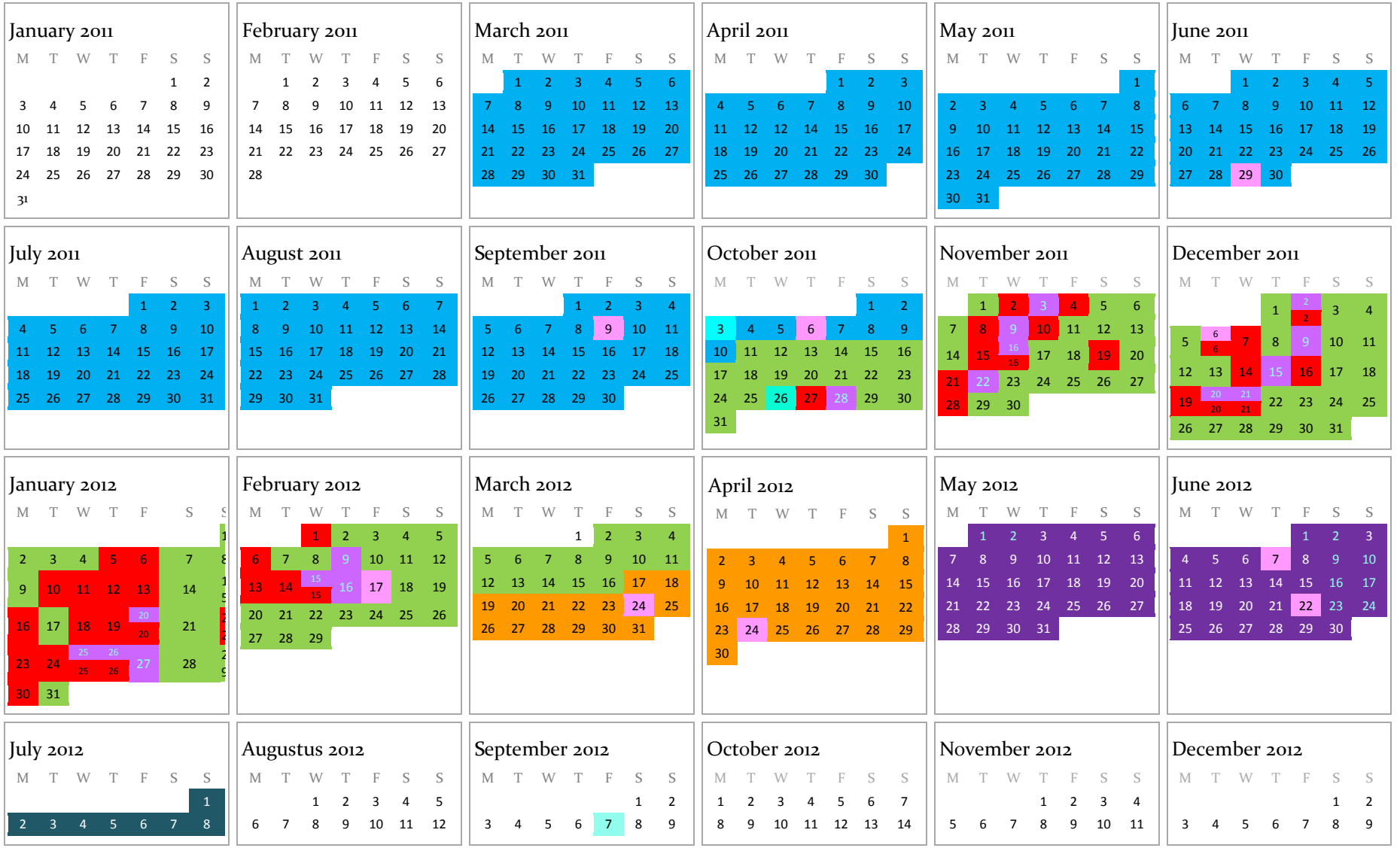
The field work organization started in the Summer of 2011, which was due to summer holidays not an optimal period to start. It took until October 2011 before schools and school management/authorities were contacted, after approval by the ethical committee was given.

The planning of the field work started in June 2011, after finalising the contracts with both field work contractors. Translation of the available protocols and SOPs was done and afterwards submitted to the ethical committees (see further). In the same month the contracts with the technical coordination contractor and the laboratory labs was finalised. The bilingualism of Belgium implied that there was more preparatory work on translation of the original documents. Since we had two field work centers and two language areas, the study was considered as multicentric and we needed to submit the project to two ethical committees in each of the language areas. Moreover, the ethical committees needed to communicate with each other, one being a central ethical committee. All these aspects caused that there was more time needed in the preparatory phase of the field work so that the field work could only be starting in October 2011.

Field work lasted 4 months until February 14, 2012. On February 16, 2012 the last samples were aliquoted. Some labs started already analysing samples during the period that field work was still running, others started analysing end of February 2012. This was decided by the different labs, seeking for an optimal scheduling of the analyses in their working scheme. All analyses were finalized by March 15, 2012. Data entry into the Computer Assisted Personal Interview (CAPI) system and cleaning was done between March 17 and April 30, 2012. The latter included checking of outliers and non-logic data or typing errors in all questionnaires and output files. The Belgian data analysis was finished by July 15, 2012. In the mean time, preparation of communication materials, writing of the Belgian overall report and reporting to EU COPHES took place from June until the end of August 2012. Communication of the Belgian individual results, communication of all results from the different countries in the EU COPHES/DEMOCOPHES consortium and finally the Belgian workshop on the Belgian results, were done in the period of September until November 2012.

Table 2: Overview of timing of some major tasks in the project:

DEMOCOPHES – TIMESCALE 2011-2012


January 2011
February 2011
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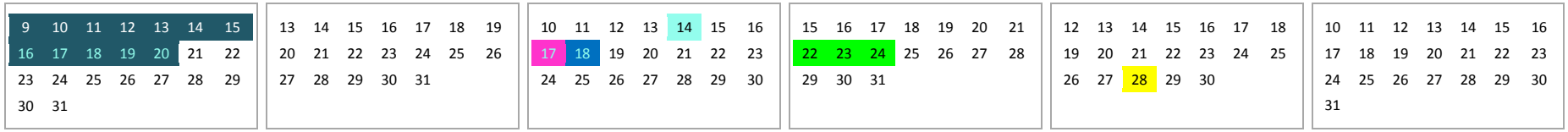


Legend

Task/activity	Begin	End
Preparation of fieldwork	01.06.2011	10.10.2011
Press releases	26.10.2011	
Fieldwork	11.10.2011	14.02.2012
Examination days (sampling and/or interview)		
Steering Committees		
Fieldwork meeting		
Aliquoting at VITO	28.102011	16.02.2012
Laboratory analysis (not indicated on calendar)	Nov 2011	**15.03.2012**

Task/activity	Begin	End
Data cleaning	17.03.2012	30.04.2012
Data analysis	01.05.2012	15.07.2012
Preparation of comm. materials(not indicated on calender)	07.06.2012	13.07.2012
Preparation of report	01.07.2012	end of 08.2012
Communication of individual results	17.09.2012	
Press release Belgium	18.09.2012	
EU Closing event in Cyprus	22.10.2012	24.10.2012
Belgian Workshop	28.11.2012	

5. Study protocol & methods

5.1 Study design

To asses pollutant concentrations in a country, an optimal study design should give a picture of the total population at a given time. It was however not possible to choose a population sample on a strictly representative basis for each participating country. A restricted sampling in numbers (aiming N=240) and population segment (children 6-11y, and their mothers with age below or equal 45y old) was performed. The sampling of the children and mothers was done via schools in a selected rural and urban area in each of the two language areas of Belgium. A cross-sectional sampling of the target population (i.e. sampling on one time moment) was the study design of choice.

5.2 Ethics

In Belgium, the ethical approval was asked by field work centers, to the 'Hospitalo-universitaire ethical committees of the University of Antwerp (UA) and the University of Liège (ULg). The submitted documents included: application form (with introduction letter), the study protocol in short, all communication materials to the participants (invitation letter, reply card, information letter mother, information brochure child, informed consent mother, assent child, letter of results) and the fact sheets used for the website.

For the Dutch speaking part of the participants, the project was approved by the ethical committee of the University of Antwerp on July 13, 2011. The committee had no remarks. For the French speaking part of the participants, the project was discussed by the ethical committee of the University of Liège on July 18, 2011. They had following remarks/questions: (1) the question was asked if the results were directly communicated to the participants, or via a medical doctor, (2) some sentences in the information letter needed to be adjusted linguistically. Liège was the coordinating ethical committee of this multicentric study and their remarks had to be integrated in all documents in Dutch and French. The DEMOCOPHES sampling was finally approved by them on September 21, 2011 (letter retrieved on November 9, 2011; approval letter). In their letter the committee asked to communicate the analysis results also to the medical doctors of the participants. Furthermore, they needed a confirmation of the approval by the committee of Antwerp, which was given by that committee on November 10, 2011.

An application form was submitted to the Commission for Protection of Personal Privacy on 09/11/2011. They returned a confirmation on November 14, 2011 and the letter was received on 2/12/2011 ('aangifte-nummer': VT005033861). An adaptation to the application was done on March 19, 2012 adding the info that the data were transferred to a European database and that the samples will be kept for 10 years in a biobank.

5.3 Study population

The Belgian study population consisted of 129 children and their mothers. 64 of them lived in the urban area of the Brussels capital and 65 pairs in a rural area. Date of birth of the children was restricted to be between 01/01/2000 and 31/12/2005 (6-11 years) and for their mothers it had to be after 01/01/1966 (≤ 45 years).

The recruitment was done via schools. There needed to be, per area (rural/urban), at least 10 children included in each of the six birth years groups, with an equal number of girls and boys (Figure 3 and Table 1) In order to reach the needednumber of participants (boys and girls) in each age group an extra inclusion criterion was introduced by the end of the recruitment period namely 'age and sex category still needed' to complete the requested study population.

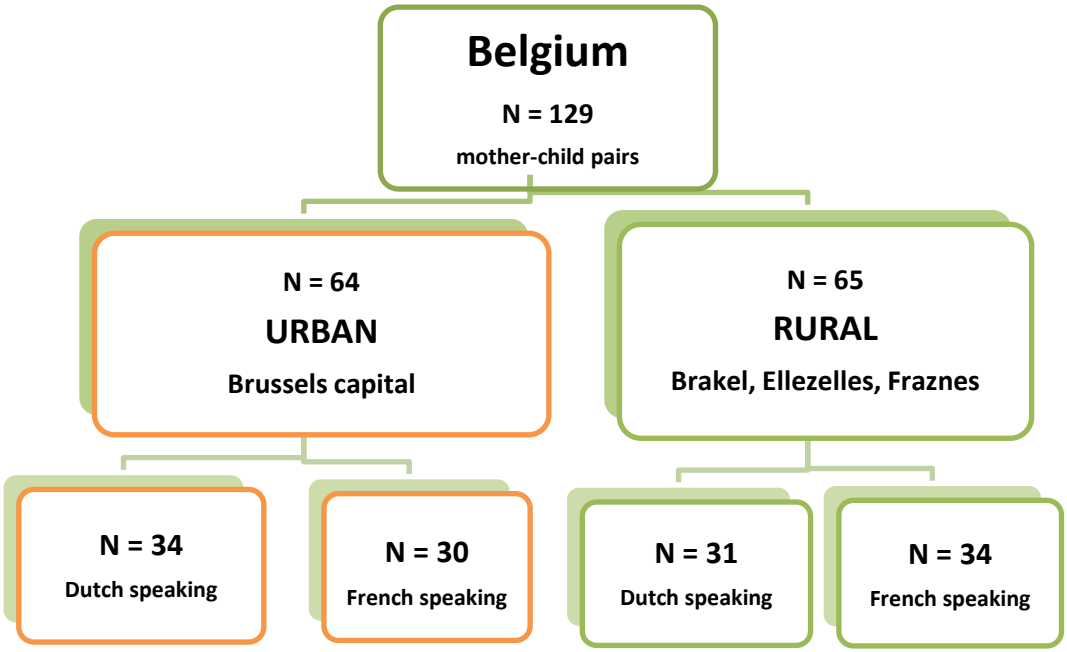


Figure 3: Belgian study population

Table 3: Overview of the number of Belgian mother child pairs in each language region and grouped according of the age group of the children.

	Flanders		Wallonia		TOTAL	
	boys	girls	boys	girls	boys	girls
URBAN						
2005	3	2	2	3	5	5
2004	3	4	2	2	5	6
2003	3	4	2	5	5	9
2002	3	2	2	1	5	3
2001	3	3	4	1	7	4
2000	2	2	3	3	5	5
all ages	**17**	**17**	**15**	**15**	**32**	**32**
RURAL						
2005	3	3	2	2	5	5
2004	2	3	5	2	7	5
2003	5	2	1	2	6	4
2002	2	5	3	4	5	9
2001	5		4	4	9	4
2000	1		1	4	2	4
all ages	**18**	**13**	**16**	**18**	**34**	**31**
TOTAL	**35**	**30**	**31**	**33**	**66**	**63**

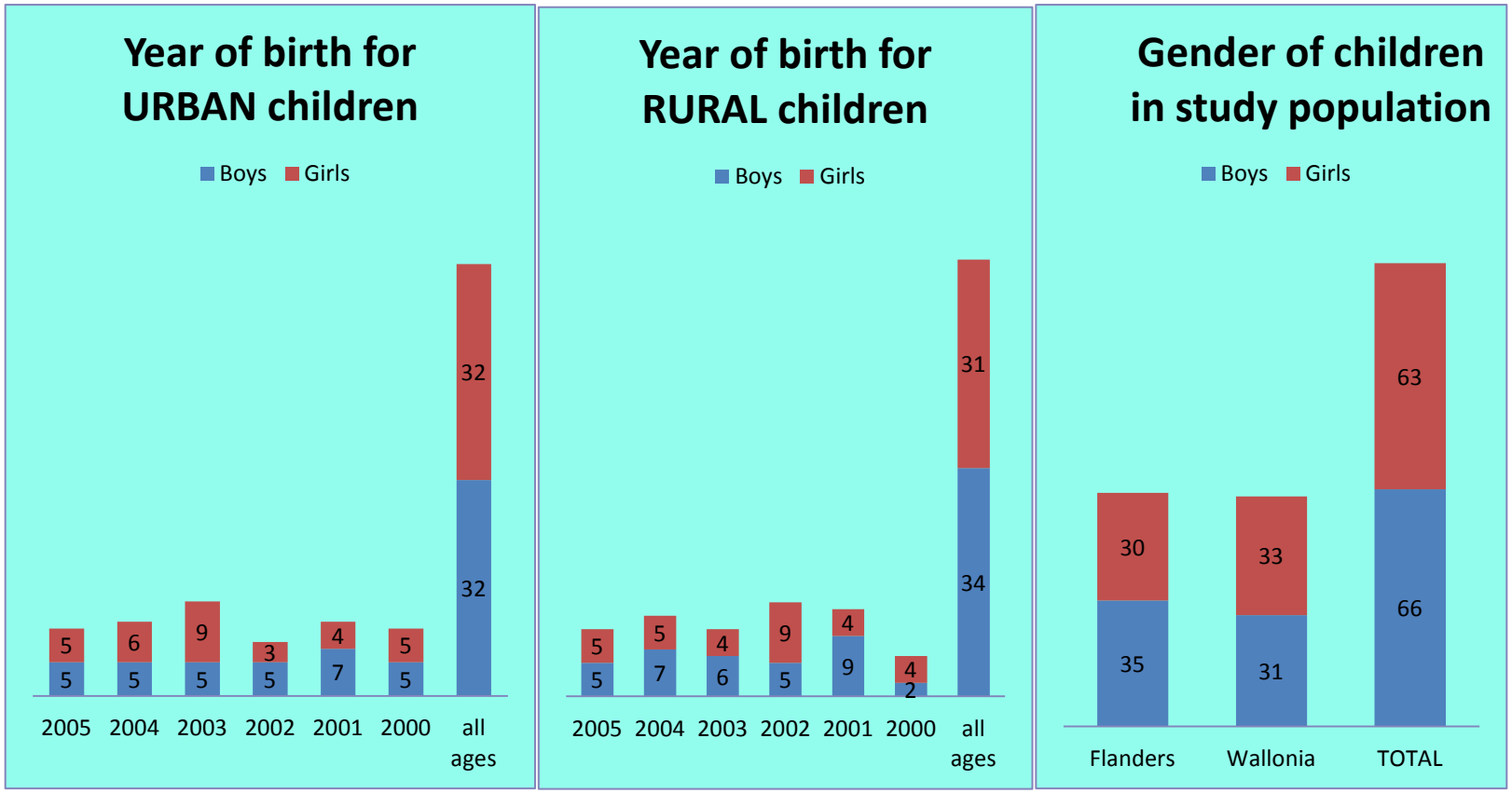


Figure 4: Year of birth and gender of children in DEMOCOPHES study population

5.4 Study areas

Each country needed to define a rural and urban area in which the participants were sampled. It was not possible to find a common definition for the degree of urbanization within Europe because the population density, municipality sizes and commuter areas of big cities were completely different in the participating countries. The two areas chosen had to represent two extremes of degree of urbanization and to be independent, which means that the rural area was for example not a commuter area of the urban area.

A. Belgian rural area

In each of the two major language regions of Belgium, a rural and an urban area have been selected. This means that there were at least two rural and one (bilingual) urban recruitment area. As rural area, the municipalities in the environment of Brakel and Ellezelles/Frasnes-lez-Anvaing were selected in respectively the Dutch and French speaking part. They met the criteria of the EU study protocol, namely <15000 inhabitants/village, <295 inhab/ km², no known sources of emission, no data on emission and immission indicating pollution, <5% industrial activities (on the basis of the surface area), >=500m from highways or N-ways. The centers of the French and Dutch speaking rural municipalities were about 10-15 km away from each other, and were situated 50-65 km south-west of the capital Brussels (Figure 6).

Table 4: Selected rural municipalities and surroundings meeting the rural inclusion criteria

MUNICIPALITY	TOTAL POPULATION	SURFACE AREA HA	AVERAGE POPULATION DENSITY PER KM^2
Dutch speaking			
BRAKEL	14.109	5646.4201	250
HOREBEKE	2.049	1119.6762	183
LIERDE	6.534	2612.5759	250
MAARKEDAL	6.418	4562.6240	141
ZWALM	8.062	3381.6982	238
French speaking			
FLOBECQ (VLOESBERG)	3.316	2300.1355	144
ELLEZELLES (ELZELE)	5.876	4469.4639	131
FRASNES-LEZ-ANVAING	11.214	11244.3977	100
MONT-DE-L'ENCLUS	3.545	2692.9875	132
CELLES	5.466	6713.5147	81
LESSINES LESSEN	18.346	7228.8950	254
LENS	4.214	4942.0195	85
LEUZE-EN-HAINAUT	13.407	7353.2205	182
CHIEVRES	6.566	4691.4343	140
BRUGELETTE	3.430	2840.2856	121

B. Belgian urban area

The Brussels-capital region was chosen to be a representative urban area, for the Dutch as well as for the French speaking part of Belgium. This region contains the largest city of Belgium (Brussels), has a high population density and is bilingual. The Brussels-capital region met the criteria (with the exception of Watermael-Bosvoorde) of having at least 150 000 inhabitants, a population density of 5000 inhab/km² and inhabitants from various Socio-Economic Status (SES).

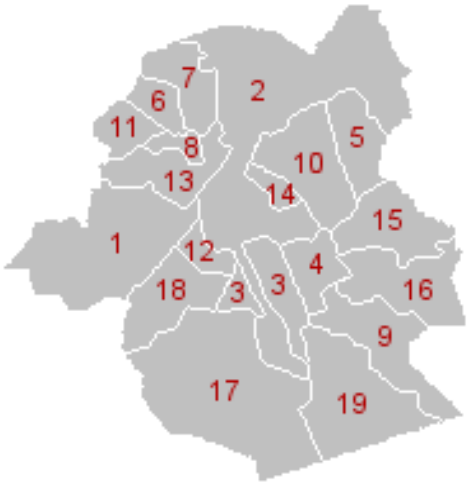


Figure 5: Brussels capital region

1. Anderlecht (1070)
2. Brussel (Brussel-Stad) (1000, 1020, 1120, 1130, 1040, 1050)
3. Elsene (1050)
4. Etterbeek (1040)
5. Evere (1140)
6. Ganshoren (1083)
7. Jette (1090)
8. Koekelberg (1081)
9. Oudergem (1160)
10. Schaarbeek (1030)
11. Sint-Agatha-Berchem (1082)
12. Sint-Gillis (1060)
13. Sint-Jans-Molenbeek (1080)
14. Sint-Joost-ten-Node (1210)
15. Sint-Lambrechts-Woluwe (1200)
16. Sint-Pieters-Woluwe (1150)
17. Ukkel (1180)
18. Vorst (1190)
19. ~~Watermaal-Bosvoorde (1170)~~

Table 5: Surface area of Brussels capital region communities (NB: Watermaal-Bosvoorde was excluded because of the lower population density)

Nr. on map	COMMUNE	TOTAL POPULATION	SURFACE AREA HA	AVERAGE POPULATION DENSITY PER KM2
1	ANDERLECHT	104.647	1774.4118	5.898
2	AUDERGHEM			
3	BRUSSELS (CITY)	157.673	3260.5926	4.836
4	ELSENE - IXELLES	80.183	634.4624	12.638
5	ETTERBEEK - ETTERBEEK	44.352	314.9323	14.083
6	EVERE	35.803	501.8112	7.135
7	GANSHOREN	22.589	245.5219	9.200
8	JETTE	46.818	504.3333	9.283
9	KOEKELBERG	19.812	117.2472	16.898
10	SCHAARBEEK	121.232	813.9966	14.893
11	SINT-AGATHA-BERCHEM - BERCHEM-SAINTE-AGATHE	22.185	294.9556	7.521
12	SINT-GILLIS – SAINT GILLES	46.981	252.4738	18.608
13	SINT-JANS-MOLENBEEK – MOLENBEEK-SAINT-JEAN	88.181	589.1792	14.967
14	SINT-JOOST-TEN-NODE – SAINT-JOSSE-TEN-NOODE	26.338	114.2297	23.057
15	SINT-LAMBRECHTS-WOLUWE – WOLUWE-	50.749	722.4840	7.024

16	**SAINT-LAMBERT SINT-PIETER-WOLUWE – WOLUWE-SAINT-PIERRE**			
17	**VORST - FOREST**	50.258	624.8132	8.044
18	**UKKEL - UCCLE**			
19	~~**WATERMAAL-BOSVOORDE**~~	24.260	1293.3011	1.876 (<5000 inhab/ km²)

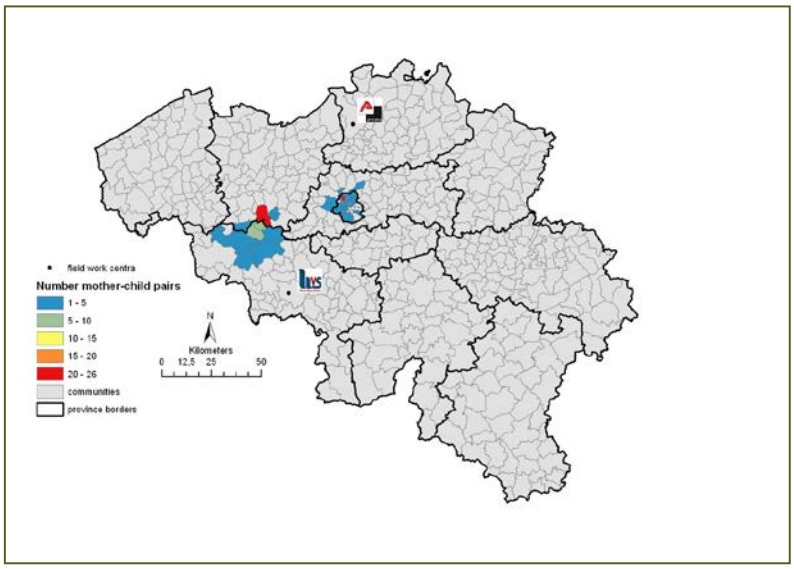


Figure 6: Map of Belgium with the two study areas (Brussels urban area and rural area in the west of the country). The areas are colored according to the number of participants in each of the municipalities. The location of the Dutch and French speaking field work centers PIH and IHP are also indicated.

5.5 Communication in the recruitment phase

5.5.1 Website and press release

A Belgian press release was launched on in the starting period of the recruitment (October 26, 2011). It was captured by one newspaper 'Avenir.

In October 2011 all information and documents of the study were made available on the national website (www.nehap.be), linked with the European COPHES/DEMOCOPHES website (www.eu-hbm.info). On the website following documents were available: Press release text, contact details of the National Management Unit, participating research teams, invitation letters, informed consent/assent form, chemical fact sheets, information leaflets, policy cornerstone paper and all questionnaires for the participants.

5.5.1 Communication with school (boards), and medical doctors

Schools

Schools and communities were contacted by phone, and thereafter received an e-mail and a letter with brief information on the study and the communication materials sent to the mothers. Some of the directors forwarded the mail to their representative school boards. Agreement for running the study in the schools was asked to the local authorities of five municipalities. If the school and local authorities agreed, the school director was visited by the field work coordinator and a field worker. The information packages and zip-lock bags with the urine vessels were then brought to the school.

Medical doctors

Medical doctors in the sampling area were informed about the study at the same time as the participants received their personal results. They got the info on the study via e-mail, using their medical organizational structure as a communication canal. A copy for the personal medical doctors was added to the results letter sent to the participants.

5.5.2 Letters for first contact during recruitment

The communication documents were mainly based on the DEMOCOPHES templates/forms. All children in each school got an invitation letter to join the study along with an information leaflet and letter addressing the mother and a colorful information leaflet addressing the children, a reply card, an informed consent form for the mother and an informed assent form for the child. The information letter contained: the scope and the aims of the study, eligibility criteria, information on the compounds which were measured in urine and hair, how recruitment and sampling was done, and the information that remaining parts of the samples were stored for 10 years in a biobank and may be used for further analyses.

Until December 2011 the six pages long - conform the COPHES model - elaborate information letter, was used. From January 2012 on, a more comprehensive and shorter version of still three pages long was given to the potential participants.
On the reply card, participants filled out if they were interested to participate and answered to questions related to the inclusion and exclusion criteria. If they decided not to participate, their address and phone number were asked to allow the field workers to contact them for a few more questions addressing non-responders. In total 828 reply cards returned with this info: 512 in the Dutch speaking part and 316 in the French speaking part of the study area.

Mother and child had to sign an informed consent and informed assent, respectively. Participants got two copies of each, of which they could keep one.

5.5.3 Confirmation letter

Participants who met the inclusion criteria got a confirmation letter to confirm their selection and to announce the date and time of the examination moment for their child. This letter was distributed via the school. The urine containers and an instruction leaflet on how to provide the urine samples were enclosed.

5.5.4 Radio and television

In the Dutch part of the country, some children and mothers were filmed and interviewed by documentary makers of the Flemish public broadcasting company VRT (CANVAS). For both contacts, a consent form was made, to ask the mothers for their agreement to interview and/or film their children.

5.6 Recruitment via schools

A list of Belgian schools in the study area was available from the Public Institutes for Education in Flanders (*http://www.ond.vlaanderen.be/onderwijsaanbod/lijst.asp?hs=211&fusie=F&p=1&app=20&sw=D*) and Wallonia (*http://www.enseignement.be/index.php?page=25932&act=search&check=&nive=111&geo_mots=&geo_prov=5&geo_cp=&geo_type=3&geo_loca=ellezelles&rese=tous&opt_spe_type=#resultats*).
Schools in the study area were picked from the list, with a preference for public and community schools.

5.6.1 Inclusion/exclusion criteria

In Belgium all EU-COPHES inclusion criteria were kept. An extra criterion of non-allowance of pregnant women was added to homogenize the study group, as there might be changes in metabolism and/or mobilization of pollutants during pregnancy.

Age
- *Mother/stepmother : born after 01/01/1966*
- *Children : born between 01/01/2000 and 31/12/2005*

Residence
- *Mother and child had to live together in the sampling location for the least 5 years.*
- *Mothers or children living in hospitals, institutions or being homeless were excluded.*
- *Only children who live most of the time (>16 days/month) with the mother were allowed to participate.*

Family

Only one child per mother (randomly selected) could be included in the study.
The mother needed to be able to do the interview in the national languages (French or Dutch).

Occupation
- *Occupational exposure was not an exclusion criteria. Occupational exposure was asked for during the interview (job titles and a description of tasks and potential exposure to chemical compounds).*

Health Status

Exclusion of participants with metabolic disturbances or abnormal urine excretion
Pregnant women were not allowed to participate

5.6.2 Selection procedure

In Belgium we did not completely follow the COPHES procedure for recruitment. On the reply cards, questions were added, asking information which allowed evaluation compliance with the inclusion and exclusion criteria. This allowed immediate selection of participants. Also the informed consent and assent forms were provided at the first written contact with the participants. The child returned the signed informed consents (of mother and child) together with the reply card.

Subsequently, the selection of the participants was confirmed via the school. Non-selected children received a letter of thanks. The selected children were provided with urine collection vials for mother and child, packed in a black plastic zip-lock bag, together with detailed written instructions on how to take the morning urine sample, a urine collection questionnaire, labels with identification numbers to be used on bags and questionnaires, and a letter which indicated the date and time of the examination moment of the child. Afterwards, the date and time for the mother's interview and hair sampling was fixed by telephone.

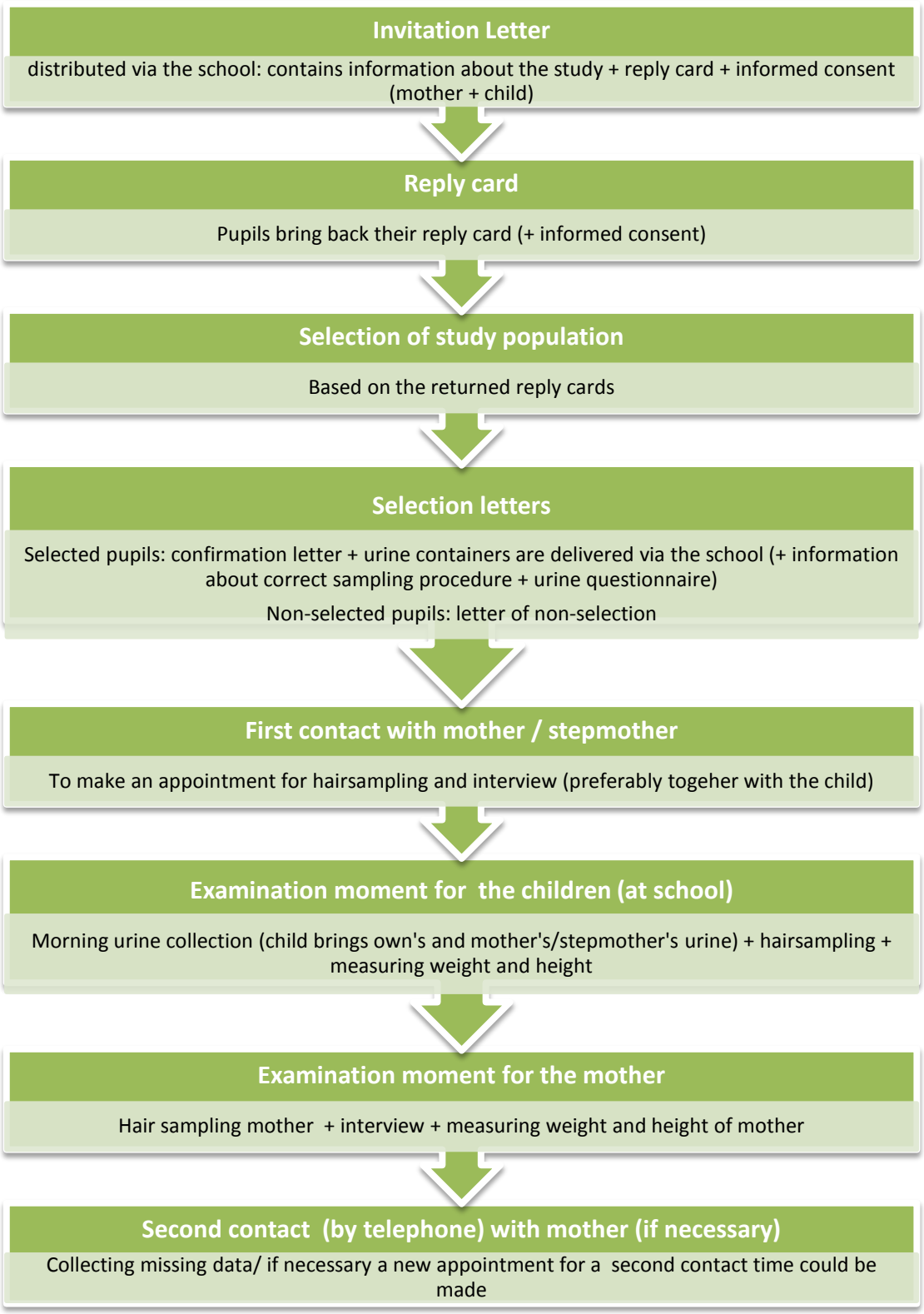


Figure 7: Procedure of recruitment via the schools

5.7 Examination procedures

All children of the same school were examined at the same day as far as possible. In the examination morning the filled urine vessels of mother and child, packed in the black ziplock bags, were handed over to the field workers the children. In case the child and/or mother had forgotten to collect or bring the morning urine, new urine vessels were given and collection was (re-)done on the day of the mother's examination. This happened with 8 children in the urban sampling location and with 6 children in the rural sampling location. On the examination day, a smaal hair segment was cut from the children and their height and weight were measured. All this, took about 15 minutes per child. All children were examined on in total 44 field work days on the 13 participating schools. A comicswas given to the participating children.

The mother's examinations mostly took place at the school. The field workers made appointments via telephone. Mothers were interviewed, hair was sampled and height and weight were measured. The mother's examination and interview took about 45 minutes in average. If possible, mothers were examined at school on the same examination days as the children.

Figure 8: Procedure of the children's and mothers' examination

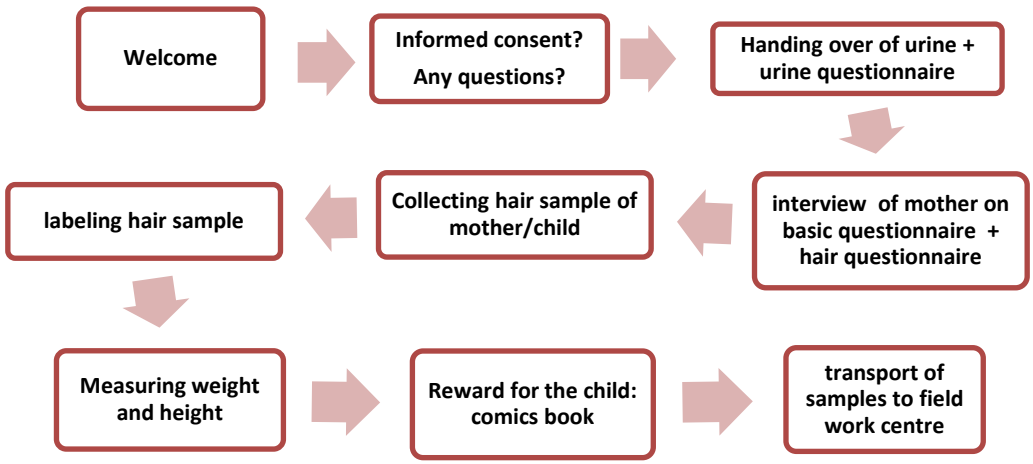


Figure 9: Procedure of the child's and mother's examination

Due to time constraints, a complete field work manual was not available at the initial phase of the field work. A smaller English field work manual was used by the field work centers as guide for their work. This included registration sheets :

- A log-book-sheetfor writing down time point of the examination and data collected during the examination
- A sample registration sheet of the incoming samples.

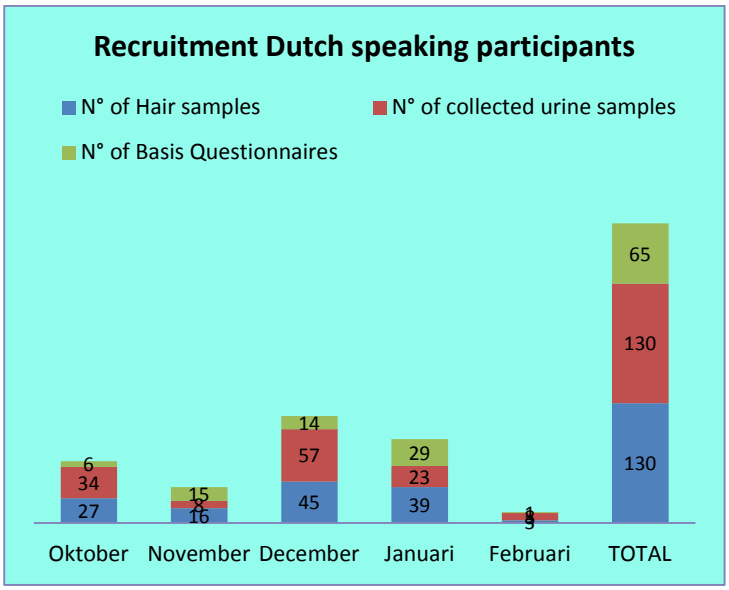


Figure 10: Recruitment of Dutch speaking study population

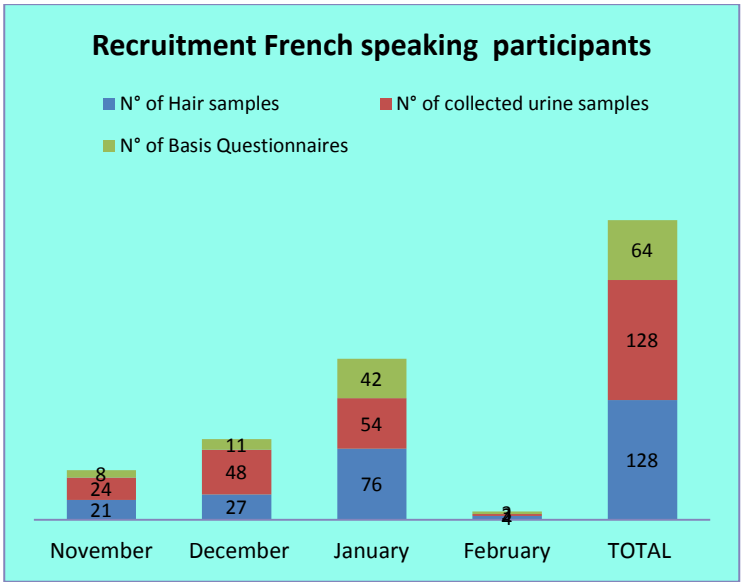


Figure 11: Recruitment of French speaking study population

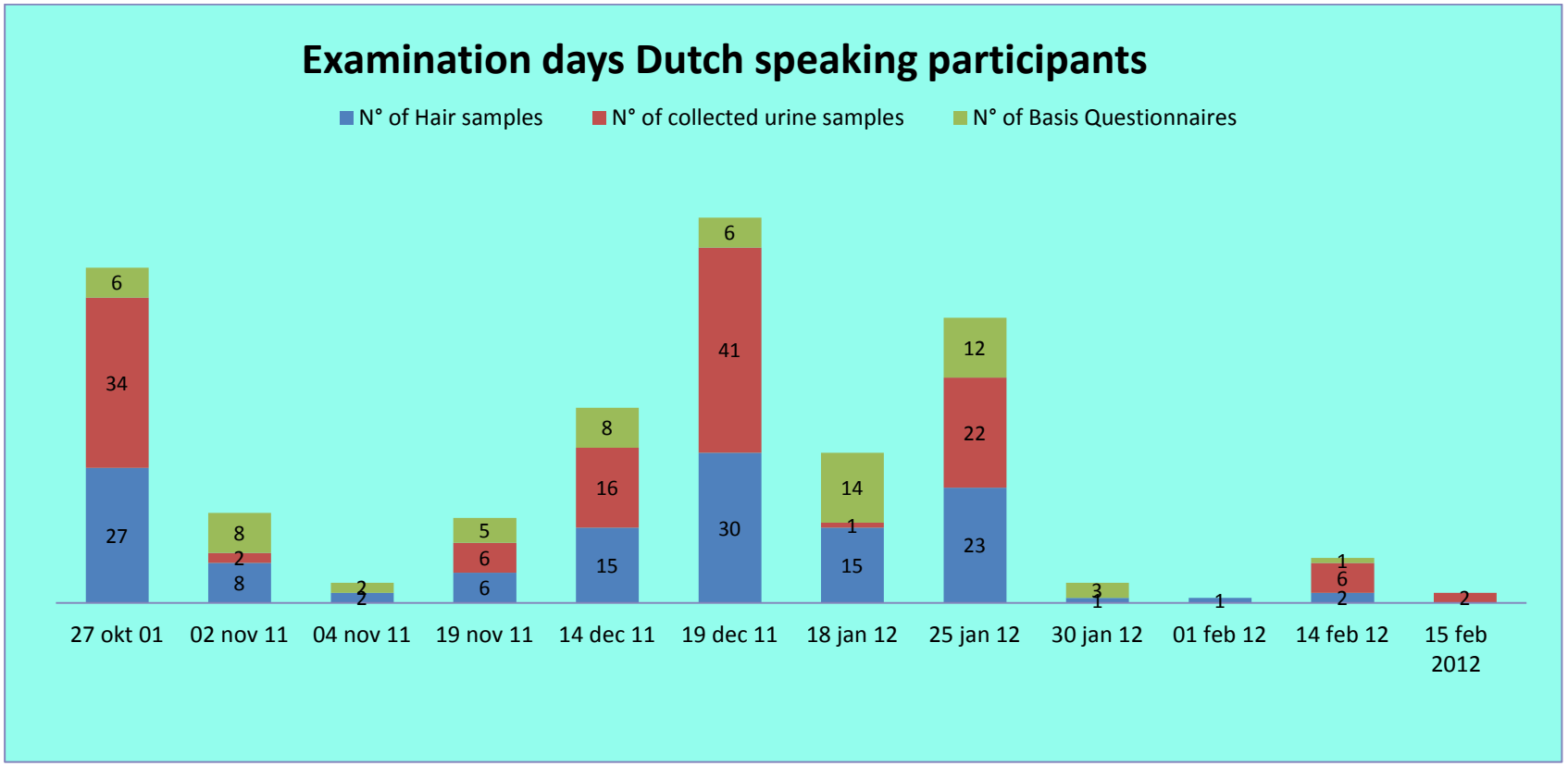


Figure 12: Examination days (in 2011-2012) for Dutch speaking participants

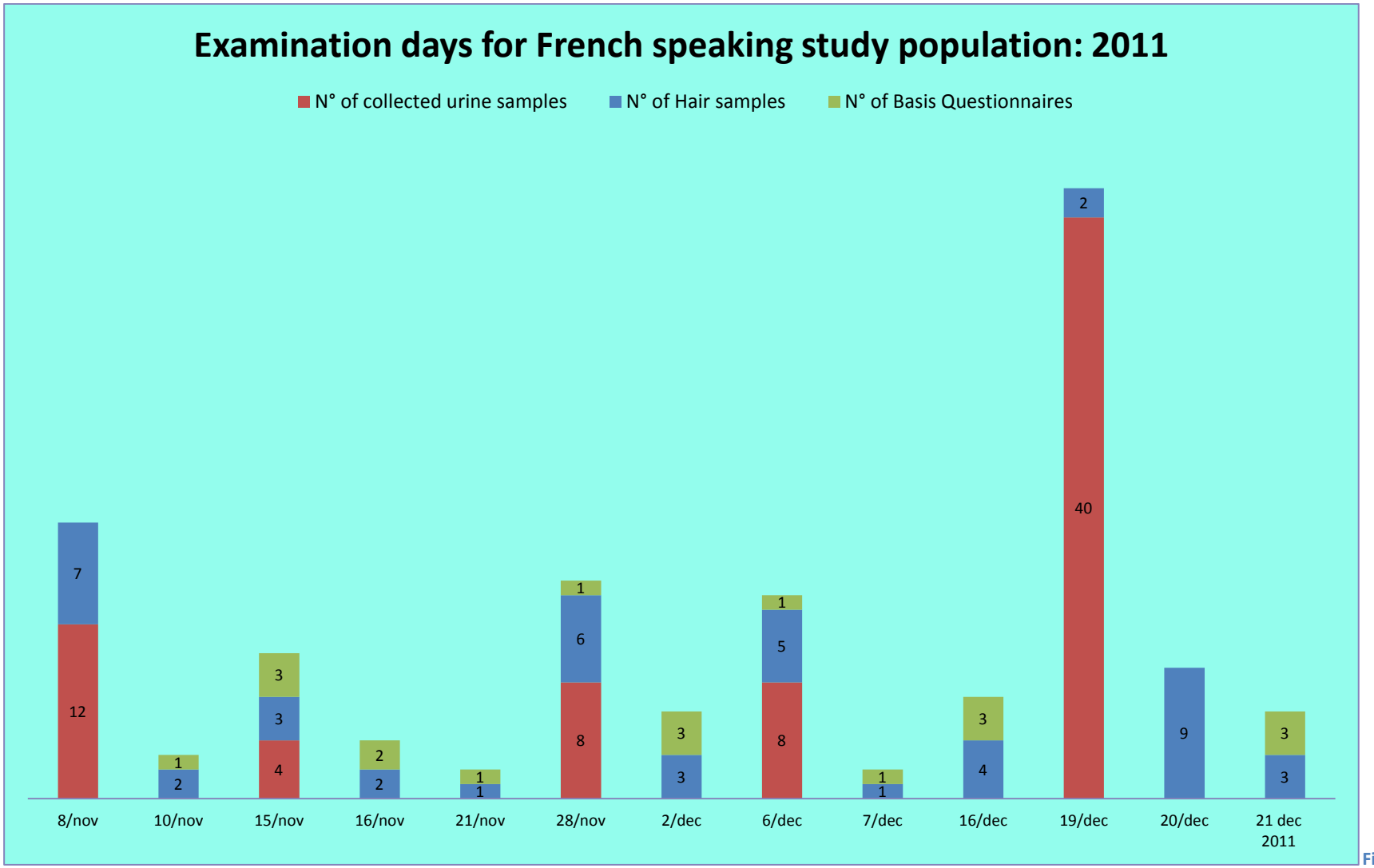


Figure 13: Examination days in 2011 for French speaking participants

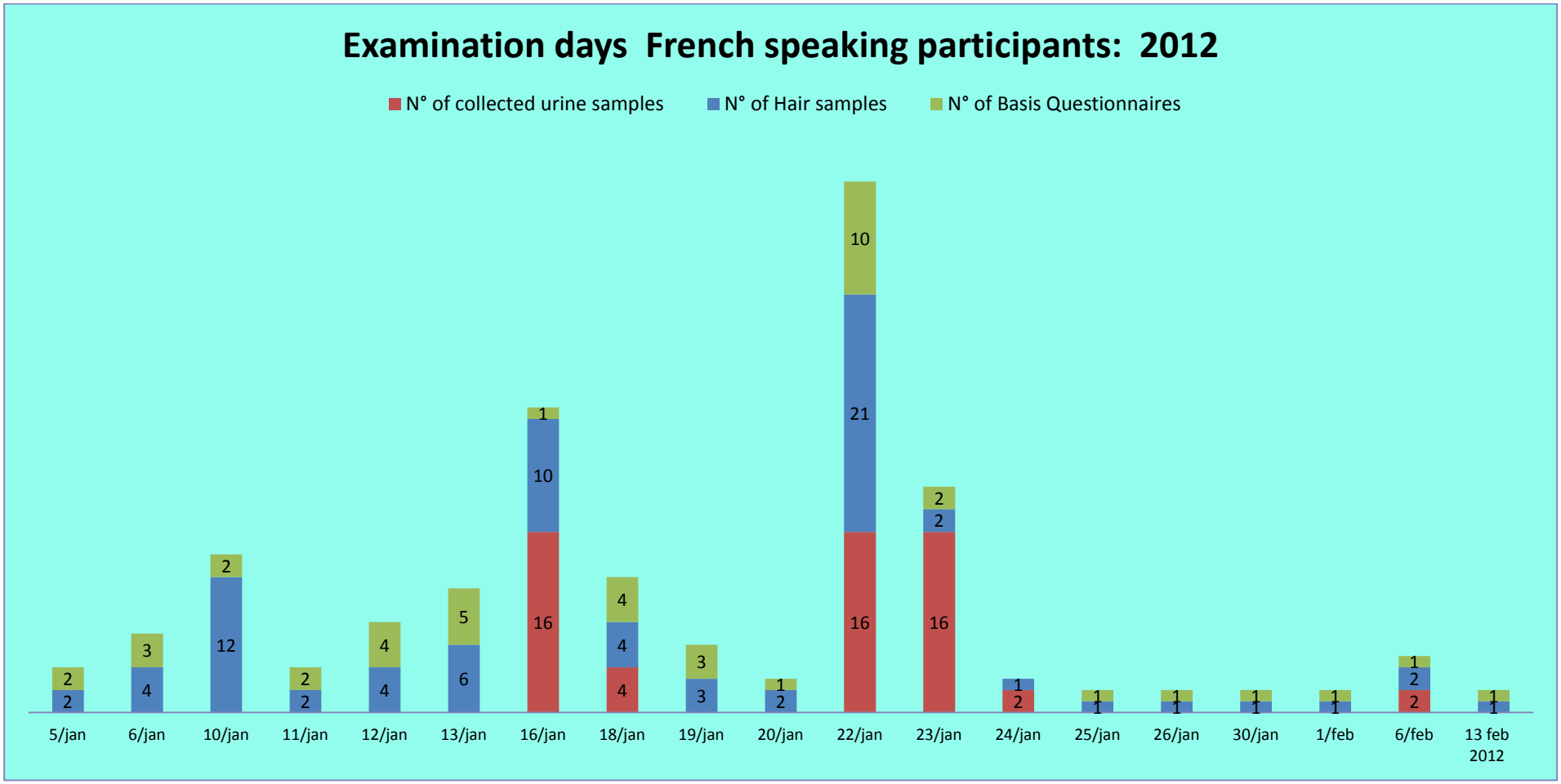


Figure 14: Examination days for French speaking participants: 2012

5.8 Collection of samples

The two coordinators of the field work centers and the coordinator of the technical coordination center attended a COPHES training in Berlin on field work methodologies. The field work coordinators trained their staff based on that information. The procedures on how to collect, label, store, transport and partition/aliquote the samples was included in the field work manual .Collected samples/questionnaires were registred on a participant's individual information sheet.

5.8.1 Urine collection

Two (for the child and the mother) - in 10% HNO_3 rinsed - urine containers were delivered to each of the participants. The vessels were of polypropylene material (250 ml, VWR International®, ref 215-5683) and labelled with a child/mother identification label. The first-morning urine had to be collected by the participants themselves both on the morning of the examination day of the child. In case they had forgotten to collect the morning urine, it was also possible to collect it later that day, provided the previous void was at least 5 hours before.. When the study centers received the samples, they checked and completed the labelling, kept the vessels at 4°C and sent them to the labs for aliquoting and analysis.

instructions *for participants on how to take the sample of morning urine:*

1. *Wash your hands with soap and water and then dry them.*
2. *Remove the primary urine containers from the ziplock plastic bag.*
3. *Open urine container by unscrewing the lid.*
4. *Discharge your* ***first morning urine*** *in the vessel until filled.*
5. *Screw the lid on tight.*
6. *Place the urine container back into the black ziplock plastic bag.*
7. *Keep the sample at 4-8ºC until you give it to the healthcare staff.*

Figure 15: Primary urine vessels with identification label of a participating mother

5.8.2 Scalp Hair collection

Hair samples of mother and child were collected by trained fieldworkers, on the examination days for mothers and children or during the home visit. Fieldworkers wore single-use disposable gloves and used titanium or stainless-steel scissors disinfected with alcohol right before use. 100 mg hair was collected by cutting two hair strings. Adhesive tape was used to fasten the hair samples of participants at distance from the root of about 3.5 to 4 cm. With ballpoint an arrow was drawn on the tape, of which thetop indicated to the root of the hair. In case the hair was shorter, more shorter strings were cut on different places at the back of the head and no tape was used to keep the strings together. Analysis of mercury was performed in the 3 cm closest to the scalp. After cutting the hair sample, it was put into a paper envelope. The paper envelope was packed into a ziplock plastic bag, which was labelled with the persons' identification number. The plastic bags were kept at at room temperature in the field work centers, until transport to the analysis lab(either via transport to the technical coordination center, or directly to the lab).

5.8.3 Measurement of height and weight

Height and weight of the participants were measured. The height was assessed using an ultrasound-type measurement, with a portable instrument called 'height rod' (Soehnle, ref Nr: 5003.01.001). For the weight a normal person's balance was used (Vogel en Halke Germany, Seca model: 750 1017009, Ser.no 2750155037549). Each of the two field work centers had their own height rod and balance.

5.9 Identification labels

The identification code of the participants was composed of 7 digits, according to the prescriptions of COPHES. The codes were composed as follows: BExyyyz, with 'BE' for the land code (Belgium), x =U (urban) or R (rural), yyy = number of individual (in the range of 100 to 199 in Flanders, and 200 to 299 in Wallonia, z = M(mother) or C(child). All primary urine containers were labeled with a tag, containing the identification code, attached at the side, near to the top of the container. For the hair samples, the labels were attached on the zip-lock bag and not on the envelop, to avoid any migration of compounds from the label adhesive/ink through the paper to the hair sample.

At the technical coordination center, the urine samples was aliquoted, and the (10% HNO_3-rinsed) secondary 15 and 50-mL polypropylene tubes (PP Falcon tubes, conical, ref. 352096 and 352098) were labelled with a label containing: (i) the identification number of the participant (see above), (ii) a biobank number i.e. a unique barcode for systematic (temporarily or long-term) storage allowing retrieval and identification of the single tubes of one person; (iii) a tube code, for easy identification and sorting out oftubes sent to external analysis labs.

5.10 Recipients' rinsing and contamination check

2076 tubes of 15mL and 551 tubes of 50mL (BD Falcon) were used for the 258 participants. All tubes were from the same lot number, respectively ref 352096 and ref 352098 for 15 and 50 mL tubes. Surplus recipients of the same lot were stored for possible later contamination check. To eliminate contamination, all tubes and urine vessels have been rinsed overnight in a bath filled with 10% nitric acid in purified water solution and further afterwards 2x rinsed with water and allowed to dry under a laminar flow. Before the start of the field work, contamination testing of the recipients was performed according toCOPHES prescriptions. The 50 and 10 mL vials were filled during 10 minutes with respectively 200 and 10 mL milliQ water. This water was then analysed for the compounds, which were measured in the urine samples:

- 11.10.2011: BPA and Triclosan were measured, using SPE and GC-ECN/MS, in rinsed tubes (10 tubes of 15 mL and 4 of 50 mL). No BPA and TCS above blanco-values (0.04 en 0.02 ng/mL water) have been detected (*LOQ for BPA and TCA in urine = 0.2 and 0.1 ng/mL urine for our methods*).
- 13.10.2011: The urine vessels and other tubes were also free from contamination with phthalates, Cd, creatinine, cotinine, (all phthalates < 0.1 ng/mL, cotinine, Cd and creatinine values < LOQ)

Surplus recipients of the same lot were stored together with the biobank samples at -80°C for possible later contamination check. This was not foreseen in the EU protocol, but it is an easy way of making it possible to test for contamination of the vessels in case later on is decided to measure other pollutants in the urine samples. Field blancs (i.e. tubes filled with water and transported as all field work recipients) were not included in the final EU protocol. It can be useful to keep some in the freezer during the study period, allowing contamination testing in case of suspected contamination.

5.11 Sample processing, transport and storage

The field workers stored and transported the samples in cool boxes from the place of examination to the field work centre. Urine was stored at 4°C and sentin cool boxes to the technical coordination center for aliquoting within not more than 4 days after sampling (Figure 16).

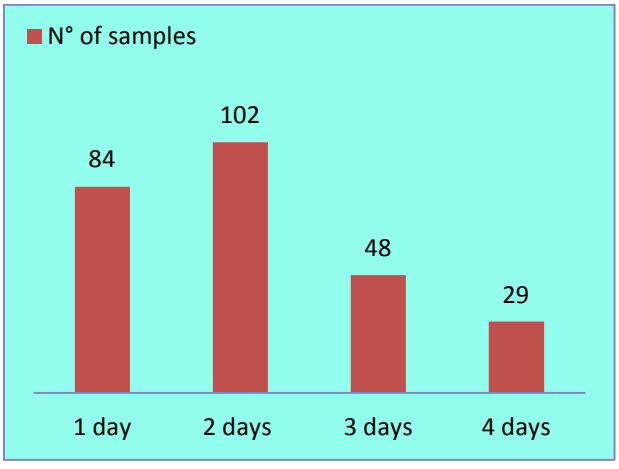


Figure 16: Overview of the time lag between reception of the urine samples by the field workers and aliquoting and storage of the aliquots at the technical coordination center

Between the Dutch speaking field work center in Antwerp and the technical coordination center in Mol (distance ca. 60 km), transport was done by a taxi company. Transport between the French speaking field work center in Mons and the technical coordination center (distance ca. 150 km) was done using the services of a clinical lab transport (MediMail Express), a transport service which operates across Belgium. Both service providers were phoned one day in advance.

Urine samples were kept in cool boxes at 4°C, as long as they were not yet aliquoted. Once arriving at the technical coordination center, the primary urine bottles were weighed to assess the approximate volume of the original sample. Aliquoting over secondary recipients was done, using 15 and 50 mL polypropylene (PP) tubes). The primary urine containers were properly stirred before decanting in secondary vessels. Aliquots were decanted directly from the primary container to avoid contamination. Weight of the primary urine container and the number of aliquots were recorded in an Excel file. All data have been further transferred into a Laboratory Information Management System (LIMS) system. Samples were kept at -20°C or -80°C. After aliquoting and storage, samples were transported batch-wise, from the technical coordination center to the labs for analysis. This transport was done using a taxi company.

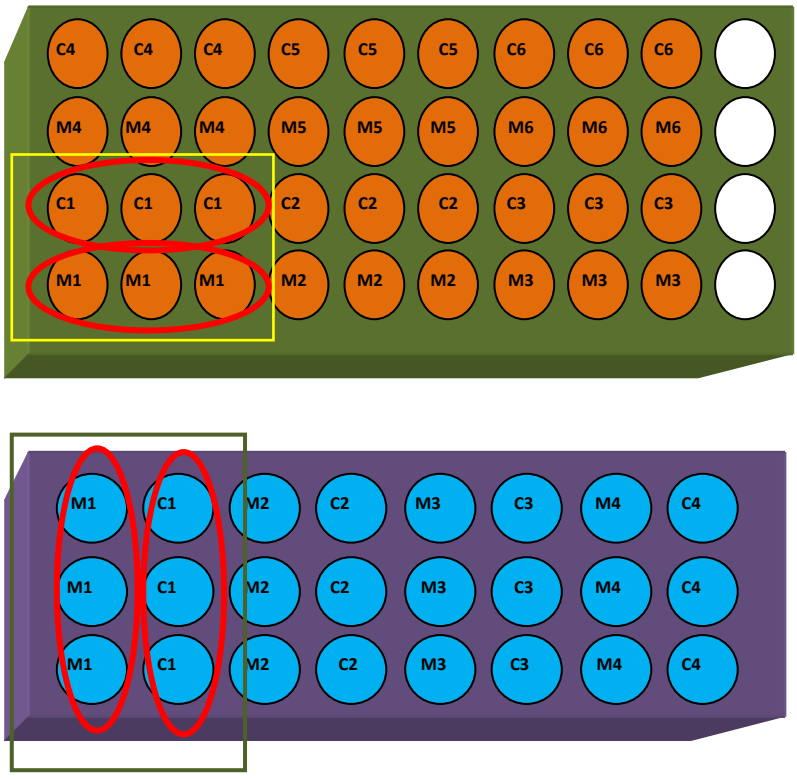
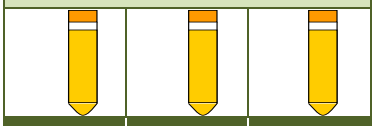


Figure 17: Way of sorting the biobank tubes with content of 15 mL (above) and 50 mL (below) in the -80°C freezer: each time 3 tubes were placed in the racks

Twenty extra empty pp-tubes of respectively 15 and 50 mL of the same lot as those used for sample aliquoting, were stored at -80°C, to allow later contamination checks of these vessels. For two children of our study population there was no matrix (urine) enough to store in the biobank. The urine samples of the biobank were sorted in the tube racks in a specific order as shown in Figure 17. The

urine biobank samples are kept in one -80°C freezer, equipped with a continuous alarm system. An extra empty -80°C freezer is available in case of technical problem. The unit 'Milieu Risico en Gezondheid' of VITO is certified according to GLP and ISO 9001 standards. According to DEMOCOPHES, storage of 10 years is foreseen for the biological material.

Table 6: Number of urine samples stored in the Belgian biobank

	biobank		
			
	10 mL	**10 mL**	**10 mL**
TOTAL N° of individuals	**259**	**258**	**254**
Children	129	128	125
Mothers	130	130	129
3 ml			2
4 ml		1	1
5 ml	1	2	
10 ml	258	255	251
15 ml			
20 ml			
25 ml			
30 ml			
35 ml			
40ml			
Total N of tubes= 771	**259**	**258**	**254**

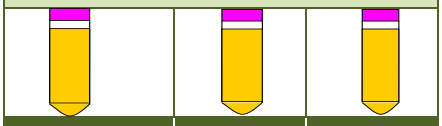
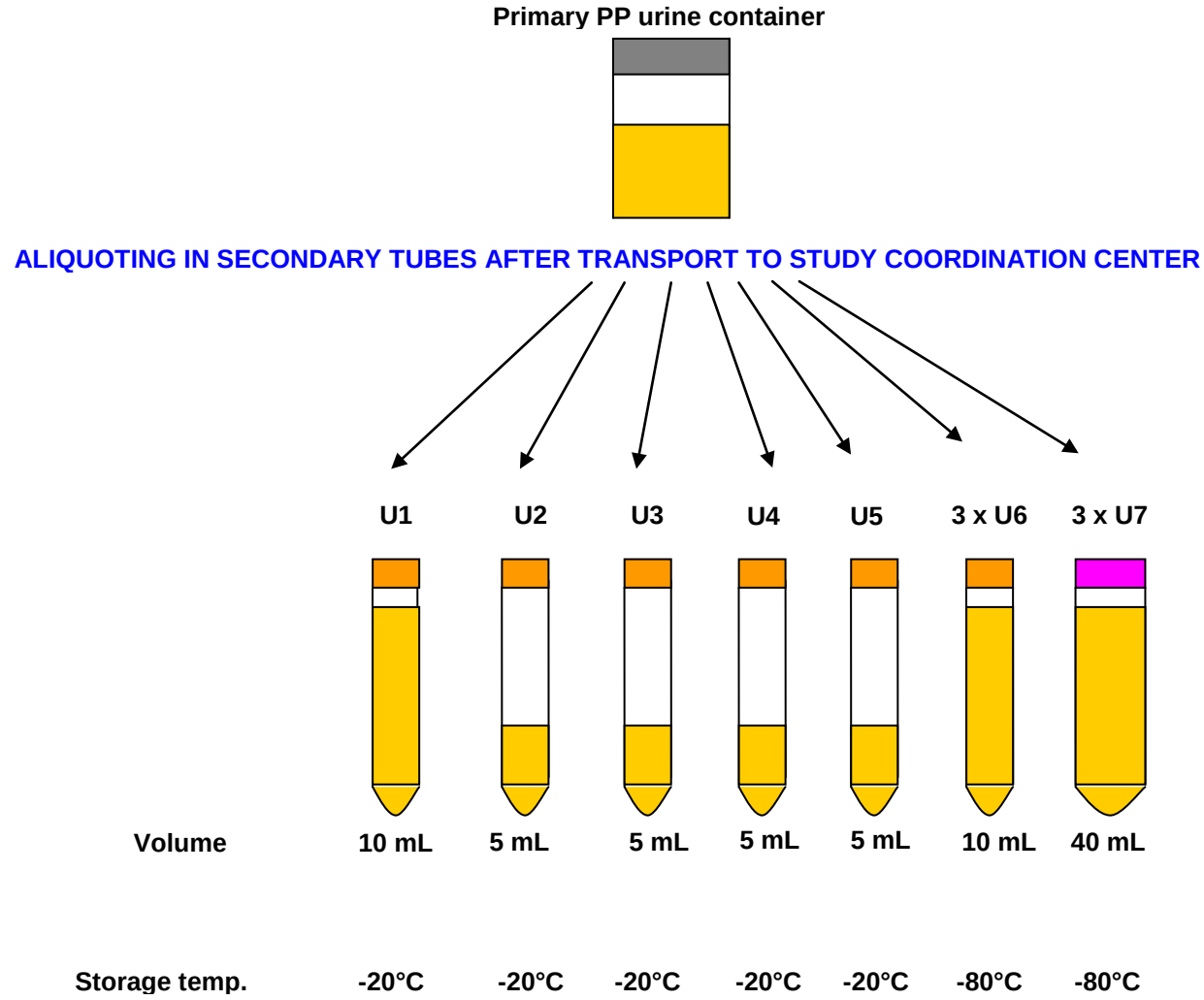
	biobank		
			
	40 mL	**40 mL**	**40 mL**
	246	**187**	**118**
	119	90	61
	127	97	57
3 ml			
4 ml			
5 ml	2		1
10 ml	3	6	3
15 ml	5	4	10
20 ml	5	15	9
25 ml	7	9	9
30 ml	7	5	5
35 ml	8	5	4
40ml	209	143	77
Total N of tubes= 551	**246**	**187**	**118**

Figure 18: Aliquoting of urine from primary urine container into secondary tubes



5.12 Questionnaires

Basic questionnaire

All participating mothers were personally face-to-face interviewed by trained interviewers in order to fill in the basic questionnaire. The interview took about 45 minutes. It included information related to: home environment and residence, nutrition during the last 4 weeks, smoking behavior, other exposure-relevant behavior, professional occupation and socio-demographic data. These questionnaires were pre-tested on 20 women before the start of the field work. Remarks on the content were centralized. Following this evaluation, following guidelines were given to the Belgian field workers to be able to complete the questionnaires in a uniform way:

- **Home environment (A2):**
  Living area of the home, garage and stock rooms excluded, i.e. including living room, kitchen, bath room, sleeping rooms, guest rooms, library,…

- **Question on alcohol (B3):**
  Remind that we ask about quantity of beer glass being 25 cL (normal size of a beer in Belgian pubs)

- **Nutrition - Cereals (B5.J):**
  Include all corn-flakes like cereals, but NOT bread

- **Nutrition - Fish (B7+9):**
  sea fish vs. fresh water fish
  -> some examples given below in 'Nederlands' and 'Français':

  Zoutwatervis / poisson de mer
  NL: ansjovis, bot, griet, heilbot, makreel, kabeljauw, koolvis, rode poon, sardien , schelvis, schar, schol, tarbot, tong, tonijn, wijting, zeeduivel.
  F: anchois, plie, barbue, elbot *, maquereau, cabillaud, colin, le grondin-perlon, sardine, églefin, limande, turbot, sole, thon, merlan, lotte de mer*

  Zoetwatervis / poisson d'eau douce
  NL: baars, forel, karper, meerval, paling, snoek, steur en zalmforel.
  F: perche, truite, carpe, silure, anguille, brochet, esturgeon, truite saumonée

- **Amalgam fillings (D3 + D6)**:
  These are the grey fillings only

- **household gloves (D15)**:
  We want to know about plastic gloves, not the rubber (latex) gloves.

latex = **rubber**: either (i) soft, high punction/shearing resistance, good quality, mat color; or (ii) thicker version used in household (with cotton filling) in different colors.
The other, plastic gloves are:
vinyl: soft, mostly shiny, not so stretchable as latex, rather cheap
nitrile: either (i) soft, mostly colored gloves used in labs, expensive, or (ii) does also exist in thicker version, also expensive, mostly used in professional context.

- **Income (F9)**:

Net total income family, all income sources added up of possibly 2 partners:

- *net salary + vacation salary from main employment*
- *salary from side activities*
- *Social benefits: child allowance, unemployment and health insurance benefit*
- *Money received from ex-partner for raising child*

Table 7: Table of income categories, included in the Belgian basic data from net monthly family income (info from statbel fgov Belgium)

Income category	***EURO***
< 50% of the MS mean net income	< 1700 euro
50 % -<60 % of the MS men net income	1700 euro - 2000 euro
60 %- < 75 % of the MS mean net income	2000 euro - 2500 euro
75 % - < 90 % of the MS mean net income	2500 euro – 3000 euro
90 % - < 115 % of the MS mean net income	3000 euro - 4000 euro
115 % - < 150% of the MS mean net income	4000 euro – 5100 euro
150 % - <200% of the MS mean net income	5100 euro – 6800 euro
>200% of the MS mean net income	> 6800 euro

http://statbel.fgov.be/nl/statistieken/gegevensinzameling/enquetes/huishoudbudget/index.jsp

The following questions were indicated by the mothers to be the most difficult to fill out:

How many square metres of living surface does your home have?		*m² without decimals*
m^2	_ _ _	
How often did you wear rubber-like plastic gloves (not latex) in the last week?		
daily	○	
less than daily	○	
never	○	
How often did your child *[name]* **play with toys consisting of or containing rubber-like plastic in the last week?** *Please also think of toys your child plays with outside your home.*		
daily	○	

less than daily	○	
never	○	
don't know	○	
Perhaps you can indicate the category your household's disposable net income belongs to. That is the gross income of all household members <u>minus</u> regular taxes on wealth, inter-household cash transfers paid, tax on income and social insurance contributions?		*Categories should be adapted according to the national income distribution of the participating country. For further explanations see Annex SOP Questionnaires and Interview Conduct.*
< 50 % of the MS mean net income	○	
50 % - < 60 % of the MS mean net income	○	
60 % - < 75 % of the MS mean net income	○	
75 % - < 90 % of the MS mean net income	○	
90 % - < 115% of the MS mean net income	○	
115 % - < 150 % of the MS mean net income	○	
150 % - < 200 % of the MS mean net income	○	
> 200 % of the MS mean net income	○	

Urine and hair questionnaire

Additional questionnaires were used to complete the information related to urine and hair sampling for both, mother and child. It included questions like: time of sampling, time of last urination/washing of the hair, time of labeling of the samples, specific characteristics of the samples, special treatments (of the hair) in the past, last meal before sampling, exposure to tobacco smoke in the last 24h, types of convenience food in the last 24h. The children had to bring both self-administered urine-questionnaires with their urine samples. The hair questionnaire was in by the field workers at the moment of the hair cutting.

Non-responder questionnaire

In Belgium non-responders were sent a mail in case we had the e-mail address and they were invited to fill out the questionnaire via a link to the CAPI SOCRATES questionnaire. The other non-responders were interviewed by phone and data were entered simultaneously in the CAPI system by the field workers. An extra question was added in order to better understand the reason of non-participation. 22 questionnaires have been completed by the non-responder themselves (queried via mail) and the majority (100 questionnaires) haven been filled in by the fieldworker (queried by telephone). Non-responders mentioned 'a language problem' and 'no time' as most important reasons for not taking part to the study.

CAPI

For data entry, the Computer Assisted Personal Interview (CAPI) system 'SOCRATOS' was used: http://www.socratos.be/home_en.html. Field workers did the data entry from paper questionnaires into the computer system. Direct data entry of the basic questionnaire during the interview was tried out on 5 individuals. The experience was that this direct data entry was difficult, mainly if questions with different options were asked. It appeared to be much easier for the mothers to point to the right answer on paper, rather than on the screen. Possibly a tablet PC with a multi touch screen would be a helpful tool.
During interviews of all other participants paper questionnaires and encoded in SOCRATOS afterwards. Encoding in SOCRATOS was not considered to have much advantage over encoding via e.g. ACCESS. Interviewing mothers was elaborate, because separate appointments were needed, although appointments were grouped as much as possible and organised at the school. 30 mother-child pairs were interviewed/sampled at their homes, all others (N=99) at school.

Belgium received an excel file with login codes for each type of questionnaire. This login code was used to get access to the Belgian questionnaires on the Socratos website. Extra test codes (test1-test10) were added to become familiar with the system http://cophes.socratos.net/login. When logging in on at the SOCRATOS website, only the Belgian questionnaires (BE_XX) were visible. All other countries had similar protected sites..

5.13 Analysis methods of the biomarkers

5.13.1 Mercury in hair

50 mg of hair - previously washed with dichloromethane, dried and cut in small pieces – to which 80µL of water was added have been hydrolysed at 40°C overnight using concentrated nitric acid (HNO3). Afterwards, water, hydrochloric acid, mixture of KBrO3/KBr, and Triton X100 was added and blended. The extract was then analysed with a FIMS (Flow Injection Mercury System, FIMS 400, Perkin Elmer), for which main parameters are: Spectrometer: FIAS 400_Perkin-Elmer(Lamp: Hg, Wavelength: 253.7n, Signal mode Type: atomic absorption, Read time: 25 sec, Loop volume: 500 µ). The inner diameter of the pump tubes was 1.52 mm for sample and reagent carrier and 1.14 mm for $KMnO_4$ and $NaBH_4$. 25 samples were run per measuring sequence.

5.13.2 Creatinine in urine

For the analysis of creatinine in urine a Cobas 6000 instrument (Roche Diagnostics) has been used. After defrosting the samples they were vortexed, centrifuged during 10 minutes at 3500rpm, transferred into m 5mL tubes and placed in a Cobas 6000 instrument (Roche Diagnostics). The Analyses were automatically performed according to the Roche procedure (Jaffé method). There were 30 samples per measuring sequence.

5.13.3 Cotinine in urine

Endogenous cotinine was measured in urine using heavy labeled cotinine-d3 as internal standard. After thawing the 5-mL samples, they were vigorously (automatically) shaken during 15 minutes, followed by centrifuging at 3000 rpm during 10 minutes, dilution (following a 1:10 or 1: 100 dilution method) and vortexing. 10µL was injected onto an online-SPE UPLC-MS-MS instrument (Acquity UPLC coupled to Xevo TQ MS-MS instrument, Waters company). The total runtime was 10 min per sample. For Data-analysis Targetlynx software (Waters company) has been used.

All samples were first measured with 1:10 dilution method. If concentration was > 100 ug/L, samples were reanalyzed using 1:100 dilution method. For the 1:10 dilution method 100 µL supernatant was added to 800 µL solvent A and 100 µL of internal standard solution. For the 1:100 dilution method 100 µL supernatant was added to 900 µL solvent A followed by vortexing during 10 sec. 100 µL of this diluted solution was added to 800 µL solvent A and 100 µL of internal standard solution followed by vortexing during 10 sec. Per sequence 90 samples were left overnight. The blanks and QC samples were included after each 25 samples.

5.13.4 Cadmium in urine

After thawing the urine samples they were vigorously automatic shaken during 15 minutes followed by centrifuging at 3000 rpm during 10 minutes. Then 1.5 mL urine was transferred to 15 mL Falcon tubes and 13.5 mL 1% HNO_3 solution+20 µL IS solution was added. Those tubes were vortexed during 10 sec. After that samples were placed in an automatic loader of an ICP-MS instrument for automatic measurement of the samples Elan DRC II ICP-MS instrument (Perkin ElmerSCIEX company) (3 replicates per sample, 4.5 min per sample). The values were transferred to secured excel-datasheet template for calculation (including corrections for MoO and Sn interferences). Per sequence 40 samples were measured. The blanks and QC samples were included after each 20 samples.

5.13.5 Phthalates in urine

The following compounds/congeners/isotopes were measured in urine: mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (5-OH MEHP), mono-2-ethyl-5-oxohexyl phthalate (5-oxo MEHP), mono-n-butyl phthalate (MnBP), mono-benzyl phthalate (MBzP), monoethylphthalate (MEP), mono-iso-butylphthalate (MiBP).

Frozen sample was thawed, vortexed and sonicated for 5 minutes. To 1 mL of sample an enzyme buffer (beta-glucuronidase E. coli type K12) and internal standards (^{13}C-MEP, ^{13}C-MnBP, ^{13}C-MBzP, ^{13}C-MEHP, ^{13}C-OH-MEHP, and ^{13}C-oxo-MEHP) were added. This mixture was incubated at 37 °C for 90 minutes. 10 µL of the solution was injected in a tandem UPLC-MS/MS (triple quadrupole, Waters Acquity UPLC-Waters Xevo TQ-S) equipped with a Acquity UPLC BEH PHENYL 1.7 µm, 2.1 x 100 mm column. Elution was done using A: Water + 0.1% acetic acid and B: ACN + 0.1% acetic acid (gradient: 95%A/5%B → 10%A/90%B, total run time 8 min). Quantification was done with an internal standard method. Calibration solutions in a range of 0.1 to 250 µg/L were made in ACN: water (1:9). 20 samples were measured per run. This procedure was conform with the COPHES SOP for DEHP phthalate metabolites, except for: ^{13}C-labeled standards were used instead of D-labeled

standards, no SPE pre-concentration on a C18-phase was applied, samples were directly injected after enzymatic cleavage.

5.13.6 BPA and Triclosan in urine

mL of urine urine was hydrolyzed adding the internal standards ^{13}C-BPA and ^{13}C-Triclosan, 50 µL β-glucuronidase/sulfatase and Na-acetate buffer 1M (pH 4.5) for 2hours at 40°C, followed by addition of 200 µL formic acid + 15 min sonication. When free BPA and TCS were determined, the same procedure was used without the addition of β-glucuronidase/sulfatase. Extraction was performed via SPE using Oasis HLB (3 mL, 60 mg). Elution was done with 5 mL methanol-dichloromethane (1:1 v/v) and the extract was evaporated till dryness. To the dry extract, 1 mL water + 2 mL hexane + 50 µL KOH + 50 µL pentafluorobenzoylchloride was added and vortexed. The hexane layer was transferred to another tube and the hexane extraction step was repeated, collecting all hexane fractions in one tube.

Clean-up of the samples was done by transferring the hexane layer to a 3mL cartridge containing 1g acidified silica (10% H_2SO_4) + 200 mg anhydrous Na_2SO_4. Elution was done using 6 mL hexane. After evaporation until dryness, the extract was reconstituted in 100 µL iso-octane. The measurements were done using Gas chromatography (Hewlett-Packard 6890 GC-) – electron capture negative ionisation mass spectrometry (Hewlett-Packard 5973 MS) (GC-ECNI/MS) equipped with a column: 15m x 0.25mm x 0.10µm DB-5MS, at 170°C (MS-source) and by injection of 1 µL, cold and splitless, using a PTV injector. The ^{13}C-TCS (m/z 494 and 299) and ^{12}C-TCS (m/z 482 and 287) (1 quantifier + 1 qualifier ion) ions were assessed. 20 samples were measured per run. The ^{13}C-BPA (m/z 628 and 418) and ^{12}C-BPA (m/z 616 and 406) (1 quantifier + 1 qualifier ion) were assessed (Geens et al., 2009)[3] Quantification was done with an internal standard method.

5.14 Statistical analysis

5.14.1 Data cleaning and descriptive statistics

The data cleaning program written in R, was provided by COPHES. It checked if the data complied with the last version of the codebook. The QC program screened the info from all questionnaires and toxicological data files. The program checked if each questionnaire contained all variables, if these variables had the correct format, if there were missing values in case the variable was obligatory, if there were numbers outside the pre-set ranges. It also performed consistency checks between some variables and it listed all given answers for the open questions. The errors reported by the QC-program were checked and if necessary input was asked from the fieldworkers which got

[3] *Geens T, Neels H, **Covaci A** (2009) Sensitive and selective method for the determination of Bisphenol-A and Triclosan in serum and urine as pentafluorobenzoate derivatives using GC-ECNI/MS. J Chromatogr B 877: 4042-4046.*

back to the paper questionnaires. For the toxicological database, additional checks were done for the values that were indicated to be below or above the limit of quantification (LOQ). First it was checked if those values were indeed < or > the LOQ, and for the values below the LOQ it was verified if they were replaced by half the LOQ. Also a table with the extreme outliers was displayed. Outliers were confirmed by re-analysis in the analysis labs. All errors were corrected in the databases, and the QC-program was run again, repeating these steps until the dataset was fully cleaned. Descriptive statistics of the biomarkers was calculated using SPSS.

5.14.2 Multiple regression models

Biomarker data were transferred to a normal distribution by natural logarithm (ln-) transformation. Multiple linear regression models were constructed for identification of the determinants of exposures. For urinary cotinine, there were less than 50% of the values above the LOQ both in children (30.4%) and mothers (34.4%). This biomarker was analysed in the multiple regression models as a dichotomous variable (above or below LOQ) using a multiple logistic regression model. The R-square (coefficient of determination) of the obtained multiple linear regression model was calculated and shows the proportion of variability in the biomarker level which is accounted for by this model. Quantitative relationships between the covariates and the biomarkers were calculated from the estimates of the multiple linear regression model, assuming that, when quantifying the relation of one covariate with the biomarker, all other covariates in the model are fixed at the population mean.

Multicollinearity, i.e. the existence of a high degree of linear correlation amongst two or more explanatory variables in a regression model (Neter et al., 1996) was examined. In the presence of multicollinearity, the estimate of one variable's impact on Y while controlling for the others tends to be less precise than if the predictors were uncorrelated with one another. Spearman rank correlation coefficients between the different explanatory variables were calculated to check correlation among the variables. If the correlation was high and the variance inflation factor in the model was larger than 10 then multicollinearity was concluded (Fox, 1991). The assumptions of normality, constancy of variance, independence (randomness) and linearity were checked with informal diagnostic plots and formal tests (White's General test for constancy of variance, Kolmogorov–Smirnov test for normality and the lack of fit test for linearity) (Neter et al., 1996). Outlying cases based on the constructed multiple linear regression model were checked. The outliers with respect to the response were assessed using the studentized deleted residuals and outliers with respect to the predictor variables were assessed using the leverages or the diagonal elements of the hat matrix. After outliers were identified, they were examined to see if they are influential cases by means of their DFFITS (influence on a single fitted value) and Cook's Distance (influence on all fitted values). Outlying observations which were also considered to be influential cases were considered for further analysis, that is fitting the model excluding them and checking whether there was any significant change in regression parameters.

The multiple linear regression models were constructed for each ln-transformed biomarker with 1) a predefined set of confounders and 2) covariates which were significantly correlated to the biomarker (univariate p-value < 0.25 as was tested in the stratified biomarker analysis by means of

an ANOVA test). The covariates and confounders tested are listed in Table 8. A final model was selected for which important covariates were identified by stepwise regression procedures in which we set p=0.25 for the independent variables to enter and p=0.05 to stay in the model.

All confounders and covariates were put in the models as categorical variables, the same categories as used in the tables of the biomarker analysis. Except for the variables 'ETS' and 'time spend in new car', as they are calculated for a subgroup of the individuals, non-smokers for ETS and people with new cars for time spend in new car, the other subjects will be lost when adding these variables in the multiple models. However, for the mothers, the variable 'tobacco smoke' was constructed as a combination of smoking and ETS; with the first category being the daily and occasional smokers followed by the categories of the ETS variables (at home, elsewhere and last 24h). As all children indicated to be non-smokers, smoking could be no confounder/covariate in these models, only ETS could be used in the models (and ETS includes all subjects). Also the covariate 'time spend in new car' is recoded as follows 1=more than one hour per day, 2=one hour per day or less and 3= never. In this way no individuals were lost when introducing the variable in the model.

Some variables were not used in the statistical models (since there were less than five observations in the yes-cathegory), or were combined or redefined. The following variables were skipped: both for mothers and children: morning urine, consumption of sea food products and offal, skin bleaching and neighborhood of metal industry. Only for the children: smoking status (as all children indicated to be non-smokers), consumption of game and hair treatment. For some variables categories were combined: for smoking status mother the category of 'daily' and 'occasional smokers' were combined since there were only 3 occasional smokers. For ETS elsewhere the category 'frequent' (only 2 subjects) and 'sometimes' were combined both for mothers and children. For wearing plastic gloves mothers the category 'daily' (only 4 subjects) and 'less than daily' were combined. For personal care products: for the mothers category 'low' (only 2 subjects) and 'moderate' were combined; for the children the category 'high' was deleted since no subjects in this category. For use of disinfection for the children the category 'daily' (only 5 subjects) and 'less than daily' were combined. For drinking water the category 'well/private water' (only 4 subjects) was combined with 'public water supply'. For PVC the category 'PVC in floors and walls' was deleted since no subjects in this category. Other variables were redefined: consumption of fresh water fish (both for mothers and children) and shell fish (only children) per week was redefined in consumption per month. The variable education was redefined in ISCED 0-4, ISCED 5 and ISCED 6, since the sampled Belgian population had a high proportion of higher educated families (tertiary education (ISCED 5-6) (84%)) and only a small proportion in ISCED 0-4 (16%). For the children, the period of urine sampling was redefined in <9, 9-11 and ≥11 hours, since there were only 8 subjects with less than 8 hours.

Table 8: Confounders and covariates used to test their influence on the levels of exposure markers measured in urine and hair

	Both for mother and child					
Confound-ers/covariates	Mercury in hair (µg/g)	Urinary cadmium (µg/L)	Urinary cotinine (µg/L)	Urinary phthalates (µg/L)	Urinary BPA (µg/L)	Urinary TCS (µg/L)
Creatinine		x	x	x	x	x
Urinary volume		x	x	x	x	x
Morning urine		x	x	x	x	x
Period urine		x	x	x	x	x
Age	x	x	x	x	x	x
Smoking	x	x	x		x	x
ETS		x	x		x	x
Food (specific items)	°Fish °Seafish °Shellfish °Fresh water fish °Seafood products °Offal	°Rice °Offal °Game °Wild mush-rooms °Chocolate °Local food		°Meat °Hazelnut spread °Fast food °Milk °Cheese °Chocolate °Ice cream °Canteen food °Chewing gum °Recent conven. food	°Meat °Fast food °Milk °Cheese °Canteen food °Chewing gum °Recent conven. food °Canned food	°Meat °Fast food °Milk °Cheese °Canteen food °Chewing gum °Recent conven. food
Water consumption	x	x		x	x	
Neighborhood of industry	x	x		x		
Fuel source		x				
House renovation		x		x	x	
PVC in house				x		
Traffic exposure		x		x		
Soldering	x	x				
Amalgame fillings	x					
Skin bleaching	x					
Broken mercury ther-mometer	x					
Broken energy-saving lamps	x					
Hair treatment	x					
Use of personal care products				x		X
Use of sunscreens						X
Use of disinfection						X
Education	x	x	x	x	x	X
Urban / rural	x	x	x	x	x	X
	Specific for child					
Gender	x	x	x	x	x	X
Use of toys				x	x	
	Specific for mother					
Use of gloves				x		

Results & Discussion

Recruitment

Non-responders

Description of study population

Belgian reference values of urinary and hair biomarkers

Belgian vs. European levels of urinary and hair biomarkers

6. Results and discussion

6.1 Recruitment

Recruitment via schools

School directors were contacted by phone to ask for their willingness of cooperation. School Directors, willing to participate, mostly accepted participation at the first contact moment. In case of willingness to participate, the local authorities, and/or the proper educational administrations were asked for their approval. The latter could take a few days, until about 2 weeks. In total we contacted five community councils or their educational administrations. Because some local authorities refused to cooperate, not all schools were able to participate. This initial step in the recruitment procedure was a slowing factor for the implementation of the field work.

At the start of the study, it was thought that 2 schools in the rural and 2 schools in the urban area would be enough to reach the number of pupils needed. During the running of the study more and more schools needed to be contacted, since the response rate was rather low, from 1-5% to 25-35%, leading to a number of candidates meeting the inclusion criteria between 0-3% en 20-25% per participating school. In the rural area 12 schools were contacted via phone. Eight of them were willing to participate. From the four non-participating schools, one was not allowed to by the educational office of the municipality and the other schools had no time or interest to allow recruitment among their pupils. In the urban area, 28 schools were contacted. Five schools could participate. The others were mainly excluded for participation because there were not enough Dutch or French speaking pupils in the school or because they were not allowed to (Table 9). In total there were 117 children recruited via 13 schools (Table 10). Another 12 children were recruited via informal contacts (friends of participants, family collegues). The latter was done by the end of the study, since that allowed more specific inclusion of children from the still needed age and sex group.

Figure 19: Map with the locations of the schools recruiting the participants.

Table 9: Schools contacted, which were not willing or not able to participate

Region	N° of contacted schools	N° of NON-participating schools	Community	Reported reason(s) for not participating
RURAL	12	4	Rural-Dutch	refusal
			Rural-French	refusal refusal by P.O.
URBAN	28	23	Urban-Dutch	Nearly no Dutch speaking pupils
				too much work
				few Belgian pupils
				not possible to collect urine in school

Region	N° of contacted schools	N° of NON-participating schools	Community	Reported reason(s) for not participating
				high class SES
			Urban-French	refusal by IP (instruction public)
				a lot of East-American, Arabian pupils
				not an optimal population
				Nearly no French speaking pupils
				low SES
				big part of mothers non-French

Table 10: Schools participating in the study and the number of participants in the schools

Region	N° of partici-pating Schools	Area	N° of letters spread in school	N° of returned reply cards (%)	N° of subjects that accepted participation (%)	Included 'eligibles' (%)
RURAL	8	Rural-Dutch	130	86 (66.2)	36 (27.6)	23 (17.7)
			149	122 (81.9)	22 (32.78)	9 (6.0)
		Rural-French	140	46 (32.9)	12 (8.6)	7 (5.0) [1]
			107	32 (29.9)	8 (7.5)	5 (4.7)
			26	18 (69.2)	7 (26.9)	7(26.9)
			221	81 (36.7)	19 (8.7)	6 (2.7)
			73	21 (28.8)	4 (5.5)	2 (2.7)
			248	106 (42.7)	23 (9.3)	7 (2.8)
			1094	**512 (46.8)**	**131 (11.97)**	**66 (6.03)**
URBAN	5	Urban-Dutch	145	117 (80.7)	53 (36.6)	24 (16.6)
			145	58 (40)	23 (15.9)	9 (6.21) [1]
		Urban-French	206	51 (24.8)	11 (5.3)	7 (3.4)
			350	59 (16.9)	16 (4.6)	11 (3.1)
			250	31 (12.4)	3 (1.2)	0 (0)
			1096	**316 (28.83)**	**106 (9.67)**	**51 (4.65)**
		TOTAL	**2190**	**828 (37.81)**	**237 (10.78)**	**117 (5.34)** [2]

[1] not including the mother-child pair of which the mother participated already via another child of her, [2] Besides recruitment via schools, 12 mother-child couples were recruited extra via personal contacts of field and project workers. In total 129 mother-child couples were includde in the Belgian study population.

Most of the participants of the DEMOCOPHES project were recruited from public schools. The selected schools were attended by pupils of different socio-economic classes. At the first contact with the school director, it was asked if the school represented more or less a good mix of Belgians and immigrants, in other words, if enough parents were able to read French and/or Dutch documents. For this reason quite some schools needed to be excluded. Because of the highly international character of Brussels, at least 13 of the 28 contacted schools in that urban area were excluded only for this reason. This was especially striking in the French speaking urban schools.

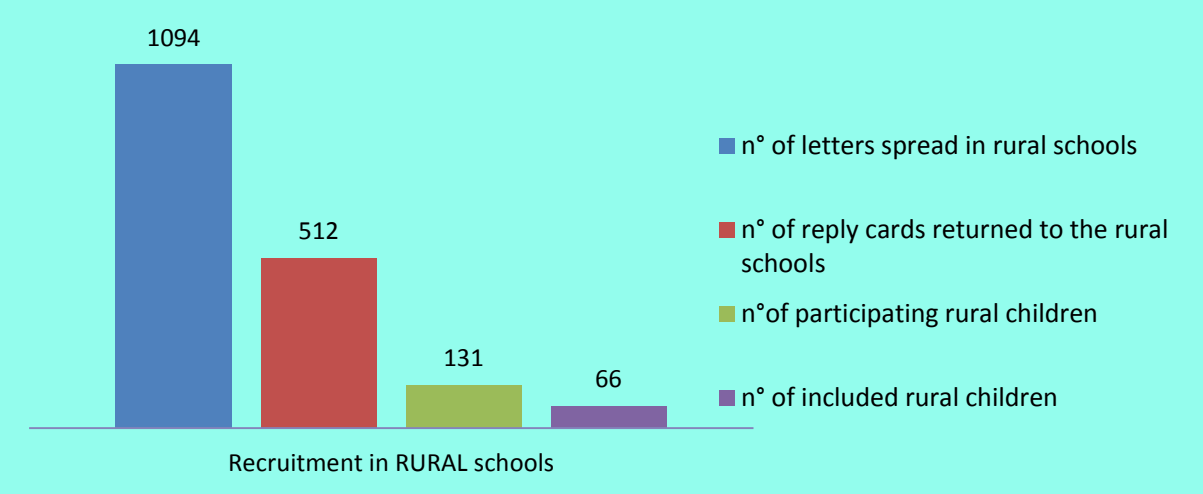


Figure 20: Recruitment in rural schools

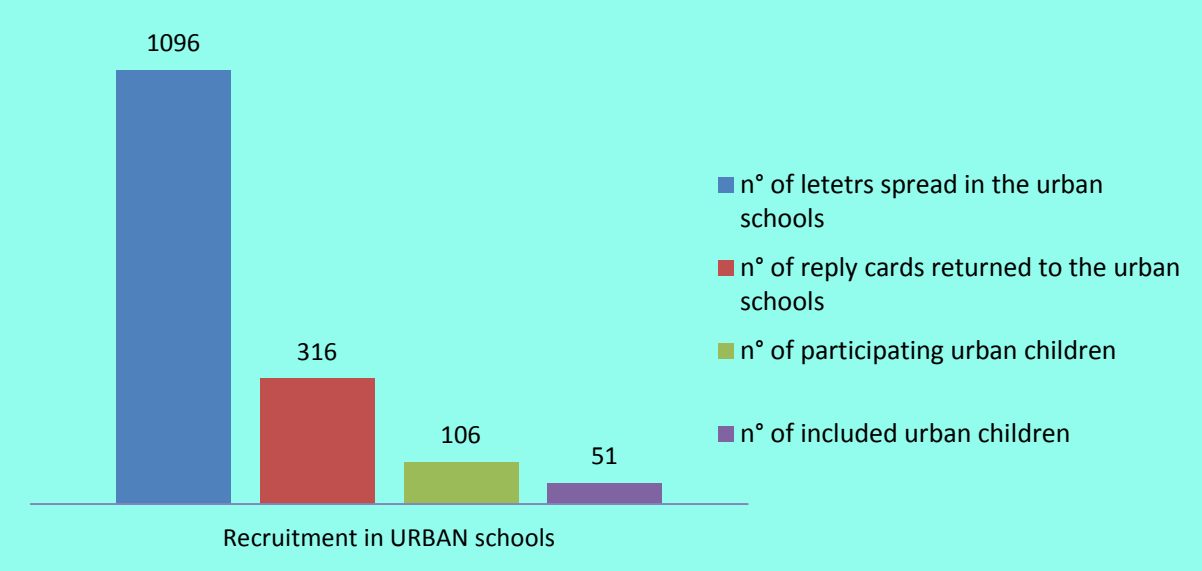


Figure 21: Recruitment in urban schools

Participation

In the schools which were willing to participate, all pupils of the basic school (6-11y) were asked for participation. The number of reply cards returned varied among schools: 12.4-81.9% (**Error! Reference source not found.**). Overall the Dutch speaking schools had somewhat more reply cards returned although participants were motivated to do so in both study areas, by giving them a small attractive stress-ball or back pack. The number of filled out and returned reply cards was higher in the rural (46.8 %) compared to the urban (28.83 %) area (Table 10). When the reply cards returned, field workers checked them on the response to participation and compliance with the inclusion

criteria. Although the incentive worked for bringing back the response card to the school, the willingness to participate to the project was rather low. The percentage of participation varied from 1.2 to 32.8% (Table 10). The participation willingness did not differ much among rural and urban areas. The degree of participation was much more dependent on the school. Overall, we received a reply card backfrom 37.8% of the invited pupils. 14.1% of the people invited to the study wished to participate, but due to stringent inclusion criteria of area, age and sex classes needed, and exclusion of the second of more children of the same mother, only 5.3% (expressing participation relative to the distributed reply cards) could participate. Most of the people wanting to participate fell out because they did not live long enough in the area, or because the mother was more than 45y old (Table 11).

Table 11: Selection of candidates by inclusion criteria

		N° of letters spread in schools	N° of possible participants	<5y in area	not in area	illness	>45y	<6Y	>1 child/mother	pregnant	Age and/or sex category not needed anymore	Suddenlynot interested anymore in participating	Total excluded	Selected participants
RURAL														
FLANDERS	Brakel	279	58	11	4	2	3		4	1		1	26	32
WALLONIA	Ellezelles	247	20	2			4				1	1	8	12
	Frasnes	568	53	4	2		2	1			22		31	22
		1.094	**131**										**65**	**66**
URBAN														
FLANDERS	Jette	290	76	1	16				12		14		43	33
WALLONIA	Jette	556	27	2			6			1			9	18
	Brussels center	250	3	2			1						3	0
		1096	**106**										**55**	**51**

Extra info:
In Flanders there was one mother with two children (brother and sister) and in Wallonia there was a mother with a twin who participated in the study. Both children got personal results, but only one of both was included in study.
12 mother-child couples were recruited extra from individual contacts of the fieldworkers (all of them met the inclusion criteria.). In total 129 mother-child couples were included.

Table 12: Overview of participating mother-child pairs recruited via schools

Participants (mother-child pairs)		number
Selected (N_1)	Invitations distributed in schools	2190 [(1)]
Non-contacts (N_2)	families that could never be reached (i.e. no answer on reply card))	1362
Contacts (N_3)	families reached (i.e. reply card returned)	828
Refusals (N_4)	families reached, but refused participation	591
Not eligible (N_5)	families reached, but not eligible	120
Participants (N_6)	families participating	117 [(1)]

Rates		Percentage
Contact rate	Cases reached proportional to selected/invited = N_3/N_1	37.8%
Cooperation rate	Participants proportional to contacted = $N_6 / (N_4 + N_5 + N_6)$	14.1 %
Response rate	Participants proportional to all selected/invited cases = N_6 / N_1	5.34 %
Refusal rate	Refusals proportional to all selected/invited cases = N_4/N_1	27.0 %

[(1)] By the end of the fieldwork period 12 mother-child couples were recruited extra from individual contacts by field and project workers (all of them met the inclusion criteria). In total 129 mother-child couples were included.

NB: The proportion reached (contact rate) is not fully correct since the inclusion criteria were included on the information document. Therefore some mothers didn't return the reply card just because there didn't fall in the inclusion criteria

Possible explanations for the low cooperation rate of 14.1% are:

- Too much information: potential participants received too large information. In the last contacted schools we used a shortened version of the information letter.Too short timing: there was no (time for) information sessions at the school for parents, children, teachers, parents committee,… needs to be put into Cooperation with parent-committees and/or medical examination centers might have been a possibility to increase the response rate.
- 'mother-child' couples were needed. This kind of recruitment strategy is much more difficult than recruiting individuals.
- Only one child per mother could be included. Brothers and/or sisters attending the same school were not allowed to participate. For some mothers this was areason for non participation.

The low response rate was most probably also partly due to the need of a face-to-face interview with the mother. An interviewing via telephone might have been a valid alternativeIn the Belgian

study, only two mothers were by exception interviewed via phone, as it was not possible to arrange a date for a face-to-face interview.

6.2 Non-responders

128 non-responder questionnaires were filled out vs. the 129 participants (responders) in the study. The main differences between responders and non-responders were higher education level and higher number of working mothers and fathers in the responders (Table 13). There was no difference in smoking habits, frequency of eating fish and number of single mothers. 36 people completed an extra question on reason of non-responding. Most important reasons for non-participation were: 'not interested', 'language problem' and 'no time' (Table 14).

Table 13: Non-responder analysis: comparison of some characteristics of responders vs. non-responders to the study. The number and percentage of individuals are indicated in the columns

	Responders N = 129		Non-responders N = 128		p-value [1]
Mothers smoking	12	9.3%	17	13.6%	p = 0.28
Households with at least one smoker	39	30.2%	39	31.5%	p = 0.83
Eating fish/shellfish at least once per week	89	69.0%	97	78.9%	p = 0.08
Single mothers	15	11.6%	22	18.0%	p = 0.15
Highest educational level in family (mother or partner):					
• Primary or lower secondary education (ISCED:0-2)	3	2.3%	10	8.1%	P=0.002
• Higher secondary or post-secondary non-tertiary education (ISCED: 3-4)	18	14.0%	33	26.8%	
• Tertiary education (ISCED: 5-6)	108	83.7%	80	65.0%	
Working mothers	118	91.5%	98	79.7%	p = 0.007
Working fathers / spouses / partners	114	94.2%	91	83.5%	p = 0.009

[1] *Proportions are compared by a Chi-square test*

Table 14: Non-responders: reasons for non-participation

Reasons for non-participation	Number of answers
Language problem	6
No time	6
Don't like it	5
<5y living in study area	3
>45y	3
Not living in study area	2
Health problems	2
Husband didn't like it	2
Urine sample issue	2

Reasons for non-participation	Number of answers
Afraid of results	1
Information letter too difficult	1
Privacy issue	1
Do not agree with the aim of the study	1
Do not know	1

6.3 Description of the study population

The median age of the 129 participating mother and children was 40 years and 8 years, respectively. The children were recruited, so that there was an equal number and sex distribution among the 6 to 11 years age groups. The basic questionnaire provided information on diet, life style, use of consumer products, and home environment (Table 15 and Table 16). 9.3% of the mothers were current smokers and none of the children reported to be smoker.

With 33% of the mothers and 36% of the children who consumed food from a local farmer, family or own growth, local food consumption was common among the Belgian participants. Fish was eaten by 30% of the mothers and 20% of the children ate fish several times per week. A large group of 50% of the children ate dishes served in a canteen.

Eighty % of mothers and only 5% of the children had teeth amalgam fillings. 57% of the mothers dyed/toned hair in the last 6 months before urine sampling. 10% of mothers and 21% of the children used anti-lice shampoo in the last 6 months. Almost all mothers had a high to moderate use of personal care products (like make-up, shampoo, eye make-up, hair styling products, body lotion, crèmes, fragrances, deodorants, nail polish and massage oil). Also about 1/3rd of the children were moderate users of those products. Most of the children played with plastic toys, however 20% of children reported to have never played with toys made of plastic.

25% of the mothers and 6% of the children spent at least one hour per day in traffic. Half (52%) of the mothers indicated that they did/had redecoration or renovation activities in their house in the last 2 years. Around 20% of the participants reported having broken an energy-saving lamp or a thermometer ever in life.

Table 15: Characteristics of the study population: mothers

Mothers (N = 129)			
Parameter	**Statistics**	**Values**	
Age, years	Total N	129	
	Median	40	
	P25 – P75	37	42
	Min.-max.	27	45
Age distribution:	Total N	129	
≤35 years	N, %	23	17.8%
35-40 years	N, %	60	46.5%
≥40 years	N, %	46	35.7%
Urinary creatinine, mg/L	Total N	129	
	Median	1152	
	P25 – P75	777	1589
	Min.-max.	205	3075
Urinary creatinine in classes	Total N	129	
< 300 mg/L (=exclusion criterion)	N, %	3	2.3%
300 – 1000 mg/L	N, %	50	38.8%
1000 – 2000 mg/L	N, %	65	50.4%
2000 – 3000 mg/L	N, %	10	7.8%
> 3000 mg/L (=exclusion criterion)	N, %	1	0.8%
Urinary volume, g	Total N	129	
	Median	153	
	P25 – P75	116	190
	Min.-max.	20	288
Urinary volume in classes	Total N	129	
< 80 gram	N, %	9	7.0%
80 – 120 gram	N, %	30	23.3%
≥ 120 gram	N, %	90	69.8%
Morning urine sample	Total N	129	
Yes	N, %	129	100.0%
No	N, %	0	0.0%
Urine sampling period (hours)	Total N	118	
	Median	8	
	P25 – P75	7	9
	Min.-max.	0	11
Urine sampling period in classes:	Total N	118	
≤5 hours	N, %	8	6.8%
5-8 hours	N, %	59	50.0%
≥8 hours	N, %	51	43.2%
Body weight, kg	Total N	125	
	Median	61	
	P25 – P75	56	70
	Min.-max.	42	105
Height, cm	Total N	123	
	Median	165	
	P25 – P75	160	170
	Min.-max.	145	179
Body-mass index, kg/m²	Total N	122	
	Median	22.53	
	P25 – P75	20.48	26.03
	Min.-max.	17.71	42.22
Smoking habits:	Total N	129	
Daily smoker	N, %	9	7.0%

Mothers (N = 129)			
Parameter	**Statistics**	**Values**	
Occasional smoker	N, %	3	2.3%
Former smoker	N, %	32	24.8%
Non smoker (never)	N, %	85	65.9%
Smoking, amount of cigarettes (in smokers only)	Total N	12	
	Median	10	
	P25 – P75	3	13
	Min.-max.	1	25
Environmental Tobacco Smoke (ETS) at home (in former and non-smokers only)	Total N	117	
Daily	N, %	5	4.3%
Less than daily	N, %	6	5.1%
Never	N, %	106	90.6%
Environmental Tobacco Smoke (ETS) elsewhere (in former and non-smokers only)	Total N	117	
Frequent	N, %	2	1.7%
Sometimes	N, %	65	55.6%
Never	N, %	50	42.7%
Environmental Tobacco Smoke (ETS) in last 24 hours (in former and non-smokers only)	Total N	117	
Yes	N, %	14	12.0%
No	N, %	103	88.0%
Alcohol consumption by mother (units/week)	N	125	
	Median	2	
	P25 – P75	1	4
	Min.-max.	0	17
Consumption of rice	Total N	129	
Several times per week	N, %	18	14.0%
Once a week or less	N, %	111	86.0%
Consumption of meat/cold meat	Total N	129	
Several times per week	N, %	117	90.7%
Once a week or less	N, %	12	9.3%
Consumption of offal	Total N	125	
Several times per month	N, %	3	2.4%
Once a month or less	N, %	122	97.6%
Consumption of game	Total N	127	
Several times per month	N, %	5	3.9%
Once a month or less	N, %	122	96.1%
Consumption of wild mushrooms	Total N	127	
Several times per month	N, %	8	6.3%
Once a month or less	N, %	119	93.7%
Consumption of hazelnut spread	Total N	129	
Several times per week	N, %	48	37.2%
Once a week or less	N, %	81	62.8%
Consumption of convenience food or fast food	Total N	129	
Several times per week	N, %	9	7.0%
Once a week or less	N, %	120	93.0%
Consumption of milk	Total N	129	
Several times per week	N, %	87	67.4%
Once a week or less	N, %	42	32.6%
Consumption of cheese	Total N	128	
Several times per week	N, %	106	82.8%
Once a week or less	N, %	22	17.2%
Consumption of cereals	Total N	129	
Several times per week	N, %	32	24.8%

Mothers (N = 129)			
Parameter	**Statistics**	**Values**	
Once a week or less	N, %	97	75.2%
Consumption of chocolate	Total N	128	
Several times per week	N, %	85	66.4%
Once a week or less	N, %	43	33.6%
Consumption of ice cream	Total N	129	
Several times per week	N, %	6	4.7%
Once a week or less	N, %	123	95.3%
Consumption of local food	Total N	129	
Several times per week	N, %	43	33.3%
Once a week or less	N, %	86	66.7%
Consumption of dishes served in a canteen	Total N	129	
Several times per week	N, %	18	14.0%
Once a week or less	N, %	111	86.0%
Consumption of chewing gum	Total N	127	
Several times per week	N, %	27	21.3%
Once a week or less	N, %	100	78.7%
Consumption of fish (all types)	Total N	129	
Several times per week	N, %	38	29.5%
Once a week or less	N, %	91	70.5%
Consumption of seafish	Total N	129	
Several times per week	N, %	14	10.9%
Once a week or less	N, %	115	89.1%
Consumption of shellfish	Total N	129	
Several times per week	N, %	6	4.7%
Once a week or less	N, %	123	95.3%
Consumption of fresh water fish	Total N	128	
Several times per week	N, %	1	0.8%
Once a week or less	N, %	127	99.2%
Consumption of sea food products	Total N	128	
Several times per month	N, %	4	3.1%
Once a month or less	N, %	124	96.9%
Consumption of convenience food in last 24 hours	Total N	124	
Yes	N, %	56	45.2%
No	N, %	68	54.8%
Consumption of canned food	Total N	129	
Several times per week	N, %	24	18.6%
Once a week or less	N, %	105	81.4%
Ever used skin bleaching	Total N	129	
Yes	N, %	1	0.8%
No	N, %	128	99.2%
Amalgam teeth fillings	Total N	125	
Yes	N, %	100	80.0%
No	N, %	25	20.0%
Hair was dyed/toned in last 6 months	Total N	128	
Yes	N, %	73	57.0%
No	N, %	55	43.0%
Hair has undergone a chemical hair structure treatment in last 6 months	Total N	129	

Mothers (N = 129)			
Parameter	**Statistics**	**Values**	
Yes	N, %	8	6.2%
No	N, %	121	93.8%
Anti lice shampoo used in last 6 months	Total N	129	
Yes	N, %	14	10.9%
No	N, %	115	89.1%
Time spend in traffic	Total N	126	
At least one hour per day	N, %	32	25.4%
One hour per day or less	N, %	94	74.6%
Time spend in new car (less than 2 years old)	Total N	30	
At least one hour per day	N, %	13	43.3%
One hour per day or less	N, %	17	56.7%
Wearing plastic gloves	Total N	128	
Daily	N, %	4	3.1%
Less than daily	N, %	17	13.3%
Never	N, %	107	83.6%
Use of personal care products	Total N	126	
High	N, %	53	42.1%
Moderate	N, %	71	56.3%
Low	N, %	2	1.6%
Use of sunscreens	Total N	128	
Yes	N, %	7	5.5%
No	N, %	121	94.5%
Use of disinfection	Total N	129	
Daily	N, %	29	22.5%
Less than daily	N, %	29	22.5%
Never	N, %	71	55.0%
Area of residence	Total N	129	
Urban	N, %	64	49.6%
Rural	N, %	65	50.4%
Highest educational level of the family	Total N	129	
Primary or lower secondary education	N, %	3	2.3%
Higher sec. or post-sec. non-tertiary education	N, %	18	14.0%
Tertiary education	N, %	108	83.7%
Industry with possible contamination of heavy metals in neighborhood of residence	Total N	129	
Yes	N, %	4	3.1%
No	N, %	125	96.9%
Industry with possible contamination of phthalates in neighborhood of residence	Total N	129	
Yes	N, %	9	7.0%
No	N, %	120	93.0%
Fossil materials as main source for heating or cooking	Total N	129	
Yes	N, %	8	6.2%
No	N, %	121	93.8%
Redecoration or renovation of house in last two years	Total N	129	
Yes	N, %	67	51.9%
No	N, %	62	48.1%
PVC in house	Total N	126	
PVC in floors and walls	N, %	0	0.0%
PVC in floors or walls	N, %	30	23.8%
No PVC	N, %	96	76.2%

Mothers (N = 129)			
Parameter	**Statistics**	**Values**	
Main source of water for drinking	Total N	129	
Public water supply	N, %	79	61.2%
Commercial producers	N, %	46	35.7%
Well / private water	N, %	4	3.1%
Mercury containing thermometer broken in the house	Total N	123	
Yes	N, %	28	22.8%
No	N, %	95	77.2%
Energy saving lamp broken in the house	Total N	112	
Yes	N, %	25	22.3%
No	N, %	87	77.7%
Soldering indoors	Total N	129	
Yes	N, %	11	8.5%
No	N, %	118	91.5%

Table 16: Characteristics of the study population: children

Children (N = 129)			
Parameter	**Statistics**	**Values**	
Gender	Total N	129	
Boy	N, %	66	51.2%
Girl	N, %	63	48.8%
Age, years	Total N	129	
	Median	8	
	P25 – P75	7	10
	Min.-max.	6	11
Age distribution:	Total N	129	
5-8 years	N, %	66	51.2%
9-11 years	N, %	63	48.8%
Urinary creatinine, mg/L	Total N	129	
	Median	1150	
	P25 – P75	880	1426
	Min.-max.	98	2573
Urinary creatinine in classes	Total N	129	
< 300 mg/L (=exclusion criterion)	N, %	4	3.1%
300 – 1000 mg/L	N, %	46	35.7%
1000 – 2000 mg/L	N, %	73	56.6%
2000 – 3000 mg/L	N, %	6	4.7%
> 3000 mg/L (=exclusion criterion)	N, %	0	0.0%
Urinary volume, g	Total N	129	
	Median	146	
	P25 – P75	103	189
	Min.-max.	31	297
Urinary volume in classes	Total N	129	
< 80 gram	N, %	13	10.1%
80 -120 gram	N, %	31	24.0%
≥ 120 gram	N, %	85	65.9%
Morning urine sample	Total N	129	
Yes	N, %	129	100.0%
No	N, %	0	0.0%
Urine sampling period (hours)	Total N	124	
	Median	11	

Children (N = 129)			
Parameter	**Statistics**	**Values**	
	P25 – P75	10	11
	Min.-max.	1	14
Urine sampling period in classes:	Total N	124	
≤5 hours	N, %	4	3.2%
5-8 hours	N, %	4	3.2%
≥8 hours	N, %	116	93.5%
Body weight, kg	Total N	111	
	Median	29	
	P25 – P75	24	34
	Min.-max.	17	65
Height, cm	Total N	110	
	Median	135	
	P25 – P75	127	142
	Min.-max.	107	155
Smoking habits	Total N	129	
Daily smoker	N, %	0	0.0%
Occasional smoker	N, %	0	0.0%
Non-smoker	N, %	129	100.0%
Environmental Tobacco Smoke (ETS) at home (in non-smokers only)	Total N	129	
Daily	N, %	7	5.4%
Less than daily	N, %	10	7.8%
Never	N, %	112	86.8%
Environmental Tobacco Smoke (ETS) elsewhere (in non-smokers only)	Total N	129	
Frequent	N, %	2	1.6%
Sometimes	N, %	59	45.7%
Never	N, %	68	52.7%
Environmental Tobacco Smoke (ETS) in last 24 hours (in non-smokers only)	Total N	127	
Yes	N, %	12	9.4%
No	N, %	115	90.6%
Consumption of rice	Total N	129	
Several times per week	N, %	21	16.3%
Once a week or less	N, %	108	83.7%
Consumption of meat/cold meat	Total N	129	
Several times per week	N, %	117	90.7%
Once a week or less	N, %	12	9.3%
Consumption of offal	Total N	127	
Several times per month	N, %	2	1.6%
Once a month or less	N, %	125	98.4%
Consumption of game	Total N	128	
Several times per month	N, %	4	3.1%
Once a month or less	N, %	124	96.9%
Consumption of wild mushrooms	Total N	128	
Several times per month	N, %	6	4.7%
Once a month or less	N, %	122	95.3%
Consumption of hazelnut spread	Total N	129	
Several times per week	N, %	96	74.4%
Once a week or less	N, %	33	25.6%
Consumption of convenience food or fast food	Total N	128	
Several times per week	N, %	7	5.5%
Once a week or less	N, %	121	94.5%

Children (N = 129)			
Parameter	**Statistics**	**Values**	
Consumption of milk	Total N	129	
Several times per week	N, %	114	88.4%
Once a week or less	N, %	15	11.6%
Consumption of cheese	Total N	127	
Several times per week	N, %	89	70.1%
Once a week or less	N, %	38	29.9%
Consumption of cereals	Total N	128	
Several times per week	N, %	75	58.6%
Once a week or less	N, %	53	41.4%
Consumption of chocolate	Total N	127	
Several times per week	N, %	84	66.1%
Once a week or less	N, %	43	33.9%
Consumption of ice cream	Total N	126	
Several times per week	N, %	9	7.1%
Once a week or less	N, %	117	92.9%
Consumption of local food	Total N	128	
Several times per week	N, %	46	35.9%
Once a week or less	N, %	82	64.1%
Consumption of dishes served in a canteen	Total N	129	
Several times per week	N, %	64	49.6%
Once a week or less	N, %	65	50.4%
Consumption of chewing gum	Total N	127	
Several times per week	N, %	18	14.2%
Once a week or less	N, %	109	85.8%
Consumption of fish (all types)	Total N	129	
Several times per week	N, %	26	20.2%
Once a week or less	N, %	103	79.8%
Consumption of seafish	Total N	129	
Several times per week	N, %	15	11.6%
Once a week or less	N, %	114	88.4%
Consumption of shellfish	Total N	128	
Several times per week	N, %	3	2.3%
Once a week or less	N, %	125	97.7%
Consumption of fresh water fish	Total N	126	
Several times per week	N, %	1	0.8%
Once a week or less	N, %	125	99.2%
Consumption of sea food products	Total N	127	
Several times per month	N, %	2	1.6%
Once a month or less	N, %	125	98.4%
Consumption of convenience food in last 24 hours	Total N	123	
Yes	N, %	52	42.3%
No	N, %	71	57.7%
Consumption of canned food	Total N	129	
Several times per week	N, %	26	20.2%
Once a week or less	N, %	103	79.8%
Ever used skin bleaching	Total N	129	
Yes	N, %	0	0.0%
No	N, %	129	100.0%

Children (N = 129)			
Parameter	**Statistics**	**Values**	
Amalgam teeth fillings	Total N	127	
Yes	N, %	6	4.7%
No	N, %	121	95.3%
Hair was dyed/toned in last 6 months	Total N	129	
Yes	N, %	0	0.0%
No	N, %	129	100.0%
Hair has undergone a chemical hair structure treatment in last 6 months	Total N	129	
Yes	N, %	3	2.3%
No	N, %	126	97.7%
Anti lice shampoo used in last 6 months	Total N	128	
Yes	N, %	27	21.1%
No	N, %	101	78.9%
Time spend in traffic	Total N	127	
At least one hour per day	N, %	8	6.3%
One hour per day or less	N, %	119	93.7%
Time spend in new car (less than 2 years old)	Total N	33	
At least one hour per day	N, %	2	6.1%
One hour per day or less	N, %	31	93.9%
Playing with plastic toys	Total N	115	
Daily	N, %	54	47.0%
Less than daily	N, %	38	33.0%
Never	N, %	23	20.0%
Use of personal care products	Total N	127	
High	N, %	0	0.0%
Moderate	N, %	45	35.4%
Low	N, %	82	64.6%
Use of sunscreens	Total N	128	
Yes	N, %	5	3.9%
No	N, %	123	96.1%
Use of disinfection	Total N	129	
Daily	N, %	5	3.9%
Less than daily	N, %	14	10.9%
Never	N, %	110	85.3%

6.4 Belgian reference values

The levels of the measured biomarkers of the participating Belgian mothers and their children are given in Table 17. The table gives an overview of the descriptive statistics of all parameters. Eight individuals needed to be excluded from the urine analyses since the creatinine levels were either too low (< 300 mg/L: 3 mothers, 4 children) or too high (> 3000mg /L: 1mother). In other words, the urine was either too diluted or too concentrated to allow accurate assessment of contaminants.

Table 17: Belgian levels of the biomarkers measured in the mothers and children

Biomarker	Matrix	Population	Unit	N	%>LOQ	GM	low CI	up CI	AM	SD	min	P_{10}	P_{25}	P_{50}	P_{75}	P_{90}	P_{95}	max
heavy metals																		
Mercury	H	mother	(µg/g)	129	95.3%	.383	.333	.441	.504	.365	.040	.142	.259	.440	.635	.987	1.236	1.969
	H	child	(µg/g)	127	80.3%	.204	.172	.242	.306	.286	.040	.040	.122	.243	.393	.582	.817	1.995
Cadmium	U	mother	µg/L	125	99.2%	.21	.18	.24	.27	.20	.01	.08	.13	.22	.31	.51	.65	1.10
			µg/g creat.	125	99.2%	.183	.162	.206	.222	.135	.006	.093	.123	.195	.281	.374	.462	.767
	U	child	µg/L	125	86.4%	.04	.04	.05	.07	.06	.01	.01	.03	.05	.09	.14	.18	.32
			µg/g creat.	125	86.4%	.039	.033	.047	.059	.050	.003	.005	.028	.050	.078	.107	.141	.323
Smoking																		
Cotinine	U	mother	µg/L	125	30.4%	-	-	-	-	-	-	-	-	.4	1.1	74.1	905.1	3708.8
			µg/g creat.	125	30.4%	-	-	-	-	-	-	-	-	.4	.8	109.0	776.5	4243.3
	U	child	µg/L	125	34.4%	-	-	-	-	-	-	-	-	.4	.8	2.9	6.0	52.0
			µg/g creat.	125	34.4%	-	-	-	-	-	-	-	-	.4	.8	3.3	5.6	58.4
Phthalate metabolites of DEHP																		
MEHP	U	mother	µg/L	125	92.8%	2.16	1.81	2.59	3.72	6.10	.25	.56	1.20	2.30	4.30	7.20	9.10	57.00
			µg/g creat.	125	92.8%	1.93	1.65	2.26	2.89	4.00	.13	.65	1.32	1.94	3.50	4.91	6.56	39.45
	U	child	µg/L	125	95.2%	2.32	1.97	2.74	3.71	4.61	.25	.89	1.30	2.20	3.80	8.70	13.00	28.00
			µg/g creat.	125	95.2%	2.08	1.77	2.44	3.37	5.39	.16	.76	1.16	1.94	3.30	7.08	10.13	50.67
5OH-MEHP	U	mother	µg/L	125	100.0%	10.97	9.50	12.67	15.61	16.27	1.20	4.20	6.30	11.00	17.00	32.00	51.00	118.00
			µg/g creat.	125	100.0%	9.79	8.70	11.01	12.32	9.75	.98	4.76	6.36	9.48	13.95	23.53	31.54	53.18
	U	child	µg/L	125	100.0%	19.77	16.87	23.18	35.11	79.21	4.00	6.60	12.00	17.00	31.00	71.00	109.00	836.00
			µg/g creat.	125	100.0%	17.66	15.11	20.65	41.51	198.79	3.19	7.65	9.96	16.06	25.91	59.61	92.59	2229.33
5OXO-MEHP	U	mother	µg/L	125	100.0%	7.63	6.59	8.84	10.87	10.97	.76	2.80	4.20	7.60	13.00	22.00	35.00	78.00
			µg/g creat.	125	100.0%	6.81	6.07	7.64	8.42	6.16	.62	3.11	4.54	6.70	9.57	16.77	21.59	35.15
	U	child	µg/L	125	100.0%	14.24	12.19	16.63	24.14	48.56	2.80	5.00	8.20	13.00	22.00	43.00	75.00	501.00
			µg/g creat.	125	100.0%	12.72	10.93	14.79	27.35	119.14	2.19	5.19	7.40	11.24	19.10	36.86	64.81	1336.00
5OH-MEHP + 5oxo-	U	mother	µg/L	125	100.0%	18.72	16.22	21.60	26.48	27.05	1.96	7.00	10.50	18.50	29.00	57.00	86.00	196.00
			µg/g creat.	125	100.0%	16.70	14.89	18.73	20.74	15.75	1.60	8.03	11.34	15.90	23.87	40.11	53.18	88.33
	U	child	µg/L	125	100.0%	34.09	29.14	39.89	59.25	127.66	6.80	12.20	20.20	30.00	51.00	111.00	184.00	1337.00

Biomarker	Matrix	Population	Unit	N	%>LOQ	GM	low CI	up CI	AM	SD	min	P_{10}	P_{25}	P_{50}	P_{75}	P_{90}	P_{95}	max
MEHP			µg/g creat.	125	100.0%	30.46	26.12	35.51	68.86	317.90	5.71	12.82	17.32	27.30	44.53	96.47	157.41	3565.33
MEHP+ 5OH-MEHP+ 5oxo-MEHP	U	mother	µg/L	125	100.0%	21.28	18.42	24.57	30.20	31.11	2.21	7.68	12.20	21.20	34.60	59.30	97.40	203.70
			µg/g creat.	125	100.0%	18.98	16.91	21.31	23.63	18.25	1.80	9.17	12.73	17.94	27.54	44.81	60.72	122.49
	U	child	µg/L	125	100.0%	36.72	31.42	42.91	62.97	130.57	7.72	13.24	21.40	32.30	55.20	115.80	209.00	1356.00
			µg/g creat.	125	100.0%	32.80	28.17	38.20	72.24	322.49	5.86	14.48	18.85	29.37	49.20	107.45	170.14	3616.00
Phthalate metabolite of BBP																		
MBzP	U	mother	µg/L	125	100.0%	6.47	5.55	7.54	10.05	16.49	.66	2.00	3.80	6.40	12.00	17.00	23.00	164.00
			µg/g creat.	125	100.0%	5.77	5.05	6.59	7.99	9.26	1.08	2.18	3.42	5.52	8.72	15.53	19.82	72.51
	U	child	µg/L	125	100.0%	8.78	7.44	10.36	14.35	21.80	.35	2.70	5.30	8.60	14.00	27.00	35.00	183.00
			µg/g creat.	125	100.0%	7.84	6.68	9.21	12.44	17.83	.31	2.53	4.65	8.01	13.58	23.34	31.94	140.99
Phthalate metabolite of DiBP																		
MiBP	U	mother	µg/L	125	100.0%	38.08	32.48	44.65	62.81	98.55	4.40	13.00	21.00	33.00	63.00	115.00	175.00	702.00
			µg/g creat.	125	100.0%	33.97	29.69	38.87	47.59	50.62	3.59	15.63	18.47	29.50	53.16	101.14	142.68	324.46
	U	child	µg/L	125	100.0%	58.16	49.29	68.63	104.64	207.49	3.80	21.00	31.00	54.00	96.00	187.00	362.00	1940.00
			µg/g creat.	125	100.0%	51.96	44.35	60.88	91.02	180.88	3.35	20.43	29.67	46.42	83.33	135.94	278.89	1634.37
Phthalate metabolite of DBP																		
MnBP	U	mother	µg/L	125	100.0%	30.86	26.88	35.43	44.03	55.37	3.70	12.00	20.00	31.00	47.00	89.00	119.00	432.00
			µg/g creat.	125	100.0%	27.53	24.57	30.85	34.84	31.52	4.00	14.06	18.68	25.34	39.77	57.37	79.31	238.54
	U	child	µg/L	125	100.0%	38.97	34.46	44.08	49.78	40.17	3.20	17.00	25.00	40.00	58.00	98.00	122.00	256.00
			µg/g creat.	125	100.0%	34.82	30.96	39.15	43.85	35.24	2.82	16.88	22.70	33.68	53.49	84.68	99.35	229.66
Phthalate metabolite of DEP																		
MEP	U	mother	µg/L	125	100.0%	36.30	29.35	44.90	76.94	130.72	3.10	7.90	15.00	34.00	80.00	168.00	240.00	892.00
			µg/g creat.	125	100.0%	32.39	26.38	39.76	67.26	114.75	3.75	6.56	13.67	32.69	70.51	156.45	221.62	759.80
	U	child	µg/L	125	100.0%	26.18	21.56	31.80	71.48	266.21	3.70	7.80	13.00	23.00	41.00	103.00	169.00	2840.00
			µg/g creat.	125	100.0%	23.39	19.41	28.19	71.84	361.79	3.79	6.87	11.95	20.88	39.69	93.48	114.18	4016.97
Triclosan																		
TCS	U	mother	µg/L	125	100.0%	2.72	1.87	3.96	53.67	186.56	.12	.35	.63	1.56	5.25	122.39	347.00	1254.15
			µg/g creat.	125	100.0%	2.42	1.67	3.53	42.05	141.61	.14	.30	.54	1.23	6.02	103.37	222.44	1104.01
	U	child	µg/L	125	97.6%	1.23	.89	1.70	27.04	113.92	.05	.24	.46	.83	1.89	7.88	121.90	806.91
			µg/g creat.	125	97.6%	1.10	.80	1.52	27.03	133.89	.05	.22	.37	.85	1.51	7.71	85.13	1235.39

Biomarker	Matrix	Population	Unit	N	%>LOQ	GM	low CI	up CI	AM	SD	min	P_{10}	P_{25}	P_{50}	P_{75}	P_{90}	P_{95}	max
BPA																		
BPA	U	mother	µg/L	125	100.0%	2.55	2.16	3.02	7.35	40.80	.30	.89	1.41	2.30	3.94	7.47	11.63	455.62
			µg/g creat.	125	100.0%	2.28	1.94	2.67	8.00	53.58	.47	.98	1.35	2.11	3.14	5.72	9.44	598.72
	U	child	µg/L	125	96.8%	2.35	1.94	2.84	6.99	39.67	.10	.76	1.40	2.27	3.76	8.15	13.44	445.24
			µg/g creat.	125	96.8%	2.10	1.73	2.54	5.44	25.77	.04	.90	1.31	2.03	3.42	6.68	11.42	288.37

With: N=number, LOQ: limit of quantification; GM: geometric mean (= antilog of mean of the logarithmic transferred values); CI: confidence interval (low CI and Up CI = lower and upper CI); SD: standard deviation; min: minimum; max: maximum; P_{10}, P_{25},….P_{95}, i.e. 10, 25,….and 95% of the population had a concentration below that value; creat: creatinine

6.5 Belgian vs. European levels of urinary and hair biomarkers

6.5.1 Mercury in hair

Background information

Mercury

What is mercury?

Mercury is a naturally occurring metal which has several forms (metallic, organic and inorganic). The metallic mercury is a shiny, silver-white, odourless liquid. If heated, it is a colourless, odourless gas.

Where is it found?

Mercury is released into the environment from a range of natural sources, such as forest fires, flooding or by natural weathering processes. Man-made sources are the incineration of waste and fossil fuels and some industrial processes. The improper disposal of mercury containing products (such as old thermometers, barometers, and energy saving light bulbs) can lead to the release of mercury in the environment. In water, mercury can be absorbed by (shell)fish, which causes food chain accumulation.

Human biomonitoring of mercury

Blood, urine or hair samples can be used to measure exposure to mercury. Blood is often used to determine organic mercury concentrations, whereas urine is most suitable for the detection of inorganic mercury. Hair analysis is an appropriate method to determine a person's history of mercury exposure through fish consumption.
Biomonitoring studies on levels of mercury allow us to determine whether people have been exposed to higher levels of mercury than are found in the general population. Finding a measurable amount of mercury in blood or urine does not mean that these levels cause an adverse health effect.

COPHES
Consortium to Perform Human Biomonitoring on a European Scale

How are we exposed?

Everyone is exposed to mercury to a small extent from air, water and food. Many people are potentially exposed to elemental mercury from dental amalgam fillings, although the amounts released are very low. Spillages of elemental mercury from broken thermometers or barometers may result in exposure to mercury vapour. Eating fish is a known source of exposure to organic mercury. Nearly all fish contain traces of mercury. In most fish this is not a problem but certain fish contain relatively higher levels of mercury, such as shark, marlin, swordfish and tuna.

How can it affect us?

Exposure to mercury can be associated with damage to the central nervous system, kidneys, stomach and also affects the immune system, blood pressure and may cause behavioural problems.
During pregnancy, mercury compounds cross the placenta and can affect the development of the foetus, and cause neurodevelopmental abnormalities.

How to reduce exposure?

- For nutritional reasons two portions of fish per week - of which one portion of fat fish type – is advisable. This also holds for pregnant and breastfeeding women and for children. However, some predetor fish such as shark, marlin, swordfish and to a lesser extend tuna, should be avoided because they may contain more contaminats such as mercury.
- Remove broken mercury thermometers and energy-saving light bulbs via container parks.
- Information on healthy food and how to remove mercury-holding products can be found via the link on www.nehap.be

Belgian levels

Mercury in hair was analysed in 129 children and 129 mothers. It was measurable in nearly all participants (Table 18). Since the hair samples were cut at approximately 2-3 cm from the scalp, the biomarker provides a measure for the exposure to mercury in the last two to three months (assuming a hair growth rate of one cm per month). The levels of mercury in hair measured in this study (P_{90} = 0. 99 µg/g hair) were in the same range as those measured in a Flemish reference population of 20-40 years old mothers (P_{90} = 0.86 µg/g hair) (Schoeters et al., 2012). Mothers had clearly higher concentrations compared to the children. The difference in concentrations between the highest (P_{90}) and lowest (P_{10}) exposed individuals was a factor 7 to 15 in mothers and children respectively (Table 18, Figure 22). This means that there is potential room for improvement within the Belgian context. There were no mothers with a hair mercury level above the JECFA-derived health based guidance value of 2.3 µg/g hair.

Table 18 Belgian hair mercury levels measured in mothers and children

Biomarker	group	Unit	N	%>LOQ	GM	low CI	up CI	P_{50}	P_{90}	P_{95}	N > health guidance (*)
Mercury	mother	(µg/g)	129	95.30%	0.383	0.333	0.441	0.44	0.987	1.236	0>2.3 µg/g
	child	(µg/g)	127	80.30%	0.204	0.172	0.242	0.243	0.582	0.817	0 >2.3 µg/g

With: N=number, LOQ: limit of quantification; GM: geometric mean (= antilog of mean of the logarithmic transferred values); CI: confidence interval (low CI and Up CI = lower and upper CI); SD: standard deviation; P_{50}, P_{90},P_{95}: 50, 90 and 95% of the population had a concentration below that value

(*) calculated based on a provisional tolerable weekly intake of 1.6 µg/kg body weight/week for *methyl mercury of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)*
(http://www.who.int/foodsafety/publications/chem/mercuryexposure.pdf)

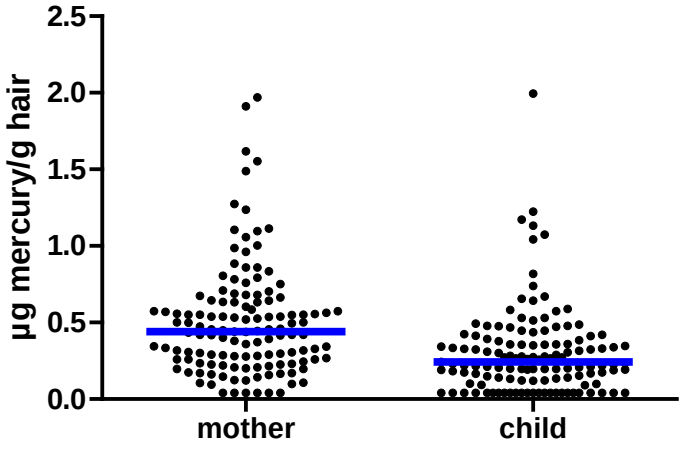


Figure 22 levels of hair mercury (µg/g hair) in the Belgian mothers (N=129) and their children (N=129). The blue line indicates the median of each age group.

Comparison of Belgian with the European levels

Mercury in hair was measured in 1839 mothers and 1836 children within 17 European countries. The weighted geometric mean (95% confidence interval, 95% CI) for mercury in hair in the European study population equaled 0.145 (0.139 – 0.151) µg/g hair in children and 0.225 (0.216 – 0.234) µg/g hair in mothers. The 90th percentile was 0.800 (95%CI: 0.698-0.917) µg/g hair and 1.200 (1.068-1.349) µg/g hair, respectively (Den Hond et al., 2012). Both mothers and children of the Belgian study population had significant higher hair mercury levels compared to the levels in the European population (p=0.001, after correction for age and gender, the latter only in the children). This may be due to the relatively high fish consumption: about 20% of the Belgian mothers and children consumed fish several times per week (see further).

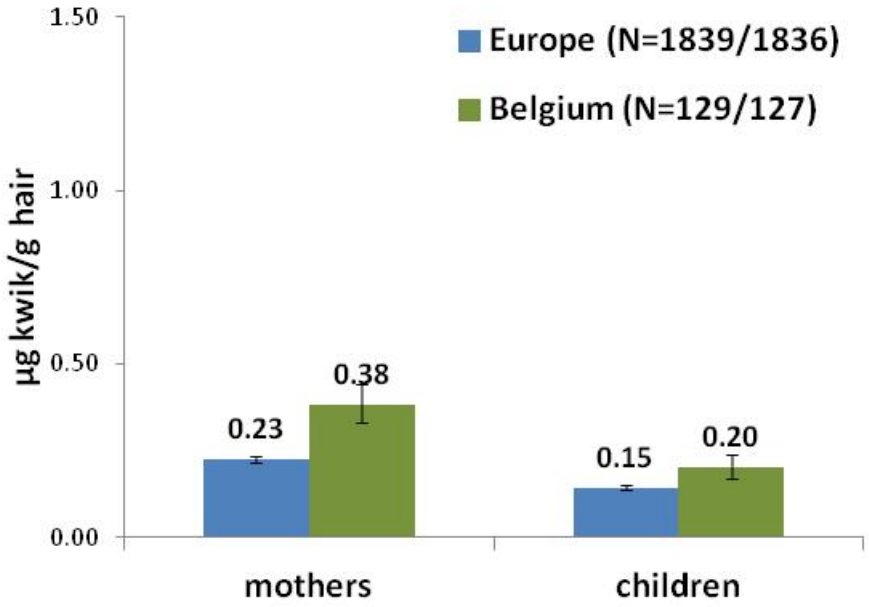


Figure 23 Mercury in hair levels measured in Belgian and European mothers and children (geometric mean±95 confidence interval). The number of mothers/children are indicated in the legend.

Determinants of exposure for the Belgian study population

Mothers and children with regular fish consumption had 1.7 times (p=0.01) to 1.5 times (p=0.007) higher mercury hair levels compared to non-regular fish consumers (Figure 24, Table 19). Regular fish consumption was defined as the summing up of the frequencies of eating fish, shellfish and fish products. Fish consumption is a well documented route of exposure to mercury. It was also observed in adolescents and adults monitored in the Flemish Environment and Health Survey 2007-2009 and in the children of the Hainaut study 2007-2009.

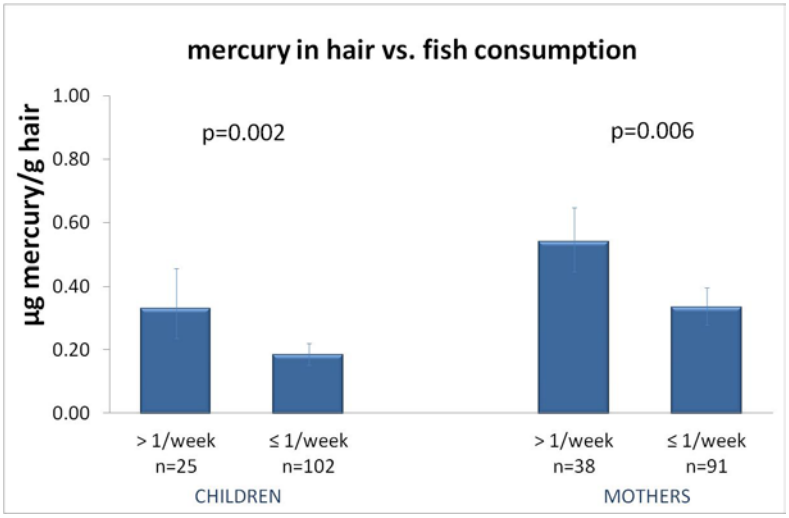


Figure 24 Mercury levels measured in hair of children and mothers consuming fish (all kinds, including seafood) more than once per week versus less than once per week (geometric means ± 95% confidence interval)

Mothers with amalgam fillings in their teeth had 40% higher mercury levels than those not having that type of fillings (p=0.05). As for urinary cadmium, also the mercury levels in children of the urban area, were higher than those of the rural area. There was no relation of mercury levels in hair with smoking, use of hair care products, use of hair stains, breaking of mercury thermometers, energy saving lamps or living in an industrial area.

Spearman correlation coefficients were calculated between the individual mercury levels in hair of the mothers and the children. The data were highly correlated (r = 0.52; p<0.001). Since fish consumption is the major determinant of mercury levels in hair, dietary fish intake was very likely the common environmental factor.

Table 19: Factors of influence on hair mercury levels of the Belgian children and their mothers. Estimate = times increase in biomarker for unit increase of the covariate/confounder

Parameter	Estimate	SE	95% CI		p	p
Mercury child: R^2 of model= 0.13						
boys vs girls	0.75	0.17	0.54	1.04	0.0819	
5-8 years vs 9-11 years	1.02	0.17	0.73	1.42	0.9246	
Fish: several times/week vs once/week or less	1.73	0.21	1.14	2.65	0.0112	
Area: urban vs rural	1.50	0.17	1.08	2.09	0.0155	
Mercury mother: R^2 of model= 0.11						
<=35 years vs 35-40 years	1.07	0.20	0.73	1.58	0.7203	0.5350
<=35 years vs >40 years	0.90	0.21	0.60	1.36	0.6146	
35-40 years vs >40 years	0.84	0.16	0.62	1.14	0.2644	
Fish: several times/week vs once/week or less	1.53	0.15	1.13	2.07	0.0069	0.0069
Amalgam teeth: yes vs no	1.42	0.18	1.00	2.02	0.0506	0.0506

With: SE = standard error; CI = confidence interval

6.5.2 Cadmium in urine

Background information

Cadmium

What is cadmium?

Cadmium is a soft, silver to white metal that is naturally present in the Earth's crust as a mineral in combination with other

Where is it found?

Cadmium can be found naturally in small quantities in the air, water and soil. Higher levels can be found in soil and water near industrial areas or hazardous waste sites. It is also found in tobacco.
Most frequently, cadmium is extracted as a by-product during the production of zinc, lead or copper. Cadmium is used in batteries, paint pigments, coatings and platings.

Cd

Human Biomonitoring of Cadmium

Cadmium can be measured in both urine and blood. Long-term exposure to cadmium can be measured in urine samples. Cadmium in blood reflects more recent exposure, about three months.
Finding a measurable amount of cadmium in blood or urine does not necessarily mean that these levels cause an adverse health effect.

How are we exposed?

Environmental exposure to cadmium occurs primarily though smoking. Non-smokers who inhale environmental tobacco smoke (also known as second-hand smoke) take up cadmium as well.
The most important source of cadmium for non-smokers is from food (especially shellfish, liver, kidney, wild mushrooms and leafy green vegetables.

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How can it affect us?

Long-term exposure to low levels of cadmium through air, water and soil can affect the kidneys and bone density. Breathing in high levels can also damage the lungs but this is more likely to occur in occupational settings rather than in the environment. High levels of cadmium can cause cancer in humans.

How to reduce exposure?

.Do not smoke tobacco products and limit exposure to second-hand smoke.
·Properly dispose of batteries and other cadmium containing products.
•Avoid eating liver, kidney, and shellfish.
·Diversify food from different origin and kind.
•Information on healthy food and how to remove mercury-holding products can be found via the link on www.nehap.be

AD1

Belgian levels

Cadmium in urine reflects long-term exposure. In the Belgian study group the mothers had on average five times higher urine levels. Nearly all urine samples contained measurable cadmium levels (Table 20). The urinary cadmium levels of the mothers were comparable to earlier measured Flemish reference values for 20-40 years old women (2003-2007) (Schoeters et al., 2012). The P_{90} of 0.51 µg/L was the same in both studies. The difference in concentrations between the highest (P_{90}) and lowest (P_{10}) exposed individuals was a factor 6 to 14 in mothers and children respectively. None of the participants of the current study had values above the HBM II (1 and 4 µg/L for children and mothers respectively) [4]. Two mothers had cadmium concentrations higher than HBM I value (0.5 and 2 µg/L for children and mothers). Both had a cadmium urine level of 1.1 µg/L (just above 1 µg/L). They were living near to the city centre of Brussels and had an age of 39 and 40 years old. These mothers were non-smokers and had no remarkable food consumption pattern. they were for respectively 2.5 and 1.5 hours per day underway in the car. One mother one had detectable levels of cotinine (2.9 µg/L) and also reported to occasionally being exposed to environmental tobacco smoke. The latter had a high school diploma, but was unemployed. The second mother worked in a hospital. None of both mothers had hobbies in which metals were involved. Both their children had urine levels which were also around the P_{90} and P_{75} of their age group (namely 0.18 and 0.10 µg/L).

Spearman correlation coefficients were calculated between the individual urinary cadmium levels of the mothers and the children. The data were positively and significantly correlated, both when expressed in µg/L ($r = 0.33$; $p<0.001$) or when expressed in µg/g creatinine ($r = 0.45$ $p<0.001$).

Table 20: Belgian urinary cadmium levels measured in mothers and children

Biomarker	group	Unit	N	%>LOQ	GM	low CI	up CI	P_{50}	P_{90}	P_{95}	N > health guidance
Cadmium	mother	µg/L	125	99.20%	0.21	0.18	0.24	0.22	0.51	0.65	2>HBM-I
		µg/g crt	125	99.20%	0.183	0.162	0.206	0.195	0.374	0.462	
	child	µg/L	125	86.40%	0.04	0.04	0.05	0.05	0.14	0.18	0>HBM-I
		µg/g crt	125	86.40%	0.039	0.033	0.047	0.05	0.107	0.141	

With: N=number, LOQ: limit of quantification; GM: geometric mean (= antilog of mean of the logarithmic transferred values); CI: confidence interval (low CI and Up CI = lower and upper CI); SD: standard deviation; P_{50}, P_{90},P_{95}: 50, 90 and 95% of the population had a concentration below that value; creat: creatinine

[4] Human biomonitoring (HBM) values. HBM-I represents the concentration of a substance in human biological material below which there is no risk for adverse health effects and, consequently, no need for action. At a concentration level higher than the HBM-I and lower than the HBM-II-value the result should be verified by further measurements. HBM-II represents the concentration of a substance in a human biological material above which – according to the knowledge and judgement of the Commission and with regard to the substance under consideration – there is an increased risk for adverse health effects and, consequently, an acute need for exposure reduction measures and the provision of biomedical care (advice). The HBM-II-value should thus be regarded as an intervention or action level.

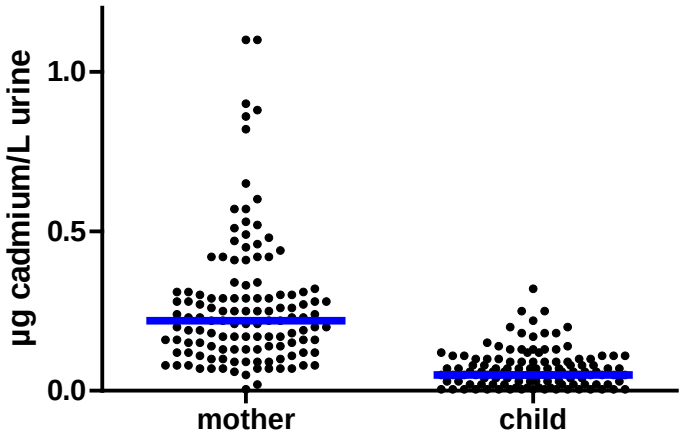

Figure 25 Urinary levels of cadmium (µg/L) in the Belgian mothers (N=125) and their children (N=125). The blue line indicates the median of each age group.

Comparison of Belgian with the European levels

In the total study group of 17 European countries, the children (N=1689) had a weighted geometric mean (95% CI) for urinary cadmium of 0.071 (0.069 – 0.074) µg/L and 0.070 (0.067 – 0.072) µg/g creatinine. The 90th percentile was 0.220 (95%CI: 0.209-0.232) µg/L and 0.189 (0.179-0.199) µg/g creatinine (Den Hond et al., 2012). In the European mothers (N=1685), the average exposure values were considerably higher compared to children, i.e. the weighted geometric mean (95% CI) equaled 0.219 (0.211 – 0.228) µg/L and 0.196 (0.189 – 0.202) µg/g creatinine and the 90th percentile was 0.620 (0.580-0.663) µg/L and 0.463 (0.436-0.492) µg/g creatinine. The Belgian urinary cadmium levels were in children significantly lower than the European average (p=0.001, corrected for creatinine, age and gender), whereas the Belgian mothers had no significantly different concentrations compared to the European average (p=0.66, corrected for creatinine and age).

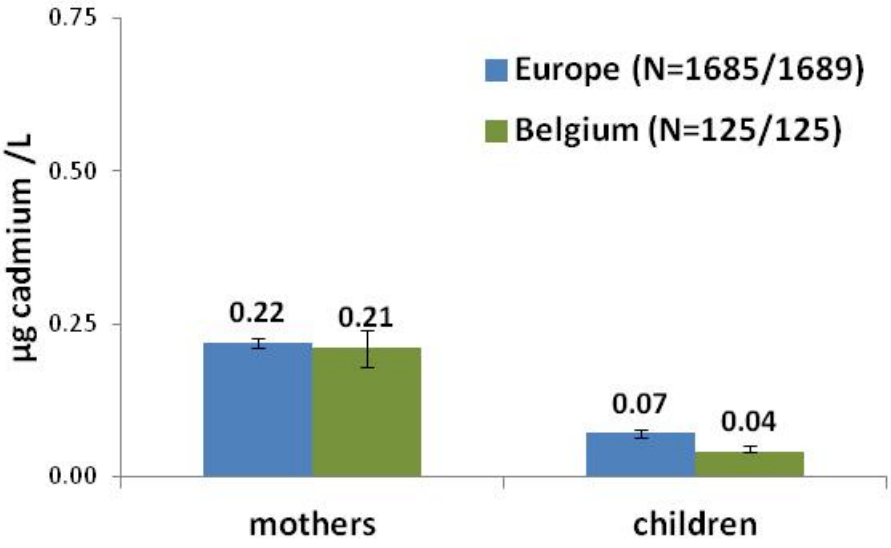

Figure 26: Urinary cadmium levels measured in Belgian and European mothers and children (geometric mean±95 confidence interval). The number of mothers/children are indicated in the legend

Determinants of exposure for the Belgian study population

The urinary cadmium levels in the Belgian mothers and children were not influence by the environmental exposure parameters, such as (passive) smoking, traffic exposure, living in an industrial area and consumption of possible cadmium containing foods (rice, offal, game, wild mushrooms, chocolate or local food). The cadmium levels in both children and mothers were however higher in the urban area compared to the rural. In the Flemish Environment and Health Survey of 2007-2009, cord blood cadmium levels also appeared to be highest in the urban regions (Figure 27).

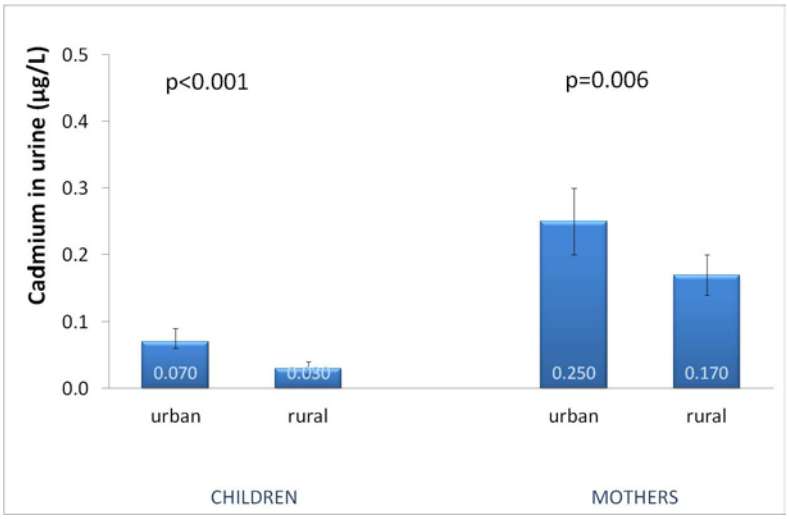

Figure 27: Geometric mean (±95% confidence interval) of urinary cadmium levels measured in both the Belgian urban and rural areas

It was also observed that urinary cadmium was about two times higher (1/0.47=2.1 or 1/0.43=2.3) in children with one or both parents having bachelor and/or master degree, compared to those with a lower school education (p=0.005). Cadmium is a heavy metal which accumulates in the body with age. In the mothers, which were between 27 and 45, there was no increase of urinary cadmium with age. This might be explained by the not so broad age-range. However, the children of the age group 9-11y had (1/0.66=) 1.5 times higher cadmium values than the children of the younger age group of 5-8y (p<0.01). Furthermore, boys had increased levels compared to girls.

Table 21: Factors of influence on urinary cadmium levels of the Belgian children and their mothers (µg/L). The estimate was expressed as fold change in biomarker for unit increase of the covariate/confounder. *Only significant covariates and fixed confounders are shown*

Parameter	Estimate	SE	95% CI		p	p
Children: R^2 of model = 0.36						
Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.77	0.18	0.54	1.09	0.1370	0.0116
Crt: 300-1000 mg/L vs 2000-3000 mg/L	0.30	0.40	0.13	0.67	0.0035	
Crt: 1000-2000 mg/L vs 2000-3000 mg/L	0.39	0.39	0.18	0.85	0.0173	
5-8 years vs 9-11 years	0.66	0.16	0.48	0.91	0.0125	0.0125

boys vs girls	1.48	0.17	1.06	2.06	0.0222	0.0222
Area: urban vs rural	2.06	0.17	1.47	2.88	<.0001	<0.0001
Education:						0.0049
Prim/second vs 1st stage tertiary	0.47	0.23	0.30	0.75	0.0016	
Prim/second vs 2nd stage tertiary	0.43	0.33	0.22	0.81	0.0103	
1st stage vs 2nd stage tertiary	0.91	0.26	0.54	1.52	0.7109	
Cadmium mother: R^2 of model = 0.36						
Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.52	0.13	0.40	0.67	<.0001	<0.0001
Crt: 300-1000 mg/L vs 2000-3000 mg/L	0.36	0.24	0.22	0.58	<.0001	
Crt: 1000-2000 mg/L vs 2000-3000 mg/L	0.69	0.24	0.43	1.10	0.1197	
<=35 years vs 35-40 years	0.98	0.18	0.69	1.40	0.9266	0.9597
<=35 years vs >40 years	0.95	0.18	0.67	1.36	0.7955	
35-40 years vs >40 years	0.97	0.14	0.74	1.28	0.8268	
daily/occasional vs former smoker	1.16	0.24	0.72	1.87	0.5311	0.4461
daily/occasional vs never smoker	1.30	0.22	0.83	2.01	0.2478	
former vs never smoker	1.11	0.14	0.84	1.48	0.4557	
Area: urban vs rural	1.39	0.12	1.08	1.77	0.0097	0.0097

With: Crt= creatinine; prim=primary school, second = secondary school, 1^{st} stage tertiary = bachelor+master degree, 2^{nd} stage tertiary = PhD degree; SE = standard error; CI = confidence interval.

6.5.3 Cotinine

Background information

Cotinine

What is cotinine?

Cotinine is a chemical that is formed in the human body after nicotine is inhaled. It is an excellent biomarker to evaluate the exposure to tobacco smoke.

Where is it found?

Cotinine is not normally found in the body, it is produced from nicotine. Nicotine comes from tobacco products such as cigarettes, and cigars.
Non-smokers who inhale environmental tobacco smoke (also known as second-hand smoke) take up nicotine as well.

Human Biomonitoring of cotinine

Exposure to environmental tobacco smoke (ETS) can be estimated by measuring levels of cotinine in blood or urine. Measuring cotinine is preferred to measuring nicotine because cotinine remains longer in the body and it can therefore be detected several days after exposure to cigarette smoke.
Finding a measurable amount of cotinine in blood or urine means you have been exposed to cigarette smoke.

Health effects from smoking

Cigarette smoke contains many toxic chemicals including nicotine. Smoking may cause lung cancer, heart disease and respiratory diseases such as bronchitis and emphysema. Non-smoking adults and children exposed to second-hand smoke face the same dangers as smokers themselves.
Children are sensitive to ETS. Children exposed to ETS have an increased risk of sudden infant death, chest infections and asthma.
Exposure to ETS during pregnancy can result in low birth weights in newborns and pre-term deliveries.

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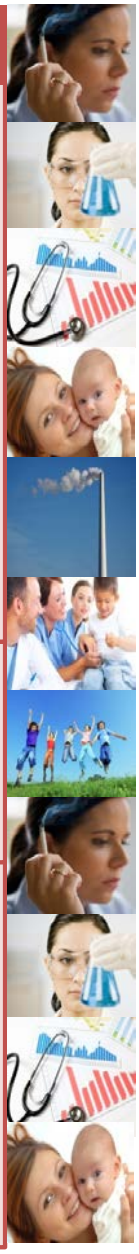
How to reduce exposure?

•Stopping with smoking to improve your health and to protect your family against smoke exposure. For help or information, contact Tabak stop at 0800 111 00 or consult our website www.nehap.be, where you find the links to relevant websites

- Limit, as much as possible, contact with second-hand tobacco smoke.
- Avoid places where smoking is allowed.



Belgian levels

In the Belgian study group, 9.3% of the mothers and none of the children were smokers. Nevertheless, in total 30.4 % of the mothers and 34.4 % the children had detectable cotinine levels in their urine. One girl of 8 years old had a cotinine level of 58.4 µg/g creatinine, which is above the theoretical level observed in smokers (50 µg/g creatinine). Her mother reported that the girl was in their home environmentally exposed 4-6 times per week to two smoking inhabitants (in total smoking 15 cigarettes per day). The mother was an occasional smoker.

Fourteen mothers had a cotinine level above 50 µg/g creatinine. Most of the children of those mothers (N=12), also had detectable levels of cotinine in their urine. Overall, the cotinine levels of the mothers and the children were highly correlated (spearman rank r= 0.60, p<0.001). Twelve of the 14 women with cotinine levels above 50 µg/g creatinine, were smokers. However, two of them were non-smokers who reported having been exposed to cigarette smoke in the last 24 hours before urine sampling. In general the 50 µg/g creatinine treshold gave a fairly good separation between smoking and non-smoking mothers (Figure 28).

Table 22 Results of cotinine in urine of Belgian mothers and their children

Biomarker	group	Unit	N	%>LOQ	GM	low CI	up CI	P_{50}	P_{90}	P_{95}	N > health guidance
Cotinine	mother	µg/L	125	30.40%	-	-	-	0.4	74.1	905.1	n.a.
		µg/g creat.	125	30.40%	-	-	-	0.4	109	776.5	
	child	µg/L	125	34.40%	-	-	-	0.4	2.9	6	n.a.
		µg/g creat.	125	34.40%	-	-	-	0.4	3.3	5.6	

With: N=number, LOQ: limit of quantification; GM: geometric mean (= antilog of mean of the logarithmic transferred values); CI: confidence interval (low CI and Up CI = lower and upper CI); SD: standard deviation; P_{50}, P_{90},P_{95}: 50, 90 and 95% of the population had a concentration below that value; creat: creatinine

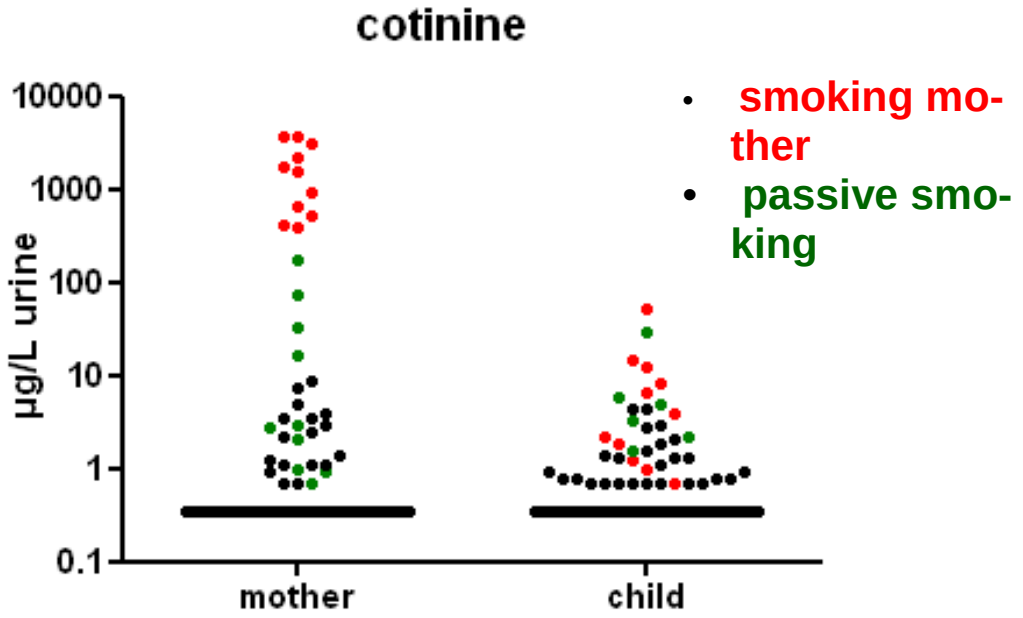


Figure 28 Urinary levels of cotinine (µg/L) in the Belgian mothers (N=125) and their children (N=125). The horizontal line indicates the median of each age group. The smoking mothers or children having a smoking mother are indicated as red dots. Non-smoking Individuals who reported to be exposed to passive smoking in the period of 24 hours before urine collection are indicated as green dots.

Comparison of Belgian with the European levels

9.3% of the Belgian mothers were smokers. In the European study population this was 21.0%. On average the levels of cotinine in the Belgian mothers and children was also lower compared to the European average (adjusted for creatinine, age and gender in case of the children)(p= 0.01 for children and p=0.002 for mothers) (Figure 29). In all countries with cotinine levels in the children below the European mean (as is the case for Belgium), less than 10% of the children (9.6% for Belgium) reported to be exposed to environmental tobacco smoke in the last 24 hours (Den Hond et al., 2012).

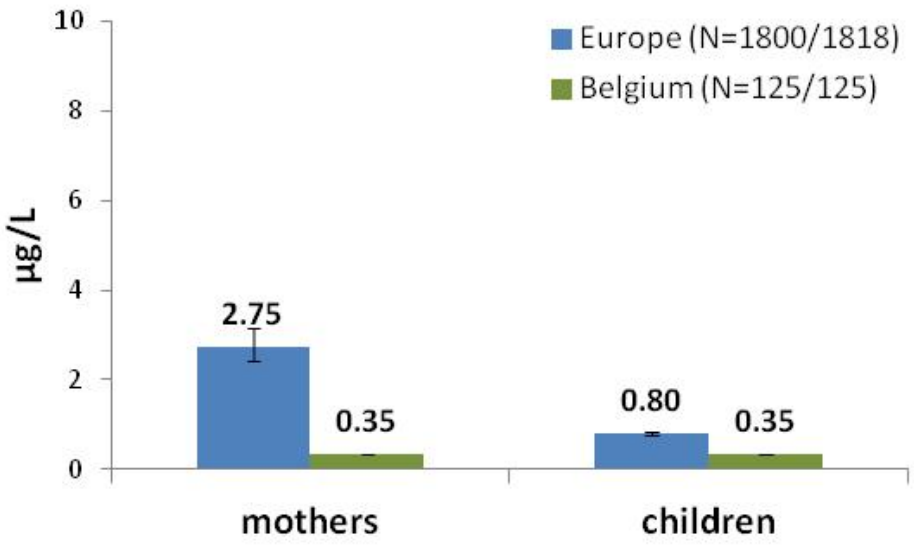


Figure 29 Urinary cotinine levels measured in Belgian and European mothers and children (geometric mean±95 confidence interval). The number of mothers/children are indicated in the legend

Determinants of exposure for the Belgian study population

For urinary cotinine, less than 50% of the values were above the LOQ both in children (30.4%) and mothers (34.4%). This biomarker was therefore analyzed in the multiple regression models as a dichotomous variable (1=above or 0=below LOQ).
Cotinine is a chemical that is formed in the human body after nicotine is inhaled. In the current study it was confirmed to be a good biomarker to evaluate the active and passive exposure to tobacco smoke. Urinary cotinine levels were higher in smoking mothers and in children exposed to environmental tobacco smoke. Children exposed to tobacco smoke in the last 24 hours had a 43 times higher chance of having measurable cotinine levels in their urine (p=0.0008) compared to children not exposed to any tobacco smoke.

There were 12 smoking mothers in our study group (12/129 =9%). All smoking women had detectable nicotine levels in their urine (median= 1238.80 µg/L) (Table 23). Almost all children of the smoking mothers (N=12) and those who reported to be exposed to any (other) environmental tobacco smoke 24 hours before urine collection (N=12), had detectable urinary cotinine levels (NB: not statistically tested because of the low numbers). The urinary concentrations of the children and mothers were good correlated, indicating that smoking behavior of the mother had its influence on exposing the child passively to smoke or possibly was associated with a different attitude in protecting the child from smoky environments (r= 0.60, p<0.001).

Table 23: Levels of cotinine (µg/L) in urine of the child and the mother stratified for the mother's smoking behaviour and for the participants reported exposure to environmental tobacco smoke (ETS) within 24 hours before urine collection. The last column indicates the number of individuals within that group, with a cotinine level below the limit of quantification (LOQ)

	number	Minimum	P_{25}	Median	P_{75}	Maximum	% below LOQ
CHILDREN with:							
all	125	-	-	0.4	0.8	58.4	65.1
non-smoking mother	117	-	-	-	0.70	30.00	70.9
reported ETS in last 24h and non-smoking mother	7	-	1.60	3.30	6.00	30.00	N=1
Reported ETS in last 24h	12	-	2.70	5.40	13.55	52.00	N=1
smoking mother	12	-	1.10	3.00	10.40	52.00	N=1
MOTHERS							
all	125	-	-	0.40	1.1	3708.8	69.8
smoker	12	219.50	471.10	1238.80	2636.25	3708.80	0.0
non-smoker	117	-	-	-	-	179.30	76.9
non-smoker and reported ETS in last 24h	14	-	-	1.60	16.30	179.30	N=4

LOQ: limit of quantification, -: below limit of quantification, N: number

Table 24 Factors of influence on cotinine levels of the Belgian children and their mothers (µg/L). Cotinine was recoded to a binary value i.e. no/yes measurable urinary cotinine. The Odds Ratio was given for a unit change in the facture of influence

Paramater	OR	95% CI		p	p
Cotinine child (no/yes): R^2 of model= 0.21					
	1.363	0.524	3.543	0.525	0.3525
	0.381	0.062	2.344	0.298	
7. Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.280	0.047	1.650	0.160	
8. Crt: 300-1000 mg/L vs 2000-3000 mg/L					
Crt: 1000-2000 mg/L vs 2000-3000 mg/L					
5-8 years vs 9-11 years	2.224	0.871	5.678	0.095	0.0948
boys vs girls	1.828	0.723	4.619	0.202	0.2023
ETS last 24h yes vs no	43.031	4.779	387.426	0.0008	0.0008
period urine: <9 vs 9-11 hours	4.554	1.094	18.953	0.037	0.0476
period urine: <9 vs >=11 hours	5.813	1.409	23.980	0.015	
period urine: 9-11 vs >=11 hours	1.276	0.462	3.525	0.638	
Cotinine mother (no/yes): R^2 of model=0.31					
	0.54	0.19	1.52	0.243	0.1839
	0.23	0.04	1.17	0.0759	
9. Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.42	0.09	1.97	0.2712	
10. Crt: 300-1000 mg/L vs 2000-3000					

mg/L					
Crt: 1000-2000 mg/L vs 2000-3000 mg/L					
<=35 years vs 35-40 years	0.86	0.23	3.28	0.8283	0.5940
<=35 years vs >40 years	1.52	0.35	6.55	0.5734	
35-40 years vs >40 years	1.76	0.59	5.26	0.309	
Smokers vs ETS exposed last 24h	8.50	0.38	191.45	0.178	<.0001
Smokers vs not ETS exposed last 24h	102.16	5.39	1935.57	0.0021	
ETS exposed last 24h vs not exposed last 24h	12.02	3.25	44.44	0.0002	

NB: In the statistical model of the mothers. all smoking variables were highly correlated: smoke status. smoke tobacco at home and smoking exposure in the last 24 hours (= all smoking exposure= own smoking + ETS);
ETS= environmental tobacco smoke (=passive smoking); crt= creatinine; SE = standard error; CI = confidence interval

10.1.1 Phthalates

Background information

Phthalates

What are phthalates?

Phthalates are a family of industrial chemicals that are used as plastic softeners or solvents in many different consumer products. Many are colourless, scentless, flavourless and have low volatility.

Where are they found?

Phthalates are widely used in the manufacture of plastics, to make them soft and flexible. They are also used in laboratory and medical devices. Some are also used as solvents. Phthalates are always incorporated with other materials (such as PVC) into an end product.

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Human Biomonitoring of phthalates

The concentration of phthalates is generally measured in human blood and urine. As most of the phthalates are rapidly broken down into simple compounds, they are quickly passed out in urine and can be easily measured with biomonitoring.
Finding a measurable amount of phthalates in blood or urine does not necessarily mean that these levels cause an adverse health effect.

How are we exposed?

Due to their versatile uses, phthalates are found everywhere. People can be exposed by eating and drinking foods that have been in contact with phthalates. They are also found in everyday items such a raincoats, shoes, and personal care products (cosmetics, shampoos, body lotions and perfumes).
Another source is contaminated indoor air (e.g. from phthalate-containing plastic products at home or work places such as vinyl flooring).
Children are more often exposed to them by sucking on plastic toys and through particles in house dust because of hand-to-mouth behaviour.

How can it affect us?

Human health effects from low-level exposure to phthalates remain unknown. However, continuous and repeated exposure to higher levels of certain phthalates may affect the hormonal system.
It is generally acknowledged that more research is needed to assess the exact health effects of exposure to phthalates.

How to reduce exposure?

- Check the labels and use phthalate-free products when possible.
- Regularly clean the house with water or with a HEPA filter vacuum cleaner to remove dust.
- Diversify food from different origin and kind.
- Information on healthy food and how to remove mercury-holding products can be found via the link on www.nehap.be

Belgian levels

Phthalates are widely used to make plastics soft and flexible. They are e.g. used in laboratory and medical devices, and some of them are applied as solvents. Phthalates are always incorporated with other materials (such as PVC) into an end product.

Phthalates in urine reflect short-term exposure of hours to days. In the Belgian study group, six different phthalate metabolites were analyzed. They were measurable in nearly all urine samples of both age groups (Table 26). For the sum of DEHP metabolites, MnBP, MBzP and MiBP, the levels in children were approximately 1.5 times those of the mothers. This was also observed by others (Kasper-Sonnberg et al. 2011; Colacino et al., 2010; Kasper-Sonnberg et al., 2011). A possible explanation for this phenomenon is a higher exposure in children, e.g. due to more intense hand-mouth contact or because of typical exposure patterns in children having more contact with toys. Furthermore, metabolism and excretion rate of these chemicals is different in children compared to adults, so that similar exposure patterns may lead to higher metabolite levels in the child's body. Only for MEP, higher mean values were detected in urine of mothers compared to children. This may be related to different exposure to e.g. personal care products. On average, MiBP and MnBP were present in the highest concentrations, followed by MEP and the DEHP metabolites (Figure 30). The concentrations in the mothers and children were rather good correlated indicating that exposure routes such as food and the home life environment are considerable (Table 25). The phthalate metabolites, MEHP, 5-oxoMEHP, 5-OH MEHP, MBzP and MnBP were also measured within the Flemish reference campaign in 20-40 years old women (2003-2007) (Schoeters et al., 2012). The levels observed in the current DEMOCOPHES study were comparable or a bit lower than those concentrations. The difference in concentrations between the highest (P_{90}) and lowest (P_{10}) exposed individuals was considerable, with a factor 10 for most phthalate metabolites, and even a factor 13-20 for MEP.

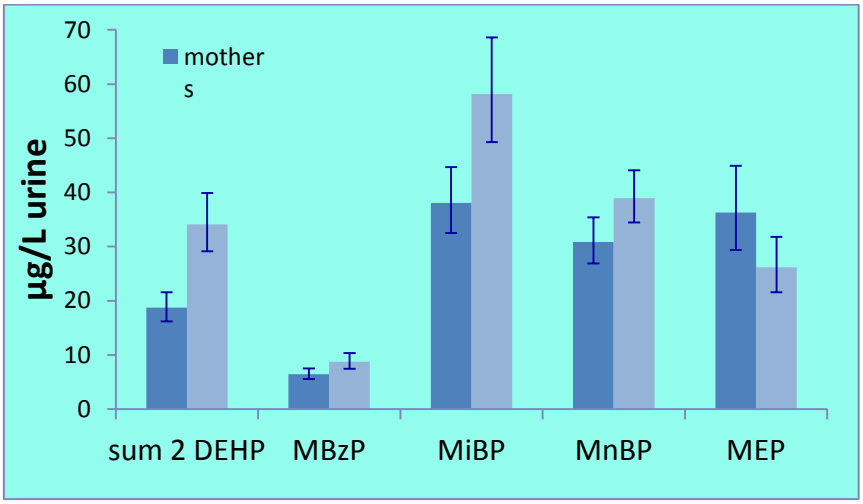


Figure 30: Geometric mean (±95 confidence interval) of urinary phthalate metabolites sum of 2 DEHP (5-oxoMEHP + 5-OHMEHP), MBzP, MiBP, MnBP and MEP in Belgian mothers and their children (µg/L)

For the sum of 5-oxo and 5-OH MEHP there is a health guidance value HBM-I of 300 µg/L for women at child-bearing age and 500 µg/L for children. One girl had a urine level of 1337 µg/L, far above the HBM I of 500 µg/L. She was 10 years old, living in the city centre of Brussels and had no special dietary habits, aside from the fact that the mother had reported that she ate game and wild mushrooms once per week. Furthermore, like many other children she consumed rice, fish and had canteen food several times per week. She did not use much personal care products, had no PVC flooring or wall paper indoors. The child was transported 30 minutes per day in rather new car of one year old. Based on this information it was not easy to explain the level, also since DEHP is mainly taken up via food

and we did not have the (proxy for the levels) in the food items consumed. Also the mother of that child had rather elevated levels i.e. 94 µg/L, which was around the P_{95} for the study group.

Table 25: Spearman rank correlations between phthalate levels of mothers and their children

BIOMARKER	N	Unit	Spearman correlation coefficient
Urinary MEHP (µg/L)	121	(µg/L)	0.25 **
		(µg/g creatinine)	0.40 ***
Urinary 5OH-MEHP	121	(µg/L)	0.15 (p=0.10)
		(µg/g creatinine)	0.35 ***
Urinary 5oxo-MEHP	121	(µg/L)	0.13 (p=0.15)
		(µg/g creatinine)	0.32 ***
Sum urinary 5OH-MEHP + 5oxo-MEHP	121	(µg/L)	0.15 (p=0.10)
		(µg/g creatinine)	0.35 ***
Urinary MBzP	121	(µg/L)	0.42 ***
		(µg/g creatinine)	0.63 ***
Urinary MiBP	121	(µg/L)	0.51 ***
		(µg/g creatinine)	0.66 ***
Urinary MnBP	121	(µg/L)	0.28 **
		(µg/g creatinine)	0.54 ***
Urinary MEP	121	(µg/L)	0.41 ***
		(µg/g creatinine)	0.50***

*p ≤ 0.05 / **p ≤ 0.01 / ***p ≤ 0.001

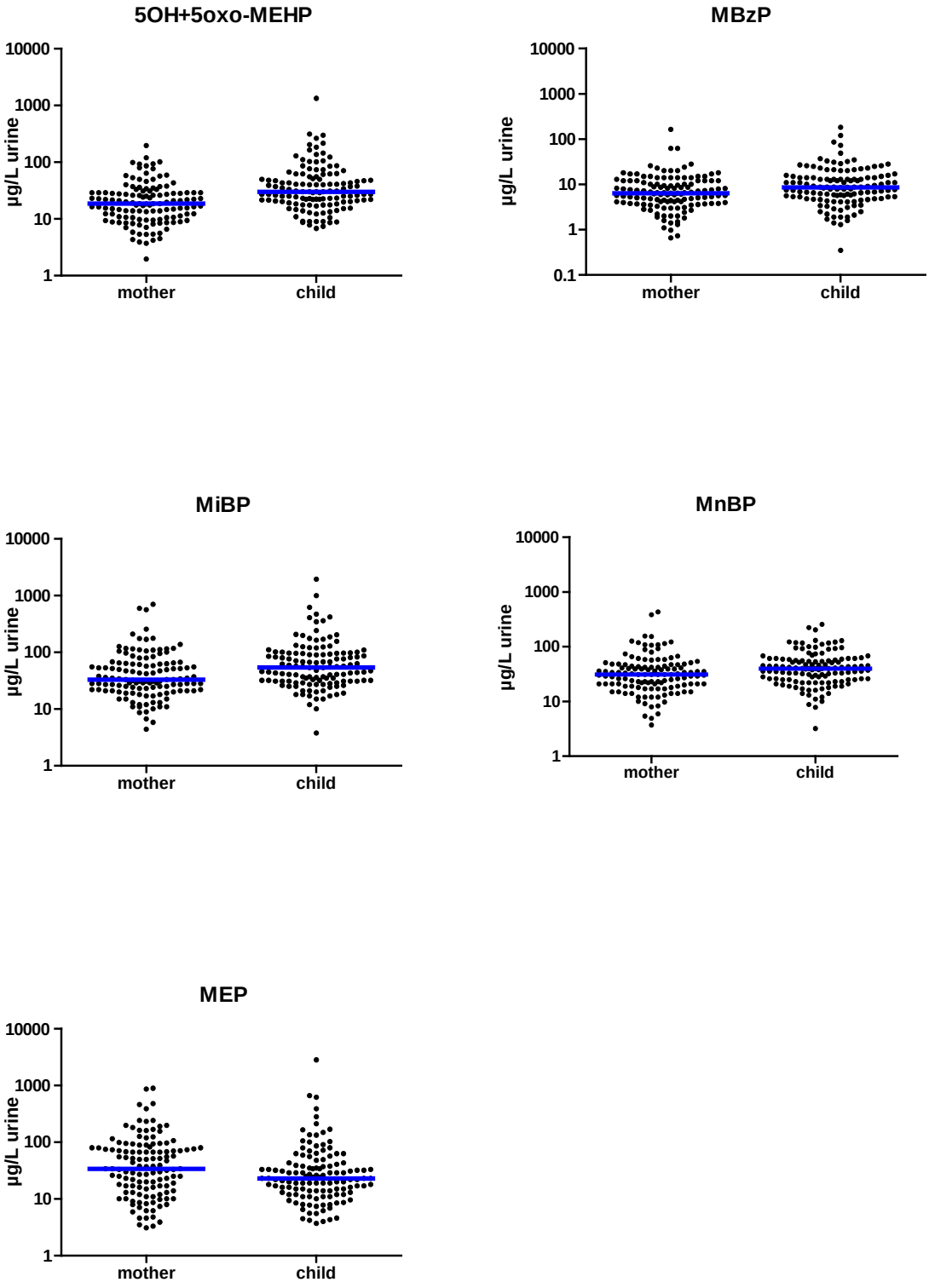


Figure 31 Individual urinary levels of phthalate metabolites (µg/L) in the Belgian mothers (N=125) and their children (N=125). The blue line indicates the median of each age group.

Table 26 Results of phthalate metabolites in urine of Belgian mothers and their children

Biomarker	group	Unit	N	%>LOQ	GM	low CI	up CI	P_{50}	P_{90}	P_{95}	N > health guidance
Phthalate metabolites of DEHP											
MEHP	mother	µg/L	125	92.80%	2.16	1.81	2.59	2.3	7.2	9.1	n.a.
		µg/g creat.	125	92.80%	1.93	1.65	2.26	1.94	4.91	6.56	
	child	µg/L	125	95.20%	2.32	1.97	2.74	2.2	8.7	13	n.a.
		µg/g creat.	125	95.20%	2.08	1.77	2.44	1.94	7.08	10.13	
5OH-MEHP + 5oxo-MEHP	mother	µg/L	125	100.00%	18.72	16.22	21.6	18.5	57	86	0
		µg/g creat.	125	100.00%	16.7	14.89	18.73	15.9	40.11	53.18	
	child	µg/L	125	100.00%	34.09	29.14	39.89	30	111	184	1> HBM-I
		µg/g creat.	125	100.00%	30.46	26.12	35.51	27.3	96.47	157.41	
Phthalate metabolite of BBP											
MBzP	mother	µg/L	125	100.00%	6.47	5.55	7.54	6.4	17	23	n.a.
		µg/g creat.	125	100.00%	5.77	5.05	6.59	5.52	15.53	19.82	
	child	µg/L	125	100.00%	8.78	7.44	10.36	8.6	27	35	n.a.
		µg/g creat.	125	100.00%	7.84	6.68	9.21	8.01	23.34	31.94	
Phthalate metabolite of DiBP											
MiBP	mother	µg/L	125	100.00%	38.08	32.48	44.65	33	115	175	n.a.
		µg/g creat.	125	100.00%	33.97	29.69	38.87	29.5	101.14	142.68	
	child	µg/L	125	100.00%	58.16	49.29	68.63	54	187	362	n.a.
		µg/g creat.	125	100.00%	51.96	44.35	60.88	46.42	135.94	278.89	
Phthalate metabolite of DBP											
MnBP	mother	µg/L	125	100.00%	30.86	26.88	35.43	31	89	119	n.a.
		µg/g creat.	125	100.00%	27.53	24.57	30.85	25.34	57.37	79.31	
	child	µg/L	125	100.00%	38.97	34.46	44.08	40	98	122	n.a.
		µg/g creat.	125	100.00%	34.82	30.96	39.15	33.68	84.68	99.35	
Phthalate metabolite of DEP											
MEP	mother	µg/L	125	100.00%	36.3	29.35	44.9	34	168	240	n.a.
		µg/g creat.	125	100.00%	32.39	26.38	39.76	32.69	156.45	221.62	
	child	µg/L	125	100.00%	26.18	21.56	31.8	23	103	169	n.a.
		µg/g creat.	125	100.00%	23.39	19.41	28.19	20.88	93.48	114.18	

With: N=number, LOQ: limit of quantification; GM: geometric mean (= antilog of mean of the logarithmic transferred values); CI: confidence interval (low CI and Up CI = lower and upper CI); SD: standard deviation; P_{50}, P_{90},P_{95}: 50, 90 and 95% of the population had a concentration below that value; creat: creatinine

Comparison of Belgian with the European levels

The phthalate metabolites MEHP, 5OH-MEHP, 5oxo-MEHP, MBzP and MEP were measured in all 17 European countries; MnBP and MiBP were measured in 13 countries only. In general, the variability between countries was relatively low (Den Hond et al., 2012). In comparison to the European data, Belgian participants had lower values of DEHP metabolites and MEP. Furthermore MBzP, MiBP and MnBP (mothers only) were higher in Belgium compared to the weighted European average, after adjustment for creatinine, age and gender (the latter only for the children).

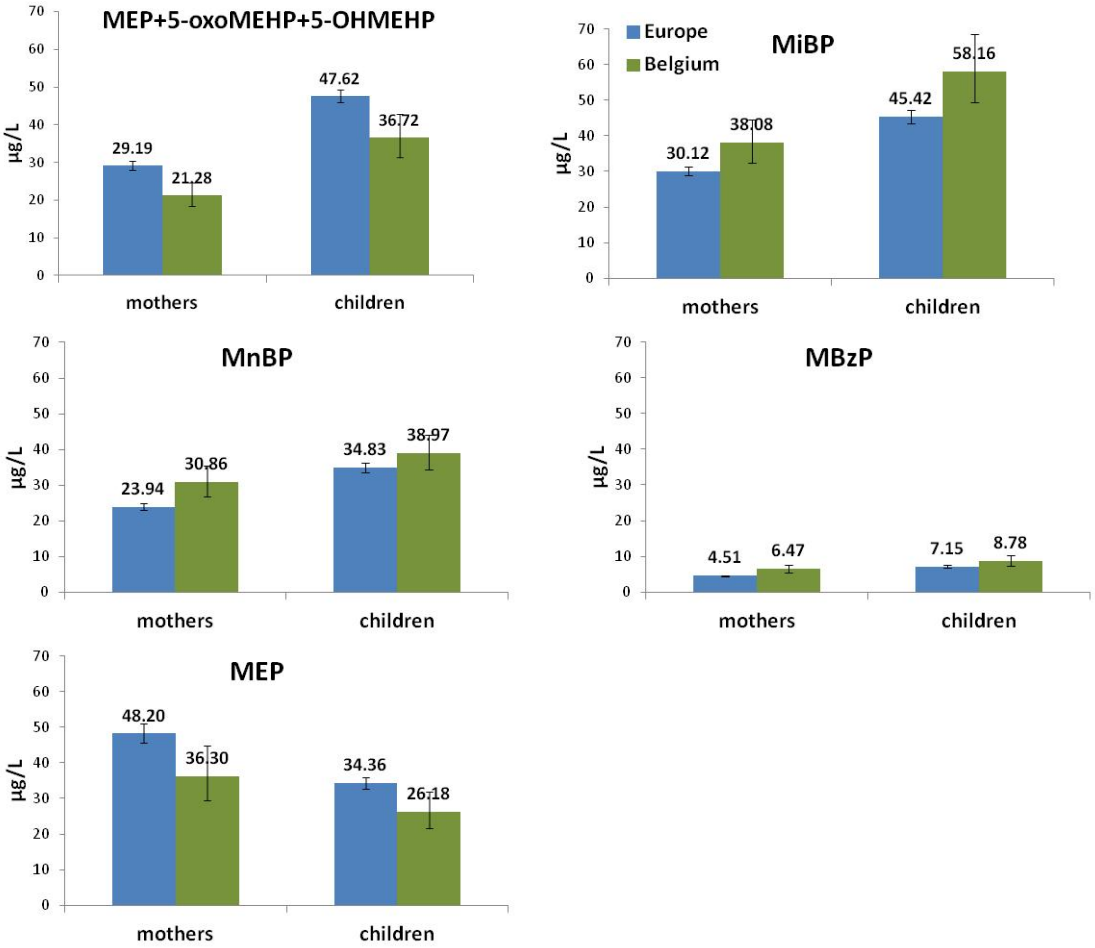


Figure 32 Urinary phthalate metabolite levels in Belgian and European mothers and children (geometric mean±95 confidence interval). Number of mothers/children in the European study group = 1800/1813, except for MiBP and MnBP which were measured in 1347/1355 mothers/children

Determinants of exposure for the Belgian study population

Ingestion of food is reported to be the most dominant pathway for exposure to DEHP, DnBP, DiBP (Wittassek et al., 2010). However for the shorter chain phthalates, DnBP, DiBP and BBP consumer products are of the same or even higher importance (Wittassek et al., 2010).

The Belgian urinary DEHP metabolite (MEHP, 5oxo-MEHP, 5OH-MEHP) levels could not be explained via the questionnaire information. Food items contain various levels of phthalates, mainly dependent on packaging and processing. It is not easy to pinpoint this exposure route, based on a general questionnaire as used in this study. The increased levels of the urinary components MBzP, MiBP and MnBP, were partly explained by the presence of PVC flouring or wall paper in the participants homes and for MiBP and MnBP by the use of personal care products. The phthalate metabolite MEP was linked with a lot of parameters, such as use of personal care products, use of toys, PVC flouring/wall paper, renovation activities in house, but also with consumption of canteen food (mothers) and chewing gum (children).

Table 27: Factors of influence on urinary DEHP phathalate metabolites (MEHP+5oxo-MEHP+5OH-MEHP) in the Belgian children and their mothers (µg/L). Estimate = times increase in biomarker for unit increase of the covariate/confounder

Parameter	Estimate	SE	95% CI		p	p
Sum MEHP+5oxo-MEHP+5OH-MEHP child: R^2 of model = 0.10						
	0.55	0.17	0.40	0.76	0.0005	0.0021
	0.64	0.38	0.30	1.34	0.232	
11. Crt: 300-1000 mg/L vs 1000-2000 mg/L	1.16	0.36	0.56	2.38	0.6894	
12. Crt: 300-1000 mg/L vs 2000-3000 mg/L						
Crt: 1000-2000 mg/L vs 2000-3000 mg/L						
5-8 years vs 9-11 years	1.09	0.15	0.81	1.48	0.5613	0.5613
boys vs girls	0.94	0.16	0.69	1.29	0.7	0.7
Sum MEHP+5oxo-MEHP+5OH-MEHP mother: R^2 of model = 0.33						
	0.45	0.13	0.35	0.58	<.0001	<.0001
	0.23	0.24	0.14	0.37	<.0001	
13. Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.51	0.24	0.32	0.82	0.0053	
14. Crt: 300-1000 mg/L vs 2000-3000 mg/L						
Crt: 1000-2000 mg/L vs 2000-3000 mg/L						
<=35 years vs 35-40 years	0.98	0.17	0.70	1.38	0.9196	0.9883
<=35 years vs >40 years	1.00	0.18	0.70	1.44	0.9909	
35-40 years vs >40 years	1.02	0.14	0.78	1.34	0.8869	

With: Crt= creatinine; SE = standard error; CI = confidence interval

Table 28: Factors of influence on urinary BBP phthalate metabolite (MBzP) in the Belgian children and their mothers (µg/L). Estimate = times increase in biomarker for unit increase of the covariate/confounder

Parameter	Estimate	SE	95% CI		p	p
MBzP child: R^2 of model = 0.13						
	0.64	0.18	0.45	0.91	0.0134	0.0168
	0.42	0.40	0.19	0.94	0.0344	
15. Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.66	0.39	0.31	1.43	0.2914	
16. Crt: 300-1000 mg/L vs 2000-3000 mg/L						
Crt: 1000-2000 mg/L vs 2000-3000 mg/L						
5-8 years vs 9-11 years	1.00	0.17	0.72	1.39	0.9895	0.9895
boys vs girls	0.86	0.17	0.62	1.21	0.3906	0.3906

PVC in floors or walls vs no PVC	1.79	0.19	1.22	2.63	0.0032	0.0032
MBzP mother: R^2 of model = 0.24						
17. Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.56	0.15	0.42	0.75	0.0002	<.0001
	0.32	0.28	0.18	0.56	<.0001	
	0.57	0.27	0.33	0.98	0.0418	
18. Crt: 300-1000 mg/L vs 2000-3000 mg/L						
Crt: 1000-2000 mg/L vs 2000-3000 mg/L						
<=35 years vs 35-40 years	0.76	0.20	0.51	1.13	0.1676	0.3282
<=35 years vs >40 years	0.75	0.21	0.49	1.13	0.1687	
35-40 years vs >40 years	0.99	0.16	0.72	1.35	0.9258	
PVC in floors or walls vs no PVC	1.60	0.17	1.14	2.24	0.0068	0.0068

With: Crt= creatinine; SE = standard error; CI = confidence interval

Table 29: Factors of influence on urinary DiBP phthalate metabolite (MiBP) in the Belgian children and their mothers (µg/L). Estimate = times increase in biomarker for unit increase of the covariate/confounder

Parameter	Estimate	SE	95% CI		p	p
MiBP child: R^2 of model = 0.21						
19. Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.67	0.17	0.48	0.94	0.0201	0.0145
	0.40	0.38	0.19	0.85	0.0173	
	0.60	0.37	0.29	1.23	0.1595	
20. Crt: 300-1000 mg/L vs 2000-3000 mg/L						
Crt: 1000-2000 mg/L vs 2000-3000 mg/L						
5-8 years vs 9-11 years	1.44	0.16	1.06	1.97	0.0218	0.0218
boys vs girls	1.08	0.16	0.79	1.48	0.6351	0.6351
Use personal care products: moderate vs low	1.48	0.17	1.07	2.06	0.019	0.019
PVC in floors or walls vs no PVC	1.85	0.18	1.29	2.65	0.0011	0.0011
MiBP mother: R^2 of model = 0.30						
21. Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.56	0.15	0.42	0.76	0.0002	<.0001
	0.24	0.28	0.14	0.42	<.0001	
	0.42	0.27	0.25	0.73	0.0021	
22. Crt: 300-1000 mg/L vs 2000-3000 mg/L						
Crt: 1000-2000 mg/L vs 2000-3000 mg/L						
<=35 years vs 35-40 years	0.84	0.20	0.56	1.25	0.3817	0.6769
<=35 years vs >40 years	0.86	0.21	0.57	1.31	0.4849	
35-40 years vs >40 years	1.03	0.16	0.75	1.41	0.8602	
PVC in floors or walls vs no PVC	1.75	0.17	1.25	2.45	0.0013	0.0013

With: Crt= creatinine; SE = standard error; CI = confidence interval

Table 30: Factors of influence on urinary DBP phthalate metabolite (MnBP) in the Belgian children and their mothers. Estimate = times increase in biomarker for unit increase of the covariate/confounder

Parameter	Estimate	SE	95% CI		p	p
MnBP child: R^2 of model = 0.16						
23. Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.68	0.13	0.53	0.88	0.0033	0.0027
	0.46	0.29	0.26	0.82	0.0089	
	0.68	0.28	0.39	1.18	0.1723	
24. Crt: 300-1000 mg/L vs 2000-3000 mg/L						
Crt: 1000-2000 mg/L vs 2000-3000 mg/L						
5-8 years vs 9-11 years	1.21	0.12	0.96	1.54	0.1075	0.1075
boys vs girls	0.91	0.12	0.71	1.16	0.4329	0.4329
Use personal care products: moderate vs low	1.28	0.13	1.00	1.65	0.0508	0.0508
PVC in floors or walls vs no PVC	1.33	0.14	1.01	1.75	0.0442	0.0442
MnBP mother: R^2 of model = 0.27						
25. Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.53	0.13	0.41	0.68	<.0001	<.0001
	0.26	0.24	0.16	0.41	<.0001	
	0.48	0.24	0.30	0.77	0.0027	
26. Crt: 300-1000 mg/L vs 2000-3000 mg/L						
Crt: 1000-2000 mg/L vs 2000-3000 mg/L						
<=35 years vs 35-40 years	0.77	0.17	0.55	1.09	0.1385	0.1128
<=35 years vs >40 years	0.68	0.18	0.48	0.98	0.0371	
35-40 years vs >40 years	0.88	0.14	0.67	1.16	0.3616	

With: Crt= creatinine; SE = standard error; CI = confidence interval

Table 31: Factors of influence on urinary DEP phthalate metabolite (MEP) in the Belgian children and their mothers (µg/L). Estimate = times increase in biomarker for unit increase of the covariate/confounder

Parameter	Estimate	SE	95% CI		p	p
MEP child : R^2 of model = 0.26						
27. Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.69	0.21	0.45	1.04	0.0757	0.0086
	0.26	0.45	0.10	0.63	0.0034	
	0.38	0.44	0.16	0.90	0.0286	
28. Crt: 300-1000 mg/L vs 2000-3000 mg/L						
Crt: 1000-2000 mg/L vs 2000-3000 mg/L						
5-8 years vs 9-11 years	0.93	0.20	0.62	1.37	0.7002	0.7002
boys vs girls	0.75	0.20	0.51	1.11	0.1531	0.1531

Chewing gum: several times/week vs once/week or less	1.74	0.27	1.02	2.98	0.0418	0.0418
Toys: daily vs less than daily	1.25	0.23	0.79	1.97	0.3307	0.0506
Toys: daily vs never	1.95	0.27	1.14	3.33	0.0151	
Toys: less than daily vs never	1.56	0.29	0.87	2.78	0.132	
Renovation: yes vs no	1.80	0.21	1.20	2.71	0.0051	0.0051
PVC in floors or walls vs no PVC	1.72	0.23	1.10	2.70	0.0191	0.0191
MEP mother: R^2 of model = 0.29						
29. Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.49 0.35 0.71	0.21 0.39 0.37	0.32 0.16 0.34	0.74 0.76 1.49	0.001 0.0082 0.3654	0.0014
30. Crt: 300-1000 mg/L vs 2000-3000 mg/L						
Crt: 1000-2000 mg/L vs 2000-3000 mg/L						
<=35 years vs 35-40 years	1.02	0.29	0.58	1.81	0.9367	0.1914
<=35 years vs >40 years	0.70	0.30	0.39	1.26	0.2329	
35-40 years vs >40 years	0.68	0.22	0.44	1.05	0.0822	
Canteen food: several times/week vs once/week or less	1.98	0.30	1.09	3.59	0.0243	0.0243
Use personal care products: high vs moderate/low	1.81	0.21	1.20	2.73	0.0048	0.0048
Renovation: yes vs no	1.83	0.20	1.23	2.73	0.0035	0.0035
PVC in floors or walls vs no PVC	1.99	0.24	1.25	3.18	0.0043	0.0043

With: Crt= creatinine; SE = standard error; CI = confidence interval

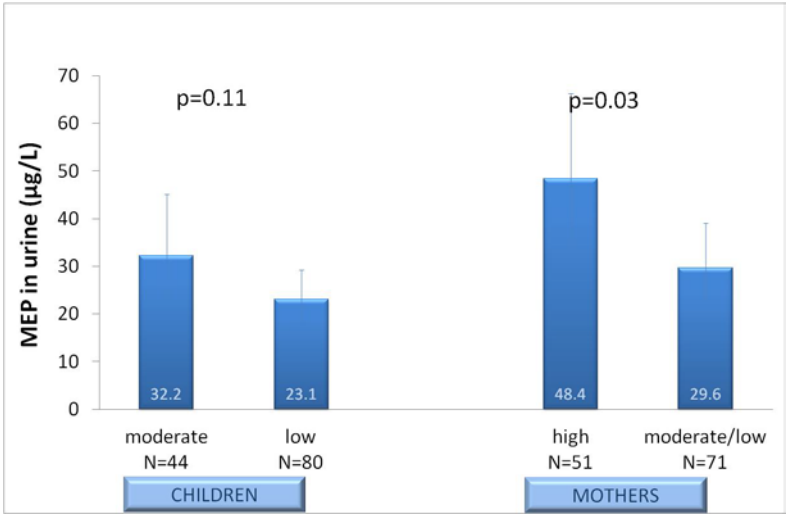


Figure 33 Geometric mean (±95% confidence interval) of MEP levels for individuals with a different frequency of personal care products' use

30.1.1 Bisphenol A

Background information

Bisphenol A

What is Bisphenol A?

Bisphenol A is a chemical compound used in the manufacture of plastics and epoxy resins used in the coating of cans.

Where is it found?

Bisphenol A is used in coating materials to cover the inner surface of cans but also in the manufacture of many plastics used for food and toys. Other typical application areas are paints, varnishes or glues, or thermal paper used for the receipt at supermarket checkouts.

COPHES
Consortium to Perform Human Biomonitoring on a European Scale

Human Biomonitoring of Bisphenol A

The concentration of Bisphenol A is generally measured in urine. Bisphenol A is rapidly metabolized in the human body and is quickly passed out in urine, where it can be measured with biomonitoring.
Bisphenol A can usually be measured in the urine of more than 90 % of the human population and finding a measurable amount of bisphenol A in blood or urine is not dangerous and does not necessarily cause adverse health effect.

How are we exposed?

We are all exposed to Bisphenol A everyday because of its widespread use in plastics and other products of daily use,
People can be exposed by eating or drinking food and drink, which have been in contact with plastic products containing Bisphenol A.

How can it affect us?

Current exposure levels to Bisphenol A are generally considered safe by various European authorities. However, human health effects from low-level exposure to Bisphenol A remains under discussion Whether continuous exposure to Bisphenol A may affect the hormonal system is currently being investigated.
It is generally acknowledged that more research is needed to assess the exact health effects of long-term exposure to low levels of Bisphenol A.

How to reduce exposure?

- Discard old plastic infant drinking bottles.
- Avoid using drinking cans for infants under 3 years old.
- Diversify food from different origin and kind.
- Information on healthy food and how to remove mercury-holding products can be found via the link on www.nehap.be

Belgian levels

BPA measured in the 6-11 years old children was slightly higher in the current study group (P_{90}=8.15 µg/L) compared to the Flemish reference population (P_{90}=6.98 µg/L), which consisted however of older, i.e. 14- 15 years old children (Schoeters et al., 2012). The levels in the mothers and the children were comparable: GM 2.55 vs 2.35 µg/L. The difference in concentrations between the highest (P_{90}) and lowest (P_{10}) exposed individuals within an age group were moderate. This points to a rather homogeneous exposure among the population. The levels of the mothers and children were correlated (r= 0.25, p<0.01). None of the participants had levels above the HBM-I threshold of 1.5 and 2.5 mg/L for children and mothers respectively.

Table 32 Results of Bisphenol A in urine of Belgian mothers and their children

Biomarker	group	Unit	N	%>LOQ	GM	low CI	up CI	P_{50}	P_{90}	P_{95}	N > health guidance
BPA											
	mother	µg/L	125	100.00%	2.55	2.16	3.02	2.3	7.47	11.63	0>HBM-I
		µg/g creat.	125	100.00%	2.28	1.94	2.67	2.11	5.72	9.44	
	child	µg/L	125	96.80%	2.35	1.94	2.84	2.27	8.15	13.44	0>HBM-I
		µg/g creat.	125	96.80%	2.1	1.73	2.54	2.03	6.68	11.42	

With: N=number, LOQ: limit of quantification; GM: geometric mean (= antilog of mean of the logarithmic transferred values); CI: confidence interval (low CI and Up CI = lower and upper CI); SD: standard deviation; P_{50}, P_{90},P_{95}: 50, 90 and 95% of the population had a concentration below that value; creat: creatinine

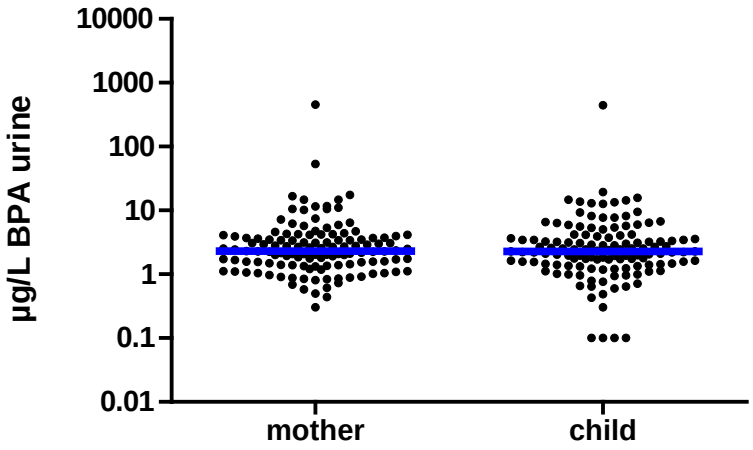


Figure 34 Urinary levels of Bisphenol A (µg/L) in the Belgian mothers (N=125) and their children (N=125). The blue horizontal line indicates the median of each age group

Comparison of Belgian with the European levels

Bisphenol A was included as an optional biomarker in the study which was measured in 654 children and 641 mothers of six European countries, namely Belgium, Denmark, Spain, Luxemburg, Slovenia,

and Sweden. Overall, there was a low inter-individual variability in urinary BPA levels: the concentration range within a country was narrow; levels were quite similar in children and in mothers; and the average levels in the different countries differed only with a factor of 1.8 in children and 2.0 in mothers (difference between the highest and the lowest average). The Belgian mothers had significantly higher values than the European average (of the six countries), but the levels were very similar in all countries.

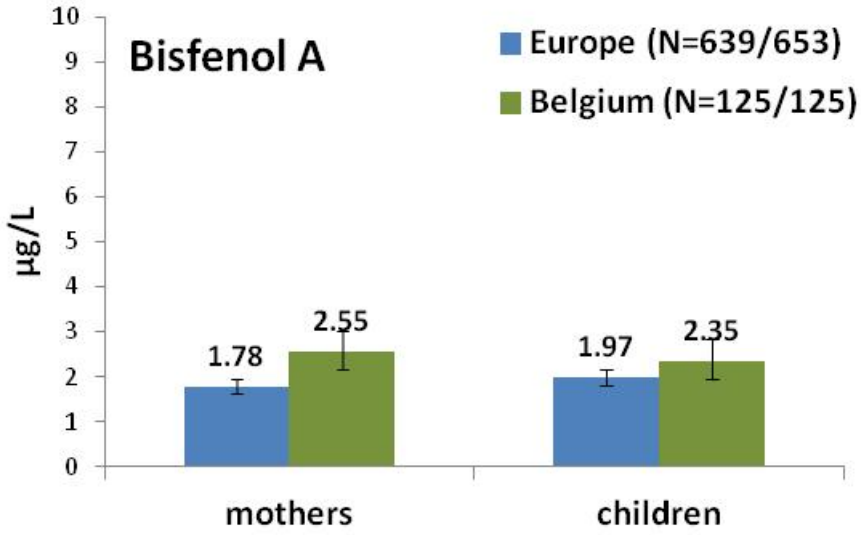


Figure 35 Urinary Bisphenol A levels measured in Belgian and European mothers and children (geometric mean±95 confidence interval). The number of mothers/children are indicated in the legend

Determinants of exposure for the Belgian study population

Bisphenol A is a chemical compound used in the manufacturing of plastics and epoxy resins used in the coating of cans. Urinary BPA levels were 1.6 times higher in mothers consuming canned food several times per week compared to those eating canned food only once or less per week (p=0.02). No other factors influenced the internal exposure.

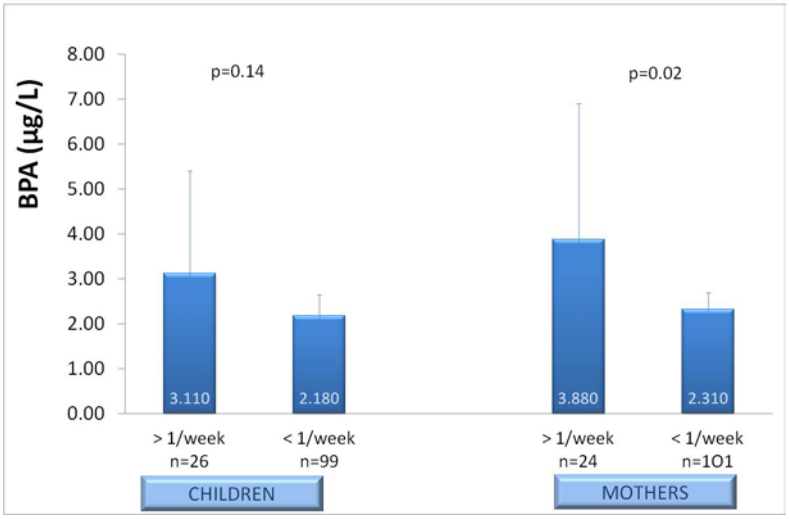


Figure 36 Concentration of BPA in urine of mothers and children in relation to consumption habit of canned food: geometric mean (±95% CI) of individuals consuming more vs. less than once per week.

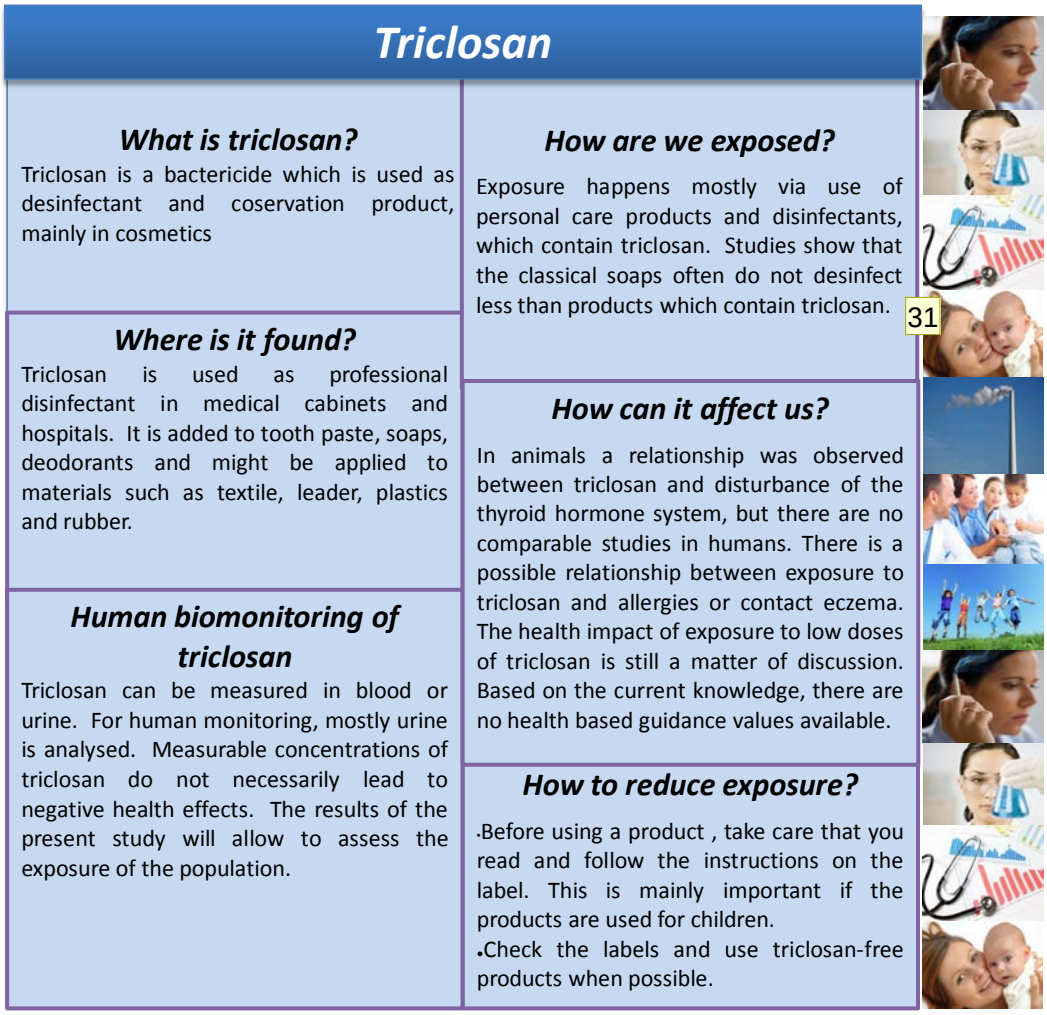
Table 33: Factors of influence on urinary bisphenol A (BPA) in the Belgian children and their mothers (µg/L). Estimate = times increase in biomarker for unit increase of the covariate/confounder

Parameter	Estimate	SE	95% CI		p	p
BPA child: R^2 of model = 0.12						
31. Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.56	0.20	0.37	0.84	0.0052	0.0008
	2.18	0.46	0.88	5.43	0.0923	
	3.89	0.45	1.61	9.41	0.0028	
32. Crt: 300-1000 mg/L vs 2000-3000 mg/L						
Crt: 1000-2000 mg/L vs 2000-3000 mg/L						
5-8 years vs 9-11 years	0.99	0.19	0.68	1.43	0.9435	0.6333
boys vs girls	1.10	0.19	0.75	1.61	0.6333	0.9435
BPA mother: R^2 of model = 0.13						
33. Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.64	0.17	0.46	0.91	0.0121	0.0061
	0.41	0.33	0.22	0.78	0.0074	
	0.64	0.32	0.34	1.20	0.1628	
34. Crt: 300-1000 mg/L vs 2000-3000 mg/L						
Crt: 1000-2000 mg/L vs 2000-3000 mg/L						
<=35 years vs 35-40 years	1.03	0.23	0.65	1.62	0.9148	0.8163
<=35 years vs >40 years	0.91	0.24	0.56	1.48	0.7113	
35-40 years vs >40 years	0.89	0.18	0.62	1.28	0.5306	
Canned food: several times/week vs once/week or less	1.62	0.21	1.07	2.44	0.0236	0.0236

With: Crt= creatinine; SE = standard error; CI = confidence interval

34.1.1 Triclosan

Background information



Belgian levels

Triclosan is a bactericide which is commonly used as disinfectant and conservation agent, mainly in cosmetics and hygiene products. It can also be added to materials, such as textiles, leader, plastics

and rubber. Urinary triclosan levels were considerably lower in the children of the current study with a P_{90}=7.88 µg/L vs 91.5 µg/L in the Flemish adolescents reference population (Schoeters et al., 2012). This might be due to the older age of the adolescents in the reference population. Adolescents generally use more personal care products than basic school pupils. The difference in concentrations between the highest (P_{90}) and lowest (P_{10}) exposed individuals was very high with a factor 350 and 35 in mothers and children respectively (Table 34, Figure 37). This reflects the strong impact of consumer habits on the levels. It was also remarkable that children and mothers' urinary concentrations of triclosan were good correlated (Spearman rank r= 0.48, p<0.001), unlike the fact that mothers might be much more exposed to personal care products and might use different kinds of those products. Mothers showed indeed, clearly higher levels of triclosan (Table 34, Figure 37).

Table 34 Results of triclosan in urine of Belgian mothers and their children

Biomarker	group	Unit	N	%>LOQ	GM	low CI	up CI	P_{50}	P_{90}	P_{95}	N > health guidance
Triclosan											
	mother	µg/L	125	100.00%	2.72	1.87	3.96	1.56	122.39	347	n.a.
		µg/g creat.	125	100.00%	2.42	1.67	3.53	1.23	103.37	222.44	
	child	µg/L	125	97.60%	1.23	0.89	1.7	0.83	7.88	121.9	n.a.
		µg/g creat.	125	97.60%	1.1	0.8	1.52	0.85	7.71	85.13	

With: N=number, LOQ: limit of quantification; GM: geometric mean (= antilog of mean of the logarithmic transferred values); CI: confidence interval (low CI and Up CI = lower and upper CI); SD: standard deviation; P_{50}, P_{90},P_{95}: 50, 90 and 95% of the population had a concentration below that value; creat: creatinine

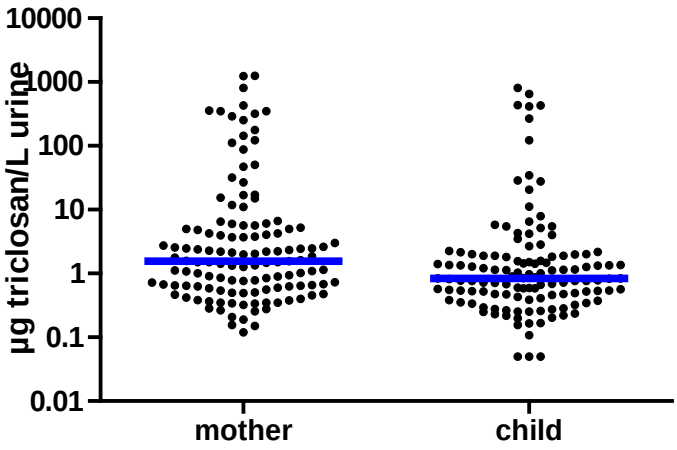


Figure 37 Urinary levels of triclosan (µg/L) in the Belgian mothers (N=125) and their children (N=125). The blue horizontal line indicates the median of each age group

Determinants of exposure for the Belgian study population

In children - but surprisingly not in mothers - triclosan levels were clearly higher if personal care products or sun screens were used moderately vs. rarely. The effect was especially clear for sunscreen users: 6.1 times higher triclosan levels compared to rarely users (p=0.02). There was no signif-

icant effect of the use of handsoaps and disinfection gels. Tooth paste use was not asked for in the questionnaire.

Table 35: Factors of influence on urinary triclosan in the Belgian children and their mothers (µg/L). Estimate = times increase in biomarker for unit increase of the covariate/confounder

Parameter	Estimate	SE	95% CI		p	p
Triclosan child: R^2 of model = 0.28						
35. Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.45 0.53 1.18	0.33 0.74 0.71	0.24 0.12 0.29	0.86 2.29 4.87	0.0166 0.3939 0.8153	0.0552
36. Crt: 300-1000 mg/L vs 2000-3000 mg/L						
Crt: 1000-2000 mg/L vs 2000-3000 mg/L						
5-8 years vs 9-11 years	0.48	0.30	0.26	0.89	0.019	0.0190
boys vs girls	0.90	0.31	0.49	1.67	0.732	0.7320
ETS last 24h yes vs no	2.95	0.54	1.02	8.54	0.046	0.0460
use personal care products: moderate vs low	2.75	0.32	1.45	5.22	0.0022	0.0022
use sunscreens: moderate vs low	6.11	0.77	1.33	28.07	0.0204	0.0204
Prim/second vs 1st stage tertiary	0.38	0.44	0.16	0.90	0.0288	0.0079
Prim/second vs 2nd stage tertiary	1.29	0.60	0.39	4.26	0.6715	
1st stage vs 2nd stage tertiary	3.41	0.48	1.32	8.78	0.0116	
Triclosan mother: R^2 of model = 0.06						
37. Crt: 300-1000 mg/L vs 1000-2000 mg/L	0.75318 0.59427 0.78902	0.39945 0.7544 0.73722	0.3415 0.13343 0.18328	1.6611 2.6469 3.3967	0.4793 0.4916 0.7484	0.6883
38. Crt: 300-1000 mg/L vs 2000-3000 mg/L						
Crt: 1000-2000 mg/L vs 2000-3000 mg/L						
<=35 years vs 35-40 years	1.7589	0.54124	0.60229	5.1366	0.2989	0.5129
<=35 years vs >40 years	1.84857	0.56334	0.60589	5.64	0.2776	
35-40 years vs >40 years	1.05098	0.4279	0.45043	2.4523	0.9077	
Urban vs rural	2.44007	0.38731	1.13327	5.2538	0.023	0.0230

With: Crt= creatinine; prim=primary school, second = secondary school, 1st stage tertiary = bachelor+master degree, 2nd stage tertiary = PhD degree; SE = standard error; CI = confidence interval

38.1.1 Correlations among the biomarkers

The group of six phthalates measured could be more or less grouped according to their correlation (Table 36, Table 37). In the mothers MiBP, MnBP and MBzP were among each other strongly correlated (r>0.60, p=0.0001) and to a bit lower extent to the DEHP metabolites (MEHP, 5OH-MEHP en 5oxo-MEHP, Spearman rank r=0.32-0.55, p=0.0001). This suggests a possible same exposure source

of exposure to those phthalates. Ingestion of food is the dominant pathway for DEHP exposure (Wittassek et al., 2010; Koch and Angerer, 2012). For DnBP, DiBP and BBP, other sources are of the same or higher importance. MEP, which is mainly used in consumer products was also correlated with the other phthalates, but clearly not so strongly (r= 0.20-0.39, p=0.02-0.0001). In children, most phthalates, were moderately to strong correlated (r= 0.30-0.64, p=0.0001), except for MEP, which was not significantly correlated with the DEHP metabolites.

Bisphenol A was correlated with all phthalates in all age groups (with MEP, however to a lesser extent). In children, also triclosan correlated well with the phthalate metabolites. In mothers and to a lesser extent in children, surprisingly urinary cadmium seemed to be rather good linked with urine phthalate metabolite levels (r=0.23-0.47, p=0.010-0.0001) and with Bisphenol A (r=0.31, p<0.001). This could be due to a similar source, which might be food.

correlation > 0.75
correlation 0.50-0.75
correlation 0.25-0.50
correlation < 0.25

Table 36: Spearman rank correlations between biomarkers measured in the mothers (µg/L urine or µg/g hair for Hg)

	Hg	Cd	Cotinine	MEHP	5OH-MEHP	5oxo-MEHP	MBzP	MiBP	MnBP	MEP	BPA	TCS
HG	1.00	0.09 (p=0.3227)	0.07 (p=0.4016)	0.15 (p=0.0941)	0.10 (p=0.2423)	0.06 (p=0.4828)	-0.06 (p=0.4997)	-0.11 (p=0.2314)	0.04 (p=0.6424)	-0.04 (p=0.639)	0.20 0.02387	0.06 0.4724
Cd		1.00	0.14 (p=0.11)	0.47 (p=0.0001)	0.40 (p=0.0001)	0.43 (p=0.0001)	0.37 (p=0.0001)	0.34 (p=0.0001)	0.37 (p=0.0001)	0.23 (p=0.0098)	0.31 0.00032	0.13 0.1492
cotinine			1.00	0.18 (p=0.04)	0.02 (p=0.79)	0.06 (p=0.50)	0.05 (p=0.54)	0.11 (p=0.2)	0.10 (p=0.24)	0.03 (p=0.77)	0.18 0.04	-0.02 0.80
MEHP				1.00	0.76 (p=0.0001)	0.76 (p=0.0001)	0.32 (p=0.0002)	0.40 (p=0.0001)	0.42 (p=0.0001)	0.25 (p=0.0036)	0.40 0.0000	0.08 0.3688
5OH-MEHP					1.00	0.96 (p=0.0001)	0.44 (p=0.0001)	0.49 (p=0.0001)	0.54 (p=0.0001)	0.25 (p=0.0044)	0.48 0.0000	0.06 0.5358
5oxo-MEHP						1.00	0.47 (p=0.0001)	0.55 (p=0.0001)	0.53 (p=0.0001)	0.20 (p=0.0225)	0.49 0.0000	0.06 0.4709
MBzP							1.00	0.60 (p=0.0001)	0.64 (p=0.0001)	0.39 (p=0.0001)	0.36 0.00003	0.13 0.1393
MiBP								1.00	0.65 (p=0.0001)	0.31 (p=0.0003)	0.39 0.0000	0.13 0.1432

	Hg	Cd	Cotinine	MEHP	5OH-MEHP	5oxo-MEHP	MBzP	MiBP	MnBP	MEP	BPA	TCS
MnBP									1,00	0,34 (p=0,0001)	0.42 0.0000	0.14 0.1249
MEP										1,00	0.21 0.0177	0.04 0.614
BPA											1,00	0.15 0.084

Table 37: Spearman rank correlations between biomarkers measured in the children (µg/L urine or µg/g hair for Hg)

	Hg	Cd	Cotinine	MEHP	5OH-MEHP	5oxo-MEHP	MBzP	MiBP	MnBP	MEP	BPA	TCS
Hg	1.00	0.09 (p=0.32)	-0.22 (p=0.01)	-0.14 (p=0.11)	-0.14 (p=0.11)	-0.17 (p=0.06)	-0.19 (p=0.035)	-0.13 (p=0.15)	-0.10 (p=0.27)	-0.11 (p=0.21)	0.04 0.62	0.05 0.57
Cd		1.00	-0.11 (p=0.20)	0.28 (p=0.001)	0.17 (p=0.05)	0.19 (p=0.03)	0.14 (p=0.12)	0.27 (p=0.002)	0.34 (p<0.0001)	0.16 (p=0.075)	0.20 0.03	0.16 0.07
Cotinine			1.00	-0.03 (p=0.71)	0.11 (p=0.22)	0.10 (p=0.25)	0.08 (p=0.38)	0.23 (p=0.008)	0.10 (p=0.24)	0.21 (p=0.02)	0.21 0.01	0.17 0.05
MEHP				1.00	0.83 (p<0.0001)	0.81 (p<0.0001)	0.40 (p<0.0001)	0.34 (p<0.0001)	0.30 (p=0.0005)	0.13 (p=0.14)	0.26 0.0032	0.28 0.001
5OH-MEHP					1.00	0.98	0.49	0.45	0.43	0.16	0.30	0.25

	Hg	Cd	Cotinine	MEHP	5OH-MEHP	5oxo-MEHP	MBzP	MiBP	MnBP	MEP	BPA	TCS
						(p<0.0001)	(p<0.0001)	(p<0.0001	(p<0.0001)	(p=0.06)	0.0005	0.004
5oxo-MEHP						1.00	0.52 (p<0.0001)	0.45 (p<0.0001)	0.46 (p<0.0001)	0.16 (p=0.08)	0.30 0.0005	0.27 0.002
MBzP							1.00	0.48 (p<0.0001)	0.60 (p<0.0001)	0.41 (p<0.0001)	0.13 0.14	0.33 0.0002
MiBP								1.00	0.64 (p<0.0001)	0.49 (p<0.0001)	0.37 0.00001	0.27 0.002
MnBP									1.00	0.50 (p<0.0001)	0.22 0.01	0.31 0.0003
MEP										1.00	0.19 0.03	0.30 0.0006
BPA											1.00	0.46 0.00000

39. Conclusions

In Belgium 129 school children (6-11y) and their mothers (≤ 45y) were selected, and morning urine and hair samples were collected. Field work was performed in the period of October 2011 until February 2012. Participants were recruited in the rural areas of Brakel, Ellezelles, Frasnes-lez-Anvaing and in the urban area of the capital Brussels. In urine the heavy metal cadmium, , the nicotine metabolite cotinine, several phthalate metabolites (MEHP, 5oxo-MEHP, 5OH-MEHP, MBzP, MiBP, MnBP) as well as bisphenol A (BPA) and triclosan were measured. Hair samples were analysed for mercury. By means of multiple linear regression analysis, the influence of life style, diet and home environment – information which was gathered via personal interview with the mothers – was analyzed for their influence on the urinary and hair exposure markers.

14.1% of the children/mothers who received an invitation for the study wished to participate. This number was most probably influenced by the fact that the inclusion criteria were indicated on the invitation letters distributed. Due to stringent selection on living area, age sex, and the exclusion of two or more children of the same mother, only 5.3% of all contacted people could take part in the study.

The levels of the contaminants measured in the Belgian study population given in the table 0 were nearly all below used health based guidance values, as far as they were available. Belgian participants had levels above the European average for mercury in hair and some phthalate metabolites in urine (MiBP, MBzP and MnBP). On the other hand, the urinary levels of cotinine, cadmium and the phthalate metabolites of DEHP and DEP were significantly lower than the European average.

The concentrations of all measured pollutants correlated good till rather good between mothers and their children. This means that the home environment and food consumption, which is (rather) similar among family members, determined a considerable part of the exposure. This study shows that several environmental contaminants in children and their mothers could be explained by information asked about life style, nutrition and home environment. Human urine and hair appeared to be good matrices to assess contaminants (chronically) present in daily life.

The results and conclusions were presented in a national symposium which took place on November 28th. This symposium was followed by a discussion on policy implications in a closed meeting and conclusions, gaps and propositions for action will be presented to the Joined Ministerial Conference on Environment and Health for political approval.

40. References

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ANNEXES

Recruitment and field work procedures

Communication materials

Fact sheets

Questionnaires

Ethical committee documents

COPHES Belgian statistics report