



# HEALS

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based on Large population Surveys

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## Abstract

The pilot European Exposure and Health Examination Survey (EXHES) is one of the milestones of the HEALS project. It is at the origin of relevant harmonized and standardized data to unravel the relationship between body burden from internal and external exposure and the onset/exacerbation of the health outcomes targeted by HEALS, i.e. asthma and allergies, neurological disorders, overweight, obesity and diabetes in childhood.

Overall, 4,888 families have joined the EXHES mother-child birth cohort by June 2019. As a whole, 5,169 children were included, which comprises 836 twins and 21 triplets. A wide variety of biological material was collected according to standardized sampling procedures in children as well as in their parents. An EXHES biobank has been created. In total, the EXHES biobank included 13,988 different biospecimens collected from the newborns, plus 7,185 and 870 biospecimens collected from their mothers and fathers, respectively.

Children and parents are presently followed-up and other biospecimens collected.

The data collected in EXHES together with the environmental data available in the HEALS EDMS database will allow the application of the HEALS paradigm to contribute to the understanding of major chronic diseases. The obtained results will be used in risk assessment.

## 1. Introduction

Environmental threats to the health of children are myriad and start since pre-conception. Several heterogeneous birth cohorts were started in early 2000 to contribute to better understanding of the underlying causes of human disorders.

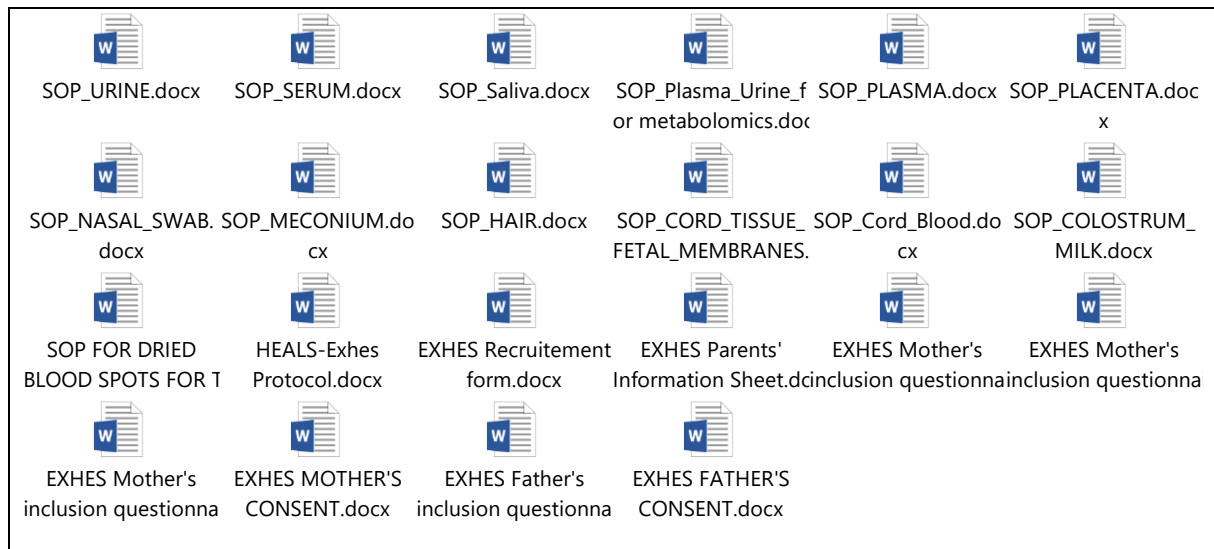
Some have been included in HEALS have been applied to study the origins of asthma and allergies, metabolic diseases and neurodegenerative disorders. The importance of birth cohorts is no longer in doubt, although there are advantages and disadvantages of mixing different-sized cohorts. Inconsistent findings may possibly be explained by the differences in exposure levels and target stressors, and by possible statistical errors. In a well-defined cohort, novel hypotheses or preliminary examinations are more easily verifiable. This is why the HEALS project has implemented the pilot European Exposure and Health Examination Survey (EXHES), the novel hypotheses being the need to apply the exposomic approach to understanding the origin of the specific diseases. The HEALS project is distinct in leveraging and building upon existing cohorts and the new EXHES birth cohorts to comprise approximately 20 000 children and prospectively investigate the role of early-life exposures and underlying biological mechanisms in childhood health and disease, specifically perinatal conditions, obesity, neurodevelopmental disorders, asthma and related pulmonary disorders as well as optimum child health.

The EXHES was organized in 10 EU countries to test the applicability of the HEALS approach for EU-wide large population surveys. EXHES will combine a longitudinal and a nested case-control phase to allow for better definition of environmental exposures and better characterization of disease and risk phenotypes over the limited duration of the project, whilst setting the foundation for post-project follow-up. The technological and computational integration proposed in HEALS will be tested through EXHES with regard to both technical feasibility and cost-effectiveness. The lessons drawn from the EXHES pilot survey will provide the basis for drafting scientific advice, protocols and, eventually, guidance for the setting up of a European Health and Exposure Survey, paving the way to EU-wide.

EXHES is at the origin of relevant harmonized and standardized data to unravel the relationship between body burden from internal and external exposure and the onset/exacerbation of the health outcomes targeted by HEALS, i.e. asthma and allergies, neurological disorders, overweight, obesity and diabetes in childhood.

## 1. EXHES Survey

The EXHES was conducted according to the protocol provided in the deliverable D17.1 titled “EXHES protocol”. It includes the execution of several steps (information, consent, questionnaire, biosampling).



### 1.1 Study design and population

Briefly EXHES comprises 2 phases:

**EXHES Phase I** is a 3 year follow-up including in each country at the beginning 100 pairs of twins (n=200 twins), 200 matched singletons (2 per pairs, on day of birth, sex, social class, region...) and 500 additional unselected singletons recruited over 18 months for a total of 900 children per centre. **600 children are expected.** Their parents will be also recruited.

- Recruitment: 0 -18 months
- 3–yr follow-up: 0 - 54 months

Children and parents will be recruited in **Croatia, France, Germany, Greece, Italy, Portugal, Slovenia, Spain, Poland and the United Kingdom.**

**EXHES PHASE II** is a nested case-control study (based on the targeted diseases and healthy controls) conducted in a sub-sample of 210 individuals (140 twins and 70 singletons) and 140 mothers in each country in which -omics analysis and geo-localisation and remote sensors will be performed **for a total of 2,100 children and 1,400 mothers.**

**1.2 EXHES PHASE I** In order to allow several levels of participation, according to the possibility of collecting and storing biological specimen<sup>1</sup>,

No major deviation from the protocol have been observed. All the mothers and fathers have been informed about EXHES and have signed a consent priori to their inclusion into the survey.

Overall, for the participants was easier to participate in the interview phase than in the biosampling collection.

Of note, it was decided to postpone the nasal swab to a later phase fo the follow-up.

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<sup>1</sup> Of note this can depend on budget availability, local authorization, parental willing to provide the specimen.

## 2. Families included in the EXHES birth cohort.

Ethical approvals were obtained in all the countries to conduct the EXHES survey. This is one of the aspects presented and discussed in the deliverable D17.2 titled “Final report on compliance with ethical review requirements”.

The recruitment of the families for the EXHES study began in 2016 and it is still ongoing.

Expecting mothers and fathers were contacted in clinic either during pregnancy or at the delivery, and accepted to be recruited in the study by providing written informed consent.

In all, 4,888 families have joined the study by June 2019 in 10 European countries (**Table 1**). Germany was the most represented country in the EXHES cohort, as nearly 2500 families from the Kuno-Kids birth cohort that were included in EXHES (Brandstetter 2019<sup>2</sup>). At the time of recruitment, all the expecting mothers (n=4,848) and about 43% (n=2149) of the expecting fathers decided to contribute to the survey either by filling in a standardized questionnaires on their health, life style, and exposure to potential risk factors and modifiers or by providing biosamples.

Overall, 5,169 children were included in the EXHES birth cohort, which included 836 twins and 21 triplets.

**Table 1:** Number of parents and births recruited in the HEALS/EXHES cohort by Country.

	Croatia	France	Germany	Greece	Italy	Poland	Portugal	Slovenia	Spain	UK	Total
<b>Recruitment</b>											
Families	289	328	2492	*	92	390	759	12	179	315	<b>4856</b>
Mothers	289	328	2492	32	92	390	719	12	179	315	<b>4816</b>
Fathers	0	180	1486	*	92	296	95	-	0	-	<b>2149</b>
Total		370		38	12					31	<b>513</b>
births	289		2492		0	407	981	11	148	5	<b>1</b>
Singletons	277	286		30						31	<b>429</b>
Twins/Triples	12	84	2330	8	64	390	485	7	142	5	<b>6</b>
			162		56	17	496	4	6	0	<b>837</b>

*\*data from Greece haven't been centralized yet.*

<sup>2</sup> Brandstetter S, Toncheva AA; Niggel J, et al. KUNO-Kids birth cohort study: rationale, design, and cohort description. Mol Cell Pediatrics 2019;6:1. <https://doi.org/10.1186/s40348-018-0088-z>



## 2. The EXHES biobank

A wide variety of biological material is collected according to standardized sampling procedures in children as well as in their parents (**Table 2**).

**Table 2:** Number of biological samples collected in the HEALS/EXHES cohort by Country

	Croatia	France	Germany	Greece	Italy	Poland	Portugal	Slovenia	Spain	UK	Total
<b>Mother samples</b>											
<b>Urine</b>	<b>286</b>	<b>258</b>	-		<b>86</b>	<b>367</b>	<b>573</b>	<b>10</b>	<b>177</b>		<b>1757</b>
<i>Singletons</i>	274	218	-		60	350	410	8	172		1491
<i>Twins</i>	12	40	-	*	26	17	163	2	5		266
<b>Blood</b>	<b>288</b>	<b>200</b>	-		<b>62</b>	<b>366</b>	<b>563</b>	<b>10</b>	<b>177</b>		<b>1666</b>
<i>Singletons</i>	276	168	-	*	43	349	394	8	172		1410
<i>Twins</i>	12	32	-	*	19	17	169	2	5		256
<b>Colostrum</b>	<b>256</b>	-	-		<b>31</b>	<b>377</b>	<b>379</b>	<b>5</b>	-		<b>1048</b>
<i>Singletons</i>	245	-	-	*	22	360	271	5	-		903
<i>Twins</i>	11	-	-	*	9	17	108	0	-		145
<b>Hair</b>	<b>288</b>	-	-		<b>61</b>	<b>381</b>	<b>653</b>	<b>10</b>	-		<b>1393</b>
<i>Singletons</i>	276	-	-	*	42	364	442	8	-		1132
<i>Twins</i>	12	-	-	*	19	17	211	2	-		261
<b>Saliva</b>	<b>288</b>	<b>230</b>	-		<b>59</b>	<b>377</b>	-	-	-		<b>954</b>
<i>Singletons</i>	276	193	-	*	41	360	-	-	-		870
<i>Twins</i>	12	37	-	*	18	17	-	-	-		84
<b>Father samples</b>											
<b>Urine</b>	-	<b>126</b>	-		<b>71</b>	<b>257</b>	-	-	-	-	<b>454</b>
<i>Singletons</i>	-	106	-	*	49	247	-	-	-	-	402
<i>Twins</i>	-	20	-	*	22	10	-	-	-	-	52
<b>Saliva</b>	-	<b>100</b>	-	*	<b>27</b>	<b>289</b>	-	-	-	-	<b>416</b>
<i>Singletons</i>	-	84	-	*	19	274	-	-	-	-	377
<i>Twins</i>	-	16	-	*	8	15	-	-	-	-	39
<b>Child samples</b>											
<b>Cord blood</b>					<b>120</b>	<b>390</b>	<b>669</b>	<b>11</b>	<b>147</b>	-	<b>2372</b>
<i>Singletons</i>	273	114	590	*	64	373	345	7	142	-	1908
<i>Twins</i>	12	36	10	*	56	17	324	4	5	-	464

<b>Meconium</b>	<b>285</b>	<b>270</b>	<b>1893</b>		<b>11</b>	<b>337</b>	<b>798</b>	<b>11</b>	-	-	<b>371</b>
<i>Singletons</i>	273	205	1805	*	61	323	382	7	-	-	305
<i>Twins</i>	12	65	88	*	56	14	416	4	-	-	655
<b>Guthrie</b>	<b>275</b>	-	-		<b>10</b>	<b>339</b>	<b>422</b>	<b>11</b>	-	-	<b>114</b>
<i>Singletons</i>	263	-	-	*	53	327	144	7	-	-	794
<i>Twins</i>	12	-	-	*	49	12	278	4	-	-	355
<b>Hair</b>	<b>286</b>	-	-		-	-	<b>806</b>	-	-	-	<b>109</b>
<i>Singletons</i>	275	-	-	*	-	-	429	-	-	-	704
<i>Twins</i>	11	-	-	*	-	-	377	-	-	-	388
<b>Cord tissue</b>	<b>288</b>	<b>86</b>	-		-	<b>303</b>		<b>11</b>	<b>117</b>	-	<b>805</b>
<i>Singletons</i>	276	65	-	*	-	288		7	113	-	749
<i>Twins</i>	12	21	-	*	-	15		4	4	-	56
<b>Placenta</b>	-	<b>50</b>	-		-	<b>383</b>	<b>708</b>	<b>11</b>	<b>117</b>	<b>17</b>	<b>144</b>
<i>Singletons</i>	-	38	-	*	-	367	368	7	113	17	106
<i>Twins</i>	-	12	-	*	-	16	340	4	4	0	376

\*data from Greece haven't been centralized yet.

Biological samples from the mothers were collected during pregnancy or during delivery. Whole blood (for genetic/epigenetic analyses, transcriptomics, protein measurements from serum and plasma) and urine samples (for metabolomic analyses) were obtained from 1,666 and 1,757 mothers, respectively, including over 250 mothers of twins. In Spain, where recruitment occurred early in pregnancy (before the 12<sup>th</sup> gestational week), maternal samples of blood and urines were collected at each gestational trimester (T1, T2 and T3) and at delivery. Maternal hair (for toxicologic analysis) and saliva samples (for metabolomic and microbiota analyses) were collected in 1,393 and 954 mothers, respectively. Samples of colostrum were collected in 1,048 women. Additionally, Guthrie cards were collected in Croatia and Italy from 367 mothers.

In Poland, Italy and France, samples of urines (n=454) and saliva (n=416) were collected also in fathers.

Biological samples obtained from the children were collected immediately after delivery, and clinical and anthropological assessments were also performed.

Cord blood and tissue were collected from 2,372 and 805 children, respectively, of whom 464 and 56 were twins. Placenta samples from 1,441 births (1065 single and 376 gemellary) were collected and stored. Meconium (newborn stool for microbiome analysis) was collected over 3,771 individuals (3056 singletons and 655 twins). Further, Guthrie cards and newborn hair samples were obtained from over one thousand children. In Germany, moreover, newborn urine samples (n=526) were collected non-invasively by catching methods, and skin (n=2,784) and buccal swabs (n=107) were also acquired.

The time period for collecting samples such as cord blood is very restricted, and mothers' informed consent is gathered only after delivery. Therefore, cord blood samples are stored from all newborns until the parents have decided for or against participation in the study. If no

informed consent to study participation and biosampling is present, stored samples are destroyed. Only samples of study participants are kept, stored at -80°C, and worked up.

In total, the EXHES biobank included 13,988 different biospecimens collected from the newborns, plus 7,185 and 870 biospecimens collected from their mothers and fathers, respectively.

### **3. Birth and Follow-up analysis**

Partial results of Environmental Wide Association Study (EWAS) analysis at birth are presented in Deliverable D17.3.

The follow-up of children is being continuing. The results will be published after the official end of the HEALS project. However, they will mention it.

The EXHES II phase has been conducted in the first year of age and data analyzed for Spain, France, Germany and Poland. Partial results of Environmental Wide Association Study (EWAS) analysis of EXHES II are presented in Deliverable D17.3

## 4. Conclusions

The HEALS project has succeeded in implementing with EXHES a substantive pan-European cohort of mother-child pairs, sometimes even a cohort of father-mother child triplets. This cohort includes twins and even triplets.

Overall, 4,888 families have joined the EXHES mother-child birth cohort by June 2019. As a whole, 5,169 children constitutes the EXHES population, which comprises 836 twins and 21 triplets. In total, the EXHES biobank included 13,988 different biospecimens collected from the newborns, plus 7,185 and 870 biospecimens collected from their mothers and fathers, respectively

The data collected in EXHES together with the environmental data available in EDMS will allow the application of the HEALS paradigm to contribute to the understanding of major chronic diseases. The obtained results will be used in risk assessment.