SIXTH FRAMEWORK PROGRAMME
PRIORITY 5 FOOD QUALITY AND SAFETY
FOOD-CT-016320-2
Instrument: Integrated Project
Thematic Priority Food quality and safety

NewGeneris
www.newgeneris.org

Publishable final activity report and Plan for using and disseminating the knowledge

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Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

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Section 1 | Project execution

1.1 NewGeneris project objectives

NewGeneris is an FP6 Integrated Project with 25 partners across Europe, and it is devoted to studying children's health in relation with food and the environment. The Project is coordinated by Maastricht University. The Executive Director, Prof. Jos CS Kleinjans, is supported by the Project Secretariat which includes a project manager, a financial officer and a project secretary.

The main goal of NewGeneris is to investigate the role of early life exposure to toxicants present in food and the environment, in the causation of subsequent health effects. In this context, the project studies maternal exposure during pregnancy to selected carcinogenic and immunotoxic chemicals and evaluates the resulting fetal (in utero) exposure and its effects on the fetus and in later childhood, particularly in relation with childhood cancer and immune disorders.The hypothetical role of relevant exposures of the fathers is also evaluated.

The main research tool employed by NewGeneris is *biomarkers*, i.e. chemicals or cellular components measured in human fluids or tissues, indicative of exposure to toxic chemicals or of their early biological effects. In the context of the project, such biomarkers are measured in biological samples (venous blood from the mothers, venous blood and semen from a limited number of fathers, and umbilical cord blood from the newborns) available in mother-child birth cohorts/biobanks in Norway, Denmark, United Kingdom, Spain and Greece (figure 1). In addition, childhood leukemia (ALL) cases will be recruited from the internationally unique Berlin-Frankfurt-Münster study in Germany, and evaluated for relevant genetic polymorphisms. The combination of these cohorts comprises a total of around 250,000 mother-child pairs, thus enabling a substantial *molecular epidemiology* study.

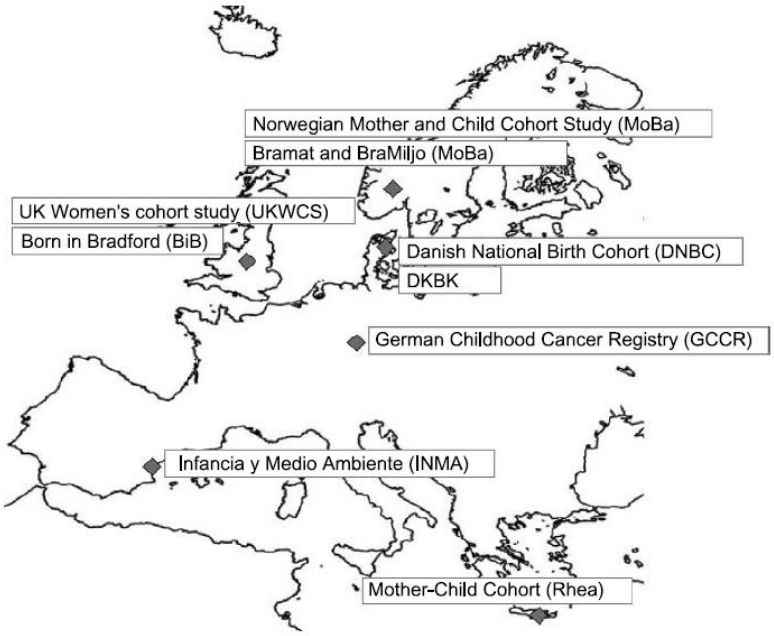

Figure 1. European mother-child cohorts contributing to NewGeneris.

NewGeneris address the following specific research objectives: (1) Dietary exposure of pregnant women to dietary carcinogens and immunotoxins are assessed using available questionnaires from existing mother/child birth cohorts; (2) Epidemiological surveys of mother/child birth cohorts are used to study

associations between maternal dietary exposure and childhood cancer risk factors and immune disorders; (3) Paternal exposure to dietary toxins is considered as an additional genetic risk; (4) An *in vitro* model of transplacental perfusion is used to better understand *in utero* exposure to selected toxins; (5) In cord blood samples from existing cohorts and newly initiated biobanks biomarkers are used to assess fetal exposure to these compounds and compared with maternal exposure; (6) In the same samples, genetic pathways indicative of risks of cancer and immune disorders in later childhood are studied by analysis of lymphocytic gene expression and proteomics profiles; (7) Inter-individual variability in responses are evaluated by genotyping of infants' DNA, and by phenotyping for DNA-repair activities; (8) Overall public health implications as well as ethical issues, will be addressed; (9) Results will be disseminated to EU food industry, advisors, regulators, and consumer organisations. Training and educational activities are carried out.

This project thus explores the currently undefined distribution of dietary genotoxin exposure in the unborn child. It increases our scientific understanding of whether and to what extent parental exposure to dietary genotoxicants and immunotoxicants induces molecular events in the fetus which may induce cancer and immune disorders in later childhood. This knowledge may be applied, by regulators and food industry, to improve children's health, in particular with regard to prevention of childhood cancer and immune disorders, by developing policy measures for reducing such dietary exposures.

The following interactions between the RTD lines are pivotal for the progress and integration of the investigations:

- In the first place, during the preparatory phase of this project, the NewGeneris partners have reached consensus on a shortlist of the most relevant dietary genotoxins and immunotoxins. This list comprises model compounds representing the most relevant sources of toxic contaminations hampering food safety, as well as optimally representing the chosen classes of toxicity, namely carcinogenesis and immunotoxicity. It also includes agents for which epidemiological or toxicological data raise concerns of possible involvement in the causation of human disease;
- In the preparatory phase of NewGeneris, it has been calculated that the volume of blood required for all biomarker analyses indicated in WP's 5-11, is too much to be possibly delivered by a single mother (and a single newborn, for that matter). This implies that blood samples from multiple children are required for analyzing the full range of foreseen biomarkers of exposure, effect and susceptibility. The availability of large population samples with well characterized exposures from the birth cohorts allows the identification of subgroups with enough subjects to allow the completion of all analyses. Biomarkers to be analyzed in specific sub-cohorts will therefore be selected on the basis of available exposure information. All data will subsequently be centralized for integrative statistical analysis (WP12);
- So, in the second place, given the limitations on the amount of biological material available from humans, especially newborns, obtained samples are of high value, and have to be taken, transported and stored with great care in order to allow for reliable biomarker analyses. Biological specimens are sampled, stored within particular Workpackages, and subsequently transported for investigation under other Workpackages. To guarantee scientific quality, white blood cells must be isolated, frozen and stored under exact conditions. For this, standard protocols on sample handling have been set up and validated, and are consistently applied by the relevant Workpackages. Although features differ, the same holds true for data handling. Therefore, also with respect to data sampling, warehousing, and, subsequently centralization of data suitable for statistical analyses, standard research protocols has been devised, in particular within WP12, and applied within other WP's. This requires effective internal communication between WP leaders and partners which is concisely supervised by NewGeneris management under WP17.

All the work has been done on these model compounds:

Chemical class	Model compound	Source	Class of toxicity
polycyclic aromatic hydrocarbons	- benzo(a)pyrene (BaP)	- environmental contamination of the food chain - formation during baking and frying - smoking and exposure to environmental tobacco smoke (ETS)	- genotoxic carcinogenesis - immunotoxicity
heterocyclic amines	- 2-amino-3-methylimidazo [4,5-f] quinoline (IQ) - 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)	- formation during baking and broiling	- genotoxic carcinogenesis
acrylamides	- monoacrylamide	- formation during baking and frying	- genotoxic carcinogenesis
nitrosamines	- dimethylnitrosamine (NDMA)	- environmental nitrate contamination of the food chain and subsequent endogenous formation	- genotoxic carcinogenesis
mycotoxins	- Aflatoxin - Deoxynivalenol	- environmental contamination of the food chain	- genotoxic carcinogenesis - immunotoxicity
organochlorins	- dioxin (TCDD) - PCB	- environmental contamination of the food chain	- cocarcinogenesis - immunotoxicity - endocrine disruption
DNA reactive aldehydes	- 4-hydroxynonenal - malondialdehyde	- macronutrients	- genotoxicity upon lipid peroxidation - immunotoxicity
alcohols	- ethanol	- life style factor	- cocarcinogenesis - immunotoxicity

NewGeneris' 5th Annual Meeting was held on January 17-18 2011 at Maastricht, the Netherlands and was attended by more than 80 collaborating senior and junior scientists coming from the 25 institutions which participate in the project Consortium.

It appeared that, all in all, NewGeneris has had a productive fifth year. It was concluded that substantial progress has been registered at all fronts. Predominantly, where the third year set out to analyze an already substantial number of samples, it could be demonstrated that biomarkers of exposure and effect did actually respond among newborns in relation to maternal exposure. This is to be considered a key milestone of the NewGeneris project which gives confidence that the project will actually be able to realize its ultimate goal, namely assessing cancer and immunotoxic risks among children as function of exposure to dietary carcinogens and immunotoxins of their mothers during pregnancy. Analytical Workpackages have developed thorough statistical analyses for which a dedicated plan is being set in place. It has been decided that in case of time and/or budgetary constraints, analyzing children should be prioritized above analyzing their mothers.

1.2 Contractors involved

NewGeneris was a large Integrated Project on children's health in relation with food and the environment, teaming up 25 contractors, academic and SME's, and more than 100 scientists in 16 countries during its 66 months duration.

List of contractors

Maastricht University, Netherlands
National Hellenic Research Foundation, Greece
Municipal Institute of Medical Research, Spain
University of Bradford, UK
University of Copenhagen, Denmark
University of Leicester, UK
Karolinska Institute, Sweden
Free University of Brussels, Belgium
German Cancer Institute, Germany
University of Oslo, Norway
National Institute for Cancer Research, Italy
University of Leeds, UK
Catholic University of Leuven, Belgium
Norwegian Institute of Public Health, Norway
Statens Serum Institute, Denmark
University of Crete, Greece
University of Eastern, Finland
Stockholm University, Sweden
Institute for Medical Research and Occupational Health, Croatia
Universitat Autonoma de Barcelona, Spain
Medical University Bratislava, Slovakia
BioDetection Systems, the Netherlands
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1.3 Work performed and approach

Workpackage 1

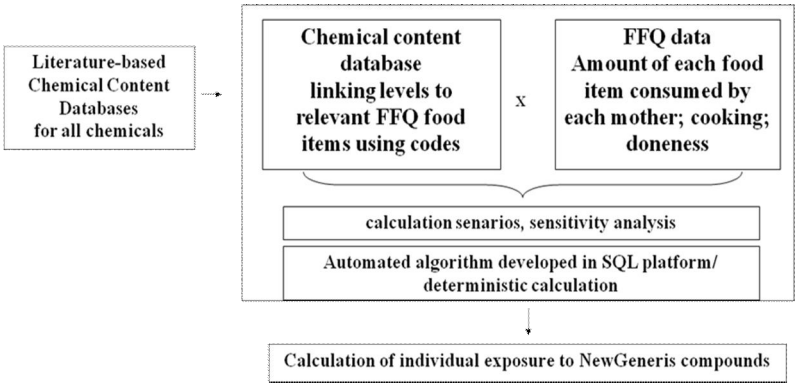
WP1 developed state-of-the art methodology for the assessment, using FFQ data, of dietary exposures to the chemicals of interest, as well to different foods of relevance to the project. The methodological approach entailed, for each exposure and for each cohort, a) identifying the main dietary sources of exposure, b) constructing Tables with the contaminant levels in different foods and c) constructing Tables with portion sizes for each food. In addition, after evaluating the coverage of the exposures of NewGeneris interest by the existing FFQs of two new NewGeneris sub-cohorts (MoBa and BiB), short supplementary questionnaires were prepared for their use in the project.




A Chemical Content Database for all food items in cohort FFQs and their corresponding content for NewGeneris chemicals of interest has been constructed. Construction of the Database took into account all available literature on food chemical content at a European or country specific level, and the variations between the different cohort FFQs. This Database, along with the cohort-specific food portion size databases, and the maternal food frequency consumption data, was eventually used to calculate the levels of the maternal chemical exposures based on chemical-specific codes and algorithms, formulated so as to provide harmonized exposure estimates comparable at a European level. To ensure that the database was fully up-to-date prior to its application in the project, WP1 organized a dedicated Revision meeting to re-evaluate all data for all chemicals in accordance with the current scientific publications and to ensure that all FFQ-related issues that arose during the construction of the database were dealt with in a harmonized manner.

The construction of a Database including all data for subjects of 5 European countries, food consumptions and chemicals (as Acrylamide, NDMA, nitrates, nitrites, ENOCs, PAHs, reactive aldehydes related fatty acids, Heterocycle Amines, DON) present in foods, is a major scientific achievement of WP1. This Database permits the calculation of individual exposure to NewGeneris compounds. In addition the introduction in this Database of biomarkers data from other WPs permits the computation of correlations of food chemical intakes with their relevant biomarkers (e.g. DNA adducts).

The performance of the exposure assessment algorithms was repeatedly evaluated and successive improvements made before it was finally employed for the final exposure assessment activities. In the context of these activities, the dietary intake of all project subjects to the chemicals of interest was calculated, and statistics of the exposure distributions, by cohort, produced, along with estimates of the contributions of different food types to each exposure, and its variation by cohort.



In this way important data on the regional variation of the dietary exposures of pregnant women in Europe to the toxic chemicals of interest and their main dietary determinants, as well as information on the dietary habits of pregnant women in different European regions, were produced. An example of the information thus generated is shown below for acrylamide together with the extended pilot study relevant to correlations of exposure with the relevant biomarkers (hemoglobin adducts), under the title of "*Acrylamide Study*".

In combination with other data (biomarkers) generated by the NewGeneris project, this work permits a detailed evaluation of the influence of dietary habits on exposure to carcinogenic and immunotoxic chemicals and associated biomarkers.

A biomarker-based pilot validation study of FFQ-based exposure assessment was conducted. The purpose of this study was to evaluate the correlation between FFQ-estimated dietary intakes and measured biomarkers of exposure in a very small sub-set of subjects mother/child pairs for:

- Acrylamide intake vs. hemoglobin-acrylamide and -glycidamide adducts: 31 matched pairs from the DK biobanks;
- PAH (B[a]P) intake vs bulky DNA adducts: 69 matched pairs from the DK biobanks;
- NOCs, ENOCs & related precursors vs O6-meG adducts: 23 matched pairs plus an additional 49 children from the Rhea cohort;
- Lipid-derived aldehydes (lipid intake) vs. malonedialdehyde adducts: 32 matched pairs from the BiB cohort;
- As this study was based on very limited information a further study was decided and is ongoing in collaboration (M. Botsivali) with WP12 statistical analysis task and the relevant in process workshops.

The results of the study using acrylamide as a model compound, conducted by WP1 (M. Botsivali) and using the full set of data are shown below:

ACRYLAMIDE STUDY

Food groups utilised for calculating acrylamide exposures

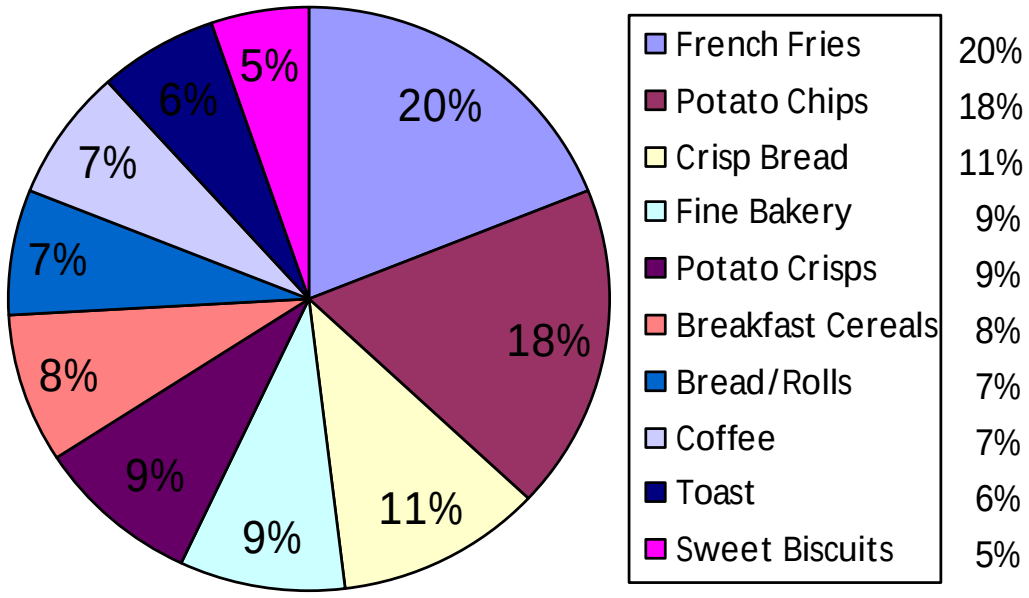
- Potato chips
- Potato crisps
- French fries
- Crisp bread
- Breakfast cereals
- Bread / rolls
- Fine bakery products
- Sweet biscuits
- Toast
- Coffee

These food items are included in the European acrylamide database (ISPRA), with their acrylamide content, as well as in European Commission Recommendations. Content levels assigned were taken from the above sources as well as from the literature. Food groups were assigned with codes which were linked with each relevant FFQ item. NewGeneris FFQs on average cover 200 food items in total, coming from the cohorts.

FFQ-calculated acrylamide intakes by cohort (μg/day)

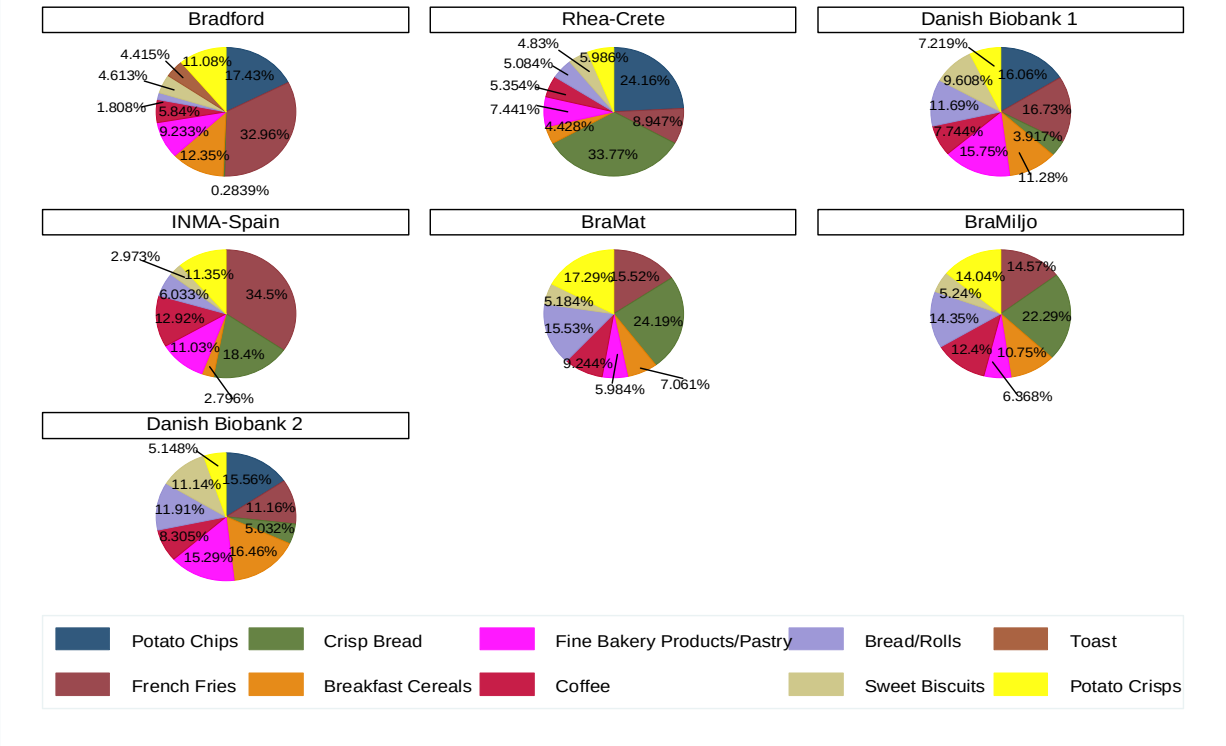
Cohort	N	mean	min	max	
All cohorts	954	21.61	0	205.56	
BiB-Bradford	149	36.39	2.83	205.56	significant variation among the cohorts (p=0.0001)
Rhea-Crete	151	16.55	3.12	115.87	
Danish Biobank 1	83	24.94	0	86.14	
Danish iobank 2	140	22.03	0	57.92	
INMA-Spain	185	17.84	0	134.86	
BraMat-Norway	135	17.58	1.98	44.27	
BraMiljo-Norway	111	16.81	3.03	37.63	

Contribution of specific food groups to acrylamide intake (pooled cohorts)



47% of acrylamide intake is due to potato products, mainly fried potatoes (French fries and potato chips) and crisps.

Contribution of specific food groups to acrylamide intake (by cohort)



Maternal ffq-estimated exposure and hemoglobin adducts

Statistical analysis for 155 mothers (with maternal/cord adduct data), including 23 smokers and 132 non-smokers

	Only food-exposed (N=132)			
	Mean	SD	min	max
- *Acrylamide exposure (µg/day)*	21.40	14.4	0	115.8
- *Acrylamide adducts (pmol/g globin)* 33.29	19.0	10.6	176.2	
- *Glycidamide adducts (pmol/g globin)*	29.46	14.9	8.8	110.2

	Smokers (N=23)			
	Mean	SD	min	max
- *Acrylamide exposure (µg/day)*	18.02	17.1	0	70.60
- *Acrylamide adducts (pmol/g globin)* 83.45	66.1	29.9	316.8	
- *Glycidamide adducts (pmol/g globin)*	94.19	80.7	29.9	376.1

Smokers have more adducts ($p<0.001$) even though their dietary intake is slightly lower

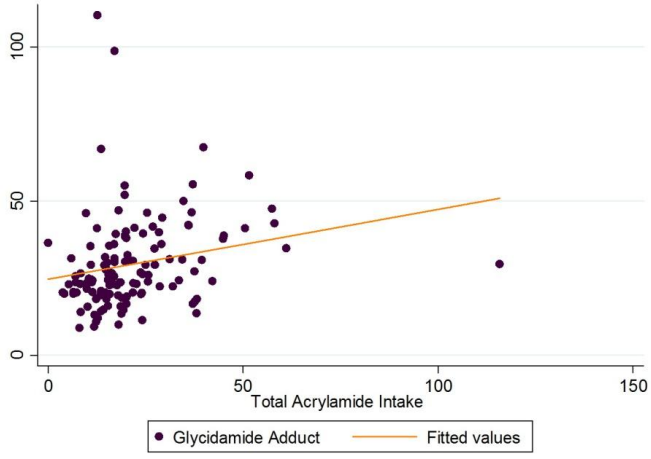
	Children of only food exposed mothers N=132			
	Mean	SD	Min	Max
- *Children acrylamide adducts*	15.72	9.3	6	83.8
- *Children glycidamide adducts*	11.38	6.6	3.6	65.4

	Children of smoking mothers N=23			
	Mean	SD	Min	Max
- *Children acrylamide adducts*	37.33	35.54	13.4	83.8
- *Children glycidamiide adducts*	30.15	27.63	8.4	115.8

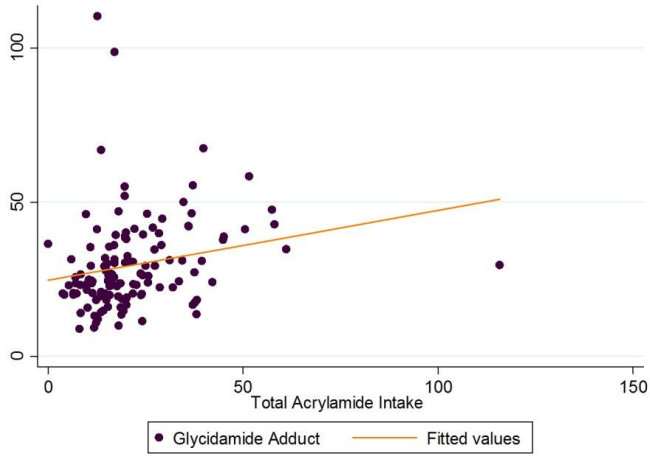
Children of smoking others have more adducts

Correlations between dietary acrylamide intake and maternal adducts in non-smokers

Acrylamide adducts Rho = 0.21; p=0.02



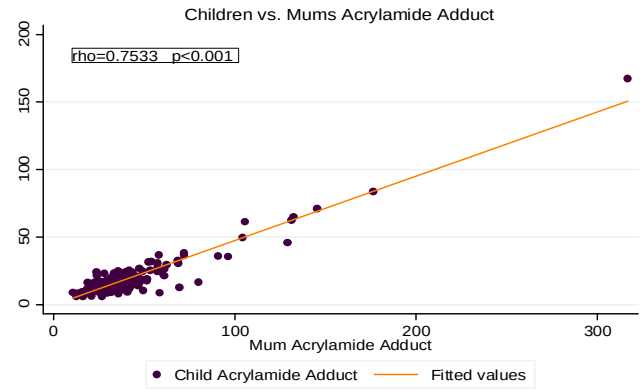
Glycidamide adducts Rho = 0.33; p=0.001



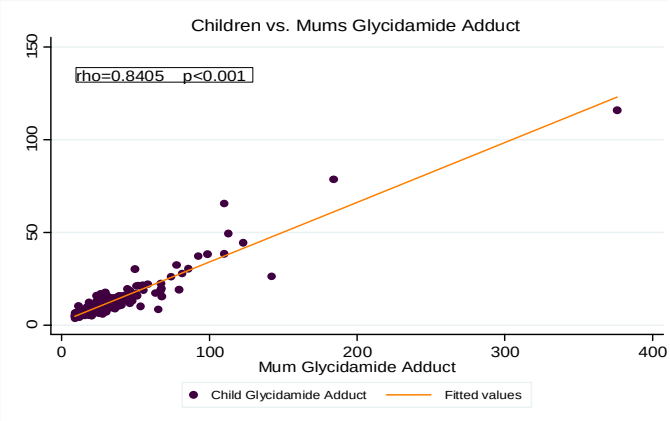
After adjusting for BMI, age, cohort and ETS, no relationship between intake and acrylamide adducts (Spearman rho=0.15; p=0.12), but significant relationship for glycidamide adducts (rho=0.56, p<0.001).

High Correlations between maternal and childrens' adducts in non-smokers

Acrylamide adducts Rho = 0.75; p<0.0001



Glycidamide adducts Rho = 0.84; p<0.001



Maternal ffq-estimated exposure and hemoglobin adducts in children

Although the link between maternal exposure and adduct levels in children is indirect, in view of the close correlation between maternal and childrens' adducts it is of interest to explore the relationship of maternal exposure with children's adducts in a larger set of subjects 913 children for which data on adduct levels and maternal FFQ-based acrylamide intake is available.

	Non-smoking (only food-exposed) mothers (N=808)			
	Mean	SD	min	max
- *Acrylamide exposure (µg/day)*	20.12	13.89	0	134.85
- *Children Acryl adducts (pmol/g globin)*	16.35	10.98	2.9	72.1
- *Children Glyc adducts (pmol/g globin)*	11.59	6.70	0	376.1

	Smoking mothers (N=105)			
	Mean	SD	min	max
- *Acrylamide exposure (µg/day)*	23.22	21.01	0	99.19
- *Children Acryl adducts (pmol/g globin)*	38.70	31.03	4.4	167.5
- *Children Glyc adducts (pmol/g globin)*	26.12	19.52	2	115.8

Overall conclusions

- Estimated acrylamide intake of NewGeneris pregnant women is lower than the mean world for the general population (only 3% are higher);
- Significant geographic variation is observed, with Bradford showing the highest exposure and Norway, Spain and Crete the lowest;
- The main contributors to dietary acrylamide exposure are potato products (french fries, potato chips and crisps) which account for roughly 50% of the exposure;
- The contribution of different sources varies regionally, with the main contributors in Bradford and Spain being french fries, in Crete and Norway crisp bread and in Denmark french fries and breakfast cereals;
- The high correlation between maternal and cord blood hemoglobin adducts, and between maternal dietary acrylamide intake and cord blood adducts indicate that higher maternal intake results in higher fetal exposure;
- The variation of fetal exposure as reflected in the levels of acrylamide adducts in cord blood, is mainly due to the variation in maternal consumption;
- More acrylamide-related adducts are found in maternal and cord blood of smoking women, indicating increased fetal exposure to acrylamide as a result of smoking.

Based on the outcome of analysis to date of NewGeneris data, reduction of maternal dietary acrylamide exposure through appropriate changes in dietary habits and avoidance of smoking results in protection of the fetus from acrylamide exposure.

Workpackage 2

The achievements of WP2 are considerable. NewGeneris is a truly multidisciplinary project and this required an impressive amount of effort between epidemiologists' participants in WP2 and researchers from basic sciences in other WPs, in order to develop a common language and common practices. This was achieved mainly due to very close contacts between WPs and the development of a jointly prepared detailed protocol. The major efforts from WP2 were focused during the first part of the project in enrolling the pregnant mothers in the project and securing adequate filed study procedures that would results in high response rates, collection of questionnaire data including Food Frequency Questionnaires (FFQ) and biological samples. For this, some cohorts included in NewGeneris had already established protocols for enrolment and questionnaire data e.g. MOBA, INMA, while other started from scratch. Even for the cohorts with already developed protocols, these were reviewed and modifications were implemented to secure the collection of questionnaire information and biological samples in the same way. A major achievement of the NewGeneris project has been the launching of new projects e.g. RHEA mother-child cohort in Crete, and through this the establishment of dynamic new groups working in molecular epidemiology in these centres. This should be considered a major achievement given that it happened only through the availability of funds and provision of know-how to centres with little previous experience. The implementation of extremely demanding protocols was a he success of NewGeneris that has led to the availability of a unique database that at present is exploited in NewGeneris but that has also given rise to additional analyses connected to other EU funded projects such as HiWate, EnviroGenoMarkers, Enrieco, Chicos and ESCAPE.

The implementation of the NewGeneris project required a very close contact between WPs and particularly between participants in WP2 (Norway, Denmark, Greece, UK and Spain). This was achieved through regular contacts via mail or telephone between participants, through the establishment of informal working groups for the development of specific protocols and through meetings at the annual meetings of NewGeneris.

The analyses of reproductive outcomes in relation to the NewGeneris biomarkers have provided, until now, among the most interesting and potentially important for public health findings from been the main scientific achievement of year 5. A complete account of the analysis on acrylamide haemoglobin adducts and glycidamide haemoglobin adducts and birth outcomes were presented that the final NewGeneris meeting in Maastricht in January 2011. A strong association was found between increased acrylamide (figure 1) and glycidamide adducts and reduced birth weight of the child. This effect was also observed in non-smokers and the magnitude of the effect is equivalent to a reduction of weight observed among smokers. The effect was observed in all five countries (figure 2) although it was less prominent in the UK. Subsample analysis by ethnicity indicated that the effect could be observed among white British mothers but was not as evident in Asian mothers that constitute approximately 50% of the population of the UK cohort. Association with reduced birth weight were also observed for bulky DNA adducts, for ethylene oxide haemoglobin adducts primarily among smokers and for a subset of mother-child pairs with Dr-Calux analyses for whom adequate quantity of lipids were available for the analysis. No association with birth weight was found for other biomarkers examined in NewGeneris. These results are innovative and, in some cases, corroborate findings in experimental animals. They are also very important for public health because risk factors for significant reductions in birth weight have rarely been identified. These results provide very solid evidence based both on questionnaires and biomarkers indicating that aspects of the diet of pregnant women are associated with significant and clinically important reductions in birth weight. In connection with analyses conducted in WP1 of NewGeneris that evaluates uptake from food of the specific contaminants, they lead to clear public health messages on diet during pregnancy.

Figure 1: Adjusted mean birth weight (g) and 95% confidence intervals per HbAA adduct quartiles in cord blood - infants of non-smokers

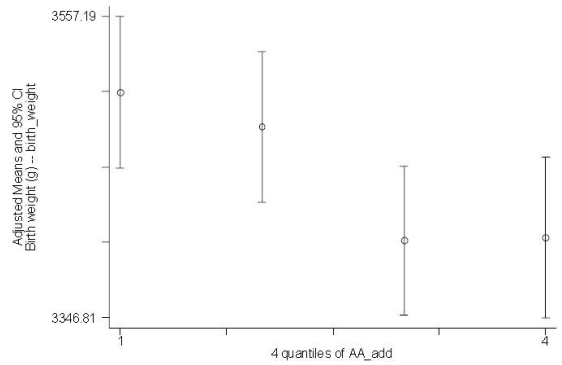
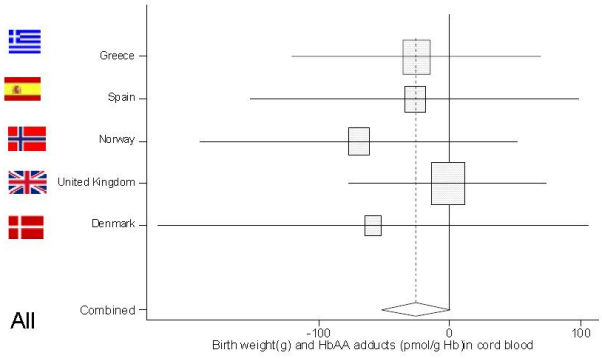


Figure 2: Birth weight (g) and haemoglobin acrylamide adducts (HbAA) by Δ10 pmol/g Hb increases. Meta-analysis by country - infants of non-smokers



Workpackage 3

Work performed at University of Bradford:

- Quantification of DNA damage with the currently most sensitive, semi-automated laser scanning confocal microscopy of γH2AX foci gave analogous values compared to the traditional Comet assay.
- γH2AX, a common and reproducible biomarker in somatic cells was successfully employed as a novel biomarker on spermatozoa samples. In combination with terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) and immunostaining for Annexin V, γH2AX was simultaneously detected in spermatozoa. This novel assay is able to detect frequencies as well as correlations of DNA double stranded breaks (γH2AX), fragmented DNA (TUNEL) and apoptosis (AnnexinV V) simultaneously. However, γH2AX cannot be used as a biomarker for in vitro exposures (to toxicants) of semen samples whereas the Comet assay can. That is, because DNA damage response is apparently not inducible in spermatozoa and may therefore represent a residual of DNA damage response which occurred earlier during spermiogenesis or spermatogenesis). However, γH2AX was able to identify environmental effectors (i.e. tobacco smoke) in cohort samples.
- Protocols for quantifying DNA strand breaks on the baseline levels and after in vitro exposure to the twelve NewGeneris test compounds on spermatozoa and lymphocytes were established using the Comet assay.
- Detection of DNA-adducts via immunostaining on spermatozoa was established.

Work performed at Norwegian Institute of Public Health:

- Protocols were established for analysis of sperm samples (baseline, and after *in vitro* exposure to chemicals) using the comet assay, measuring both DNA strand breaks and oxidised purine bases;

- Flow cytometric analysis revealed associations with Comet assay DNA lesions, in sperm from fertile men;
- Sperm integrity appears to be resilient to chemical exposure and ionizing radiation in vitro, probably reflecting high packaging of protamine-associated chromatin, and an absence from DNA of water molecules;
- The established Comet assay protocols are capable of discriminating between individuals' sperm chromatin integrity, even in a small cohort of fertile men. This may reflect individual differences in exposure to environmental xenobiotics.

Work performed at Maastricht University:
- BPDE adducts (^{32}P postlabelling) were induced *in vitro* at very high levels, but this was associated with only minor increases in BDPE-induced DNA lesions as assessed by the comet assay;
- Gene-expression profiles on spermatozoa samples could be established using Agilent microarray or qRT-PCR platforms, showing its feasibility to elucidate gene-environment interactions in the human germ cells. Hence, spermatozoa may represent an easily accessible tissue, in contrast to the testis, for large biomonotoring studies;
- Minisatellite data was generated in 81 mother-father-newborn triads showing a greater mutation frequency in the male and possible association between mutation frequency and the intake of caffeine and exposure to cigarette smoke;
- A methodology to detect Vitamin C (a free radical scavenger) radicals in spermatozoa was developed.

Workpackage 4

The human placental perfusion system has been used supplemented with BeWo cell cultures and including studies with inhibition of transport. The systems are used by other groups with comparable set-up. Included is the list of substances studied and the corresponding publications.

STUDIES	Publications	Perfusion facility	Concentrations tested (uM)	Number of perfusions	FM ratio (2½hr)
NDMA	Annola et al. 2009	Kuopio, FIN	1 and 5	12	0.98±0.051
NDMA	NO (Annola et al. 2009)	DK	1.0	3	0.71±0.26
DON	Nielsen et al. (in press)	DK	0.34	5	0.54±0.086
Ethanol Bisphenol A	Moerck et al. 2010	DK	2‰ 0.5	5 9	1.03±0.074 1.00±0.223
Ethanol	Veid, Karttunen et al. (submitted 2011)	Kuopio, FIN	0.5 and 2‰	25	0.98±0.06 (3h)
B(a)P	Mathiesen et al. 2009 (Myllynen et al. 2010)	DK	0.1 and 1.0	15	0.6±0.15
B(a)P	Karttunen et al. 2010 (Myllynen et al. 2010)	Kuopio, FIN	0.1 and 1.0	8	0.22±0.1
IQ	Immonen et al. 2010 (Myllynen et al. 2010)	Oulu, FIN	0.5	16	0.95±0.19
IQ	Immonen et al. 2010 (Myllynen et al. 2010)	DK	0.5 and 1.0	7	0.77±0.06
PhIP	Myllynen et al. 2008 (Myllynen et al. 2010)	Oulu, FIN	2	16	0.50± 0.11
PhIP	Myllynen et al. 2010 (Myllynen et al. 2010)	DK	0.2 and 2.0	6	0.6
TCDD	Pedersen et al. 2010	DK	6.0pg/mL	5	No transfer
PCB52 PCB180	Carreira et al. 2011	DK	1.5	4 5	0.23±0.07 0.70±0.17
Acrylamide Glycidamide	Annola et al. 2008	Kuopio, FIN	70.3 (n=4) and 140.7 (n=9) 57.4 (n=4)	17	1.060.87
Aflatoxin B1	Partunen et al. 2009	Kuopio, FIN	0.5 and 5	8	0.58

Major results WP4:

1. Setting up common logistics for obtaining placental tissue, cord blood, parental blood and exposure information from study families; Performed and published - pilotproject DK Biobank 1;
2. Establish a validated ex vivo/in vitro system for study of transport of selected genotoxicants and immunotoxic substances across the placental barrier; Performed and published (PAH, PhIP, IQ and antipyrine);
3. Study placental transport of 4 substances from the list in B4, using placental perfusion and compare data with exposure and effect data in other in vitro system; Performed and published (Exposure: PAH, In vitro with BeWo cells: BPA, DON, PCBs);
4. Provide tissue for other WPs for studies of exposure and effects in cord blood and tissue from placenta as well as parental tissues; Performed and published DK Biobank1+2;
5. Study further compounds with relevance to NEWGENERIS summing up to totally 10 compounds, however depending on the analytical facilities. Performed and published showing fetal exposure of the studied compounds (Acrylamide, glycidamide, Aflatoxin B1, Agflatoxicol, benzo(a)pyrene, NDMA, PhIP, IQ, DON, ethanol, Bisphenol A, TCDD, PCB52 & 180) and including some mechanistic data (PhIP and IQ).

Conclusions

Fetal exposure demonstrated within the NewGeneris:

1. From Maternal/Umbillical cord blood measurements *in vivo*
   - Benzo(a)pyrene (DNA adducts)
   - Acrylamide Hb-adducts
   - Calux
2. From human placental transport studies *ex vivo*
   - All studied except TCDD, which could not be detected probably due to low sensitivity of Calux assay
3. From placental transport model studies in vitro with BeWo cells
   - Bisphenol A
   - PCBs
   - DON

The placenta barrier is not protective towards exposures to the studied compounds.

<u>Workpackage 5 and 6</u>

WP6 started off with establishment of an SOP for DNA extraction from buffy coats. After a lot of discussions it was decided to use a commercial kit-based method with modifications elaborated and tested within the WP for DNA isolation, rather than the classical phenol/chloroform extraction procedure and a training session with all involved WP6 partners took place in Heidelberg. The task of isolating DNA from almost 1000 cord and about 400 maternal blood samples and the distribution of DNA samples among the partners within WP6 for biomarker analyses and to partner 9 for GWAS analysis demanded a lot of time and resources from the partners involved, 6, 7 and 19 (later 26) and put a particularly heavy burden on partner 2. It would have been preferred, mainly for logistic reasons, that the DNA extraction would have been carried out by one partner or by a commercial enterprise, but since this job was not a specific task (and had no separate budget) it was decided to divide up the work among the involved WP6 partners.

Three of the biomarkers applied in WP6 were the hemoglobin adducts of acrylamide, its metabolite glycidamide and of ethylene oxide. All analyses were carried out by partner 18 using liquid chromatography combined with tandem mass spectrometry. The two former adducts originate from dietary intake of acrylamide formed from sugars and certain amino acids during heating of food. For the levels of these adducts differences related to country as well as ethnicity were noted, probably reflecting

differences in dietary habits and in food preparation. The ethylene oxide adduct analysed reflects primarily endogenous exposure to ethylene, an end product in the lipid peroxidation pathway. Both acrylamide and ethylene are present in tobacco smoke and in smokers this source is more important than dietary or endogenous production. In addition there was, for all three adducts, a strong correlation between levels in the cord blood and in the maternal blood. Furthermore, if taking differences in reactivity between foetal and adult blood into account, the dose (concentration over time) of the alkylating agents were about the same in cord and maternal blood. These data indicate that the metabolism of acrylamide and ethylene to the reactive intermediates glycidamide and ethylene oxide is taking place mainly in the mother and easily transported to the placenta, which is supported by the observations made with placental perfusion in WP4. Furthermore, the data indicate that the Hb adduct levels measured in cord blood reflect the exposure during the whole third trimester of pregnancy.

Three other exposures towards the analyses were directed were dioxin and dioxin-like chemicals, estrogens and androgens. All of those biomarkers were analysed by partner 23 using dioxin receptor mediated assays (called DR-, ER-, and AR-CALUX assays, respectively). Clear differences between countries were observed for exposures to dioxins and the reason for that is currently being analysed. Furthermore, it was noted for DR-CALUX that maternal blood had higher levels than the cord blood and there was no correlation between cord and maternal. Possible reasons for these findings are currently being investigated by other NG workpackages.

The isolated DNA samples were used for analyses of bulky DNA adducts including polycyclic aromatic hydrocarbons by using the ^{32}P-postlabelling method (partner 1, 7 and 19 (later 26)) and more specifically, polycyclic aromatic hydrocarbon-DNA adducts by a newly developed immunoassay with antibody elicited against the most common adduct of benzo(a)pyrene dihydrodiol epoxide (partner 2). For the samples analysed with the postlabelling assay there were clear differences between countries and with a general trend towards higher adduct levels in the countries of southern Europe (an observation also made in other studies). In addition, the adduct level in the cord and the maternal DNA was very similar and there was a statistically significant medium strong positive correlation between adduct levels in the cord and in the maternal DNA. For the immuno assay of PAH-like DNA adducts in cord DNA there were also differences between countries, adduct levels of the mother and child were very similar and there was a good correlation between adduct levels in the cord and in the maternal DNA.

The DNA samples were also used to analyse the DNA adduct called M_1dG. This adduct has two sources of origin, it is formed when malondialdehyde (a major end product in lipid peroxidation) interacts with DNA, but it could also be formed from reactive oxygen species, i.e. when these radicals react with deoxyribose of DNA a compound called base propenal is formed which reacts with DNA, forming M_1dG. This adduct was analysed by partner 6 using an immuno slot blot assay. The data obtained showed that there were substantial differences between countries in adduct levels. Furthermore, the levels of the paired cord and maternal samples were very similar, except for the last collection of samples in Denmark and there was a correlation between levels in the cord and the maternal samples.

Finally, DNA was also used for analyses of O^6-methyldeoxyguanosine, a reaction product with DNA of methylating agents like dimethylnitrosamine. This DNA adduct was analysed by partner 2 using the a similar immunoassay as mentioned above for the benzo(a)pyrene-like DNA adducts but with a specific antibody. With this assay it was observed that the levels in different countries were similar as well as the levels in mother and child and the correlation between maternal and cord adduct levels was relatively weak, but significant.

For most biomarkers there were clear differences between countries in biomarker levels, indicating differences in exposures. Most biomarkers showed a correlation between cord and maternal samples and the levels in child and mother were similar. The data obtained in WP6 are currently being combined with other biomarker data and with food frequency and other type of questionnaire data, generated by other WPs, to an integrated statistical analysis.

Workpackage 7

1. Development of an automated image analysis system for the scoring of MN

An automated image analysis system for the scoring of MN has been developed. This image analysis for semi-automated lecture of MN has been validated by the VUB laboratory in collaboration with IMSTAR and has been used for the high throughput analysis of the MN slides from the different cohorts. In addition an optimized MN protocol for whole blood cultures from adult and umbilical cord blood for human biomonitoring was developed and established in the partner laboratories. Agreements were made on the processing and the analysis of the samples.

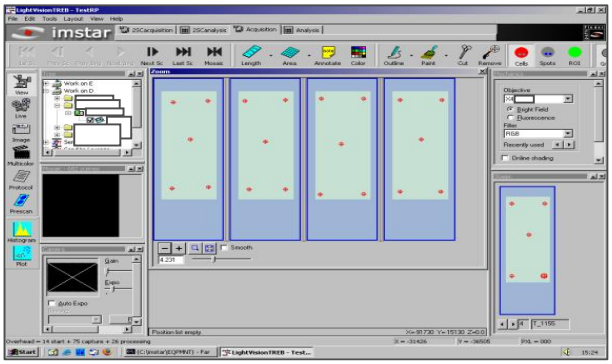
Comparison with other automated image analysis systems available on the market reveals that the main difference lies in the detection algorithms. We developed specific algorithms starting from the cell as a detection unit. The whole detection and scoring process was separated into two distinct steps: in the first step, the cells and nuclei are detected and then in the second step, the MN are searched in the detected cells. This approach allows to score MN in both mono- and binucleated cells and to obtain the CBPI from the analysed samples. Moreovern MN scoring occurs according to HUMN scoring criteria and is thoroughly validated with false positive and false negative rates as low as possible. This resulted in two publications:

- *Automated image analysis of cytokinesis-blocked micronuclei: an adapted protocol and a validated scoring procedure for biomonitoring* (2009). Ilse Decordier, Alexander Papine, Gina Plas, Sam Roesems, Kim Vande Loock, Jennifer Moreno-Palomo, Eduardo Cemeli, Diana Anderson, Aleksandra Fucic, Ricardo Marcos, Francoise Soussaline and Micheline Kirsch-Volders. *Mutagenesis*, 24: 85-93.
- *Automated image analysis of micronuclei by IMSTAR for biomonitoring* (2011). Decordier I, Papine A, Vande Loock K, Plas G, Soussaline F and Kirsch-Volders M. Mutagenesis 26(1): 163-168.

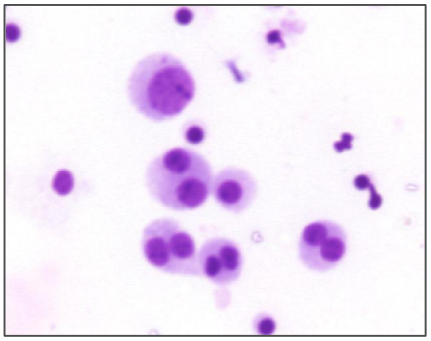
Photos illustrating the development of the automated MN image analysis system:

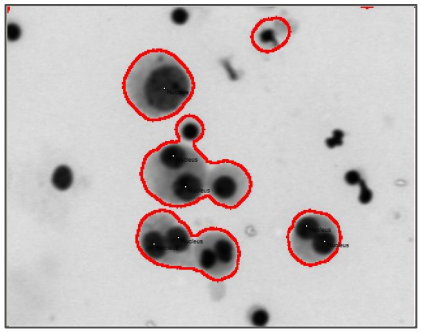
Choice of the slide zone

- Automatic focus (landmark-based);
- Optional slide feeder device.

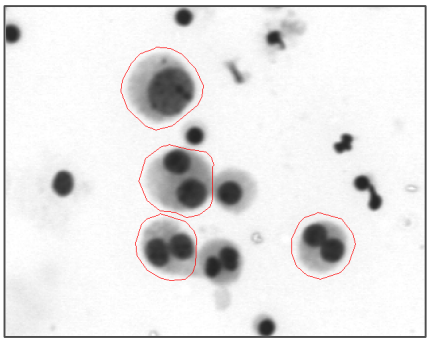


Modeling the cells and nuclei





Modeling the micronuclei



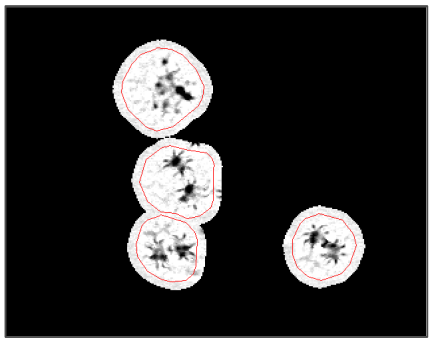
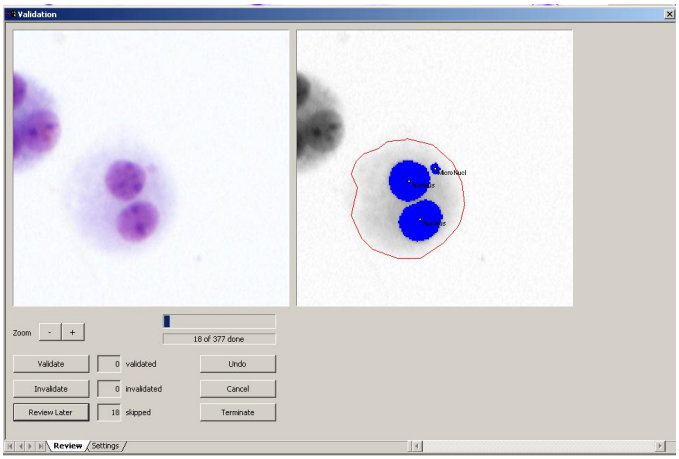
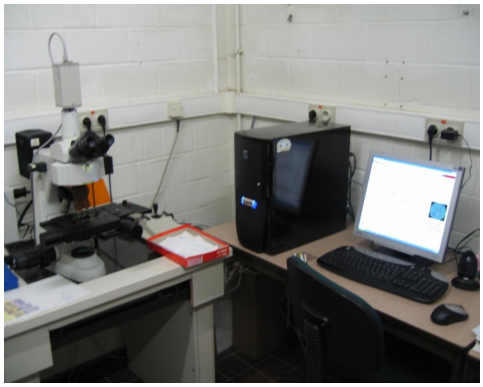
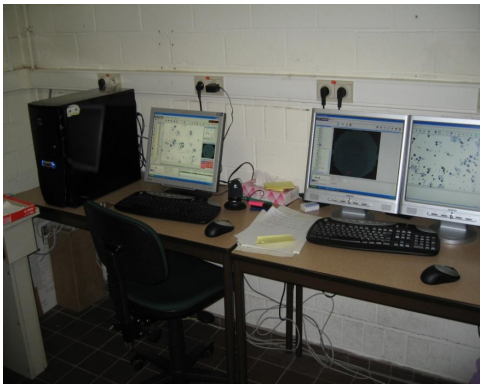


Photo of the automated MN image analysis system







2. Use of the automated MN scoring of the newborn and maternal samples from the different cohorts

The automated system was used to score the slides from the different cohorts. First the different partners involved in the biomonitoring studies of the selected cohorts were trained to prepare MN slides according to the adapted protocol at the VUB or at the individual labs. In total, data were obtained for 631 newborns.

To the best of our knowledge, this is the first large study (project) analyzing MN frequencies in peripheral and umbilical cord blood from mothers and newborns using a semi-automated image analysis system. Unique to this study is the evaluation of MNBN, MNMONO, CBPI in newborns. To analyse and compare in depth the genotoxic responses at the level of micronuclei formation of children of different countries with similar or different diet and environment, a thoroughly statistical analysis is needed including all the

different biomarkers assessed within NewGeneris. However, based on the largest data set obtained per cohort, i.e. the Rhea cohort in Crete a first analysis based on MN data and demographic data was performed and resulted in a publication in Environmental Health Perspectives.
This analysis included 251 newborns and 223 mothers, including 182 mother-child pairs. We hypothesized that gestational factors and delivery type would influence MN levels in newborns, in addition to maternal smoking and the child's age and gender. The manuscript was entitled: "Maternal and Gestational Factors and Micronuclei Frequencies in Umbilical Blood: the NewGeneris Rhea Cohort in Crete" by Vande Loock et al., and was accepted for publication in this journal: doi: 10.1289/ehp.1003246 (available at http://dx.doi.org/) Online 27 May 2011.
Based on the data obtained from the Rhea cohort, we can conclude that although confirmation in a larger study population is needed, multivariable analysis revealed the importance of taking into account gestational age when studying MN frequencies in newborns. In addition, our results indicate the importance of assessing both MNMONO and MNBN for biomonitoring of newborns, since the first reflects damage expressed during *in vivo* cell division and accumulated *in utero* and the latter includes additional damage coming into expression as MN during the *in vitro* culture step. Due to physiological differences and the age of circulating T-lymphocytes, it is not clear yet whether MN frequencies in newborns can be interpreted in the same way as in adults, whether they are predictive for cancer and childhood cancer in particular. The results obtained can be used for the general analysis and comparison of MN in newborns and their mothers from the different NewGeneris cohorts.

3. Characterization of the genotoxicity of a selected number of potential food carcinogens with the *in vitro* micronucleus (MN) cytokinesis-block assay

Within WP7 the compounds ethanol (EtOH), 4-hydroxynonenal (4-HNE), malondialdehyde (MDA), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 3,3',4,4'-tetrachlorobiphenyl (PCB 153), benzo[a]pyrene (BaP), 2-amino-3-methylimidazol[4,5-f]quinoline (IQ), 2-amino-1-methyl-6-phenylimidazol[4,5-b]pyridine (PhIP), N-Nitrosodimethylamine (NDMA) and acrylamide (AA) were evaluated in an interlaboratory comparison by UB and IMROH with the *in vitro* cytokinesis-block micronucleus assay (CBMN) with objective of assessing the induction of micronuclei, buds and nucleoplasmic bridges in dose responses. Statistically significant increase in MNBN frequency in binucleated cells was recorded by both laboratories for the compound PhIP (2.5µM). The compounds PCB (250 microM) and AA (500 microM) induced statistically significant increase of MNBN although it was recorded by one of the two laboratories. Induction of buds and nucleoplasmic bridges was only observed for BaP (100 microM) and AA (500 microM) by one of the laboratories. Data generated in this study may assist in the interpretation of the mother/newborn biomonitoring study being carried out within project NewGeneris and will contribute to overall knowledge on the genotoxic potential of dietary/ environmental toxicants. This resulted in a publication entitled: "Evaluation of the genotoxicity of 10 selected dietary/environmental compounds with the in vitro micronucleus cytokinesis-block assay in an interlaboratory comparison" by Katic et al., 2010, Food Chem Toxicol, 48, 2612-2623.

Workpackage 8 and 9

The following achievements were obtained: *In vitro* gene expression studies on human PBMCs for the 12 NewGeneris compounds, resulted in the identification of genes that are deregulated by specific compounds as well as groups of related compounds (genotoxic carcinogens, non-genotoxic carcinogens) and genes that correlate with micronuclei levels. The same in vitro studies also led to the dentification of gene profiles that are deregulated by groups of related compounds (immunotoxic carcinogens, non-immunotoxic carcinogens) and genes that correlate with immune functionality measured as suppression of proliferation and cytokine release.
Whole genome gene expression analyses on 120 samples from the BraMat subcohort were analyzed by numerous uni- and multivariate statistical tools to identify genes deregulated by combined exposure or by exposure to specific compounds. Genes have been identified that are indicative of genotoxic carcinogen exposure based on exposure calculations from the FFQ. Furthermore, genes were identified

that correlated with exposure data (e.g. Calux data and haemoglobin adducts) and effect data (micronuclei). Similar analyse led to the identification of genes whose expression is indicative of immunotoxic exposure, based on calculations from the FFQ data. Other parameters related to immunotoxic exposures or effects are not available. Eventually this resulted in the selection of 48 genes; a combination of genes responsive to genotoxic or immunotoxic exposure (calculated from the food frequency questionnaires), and genes associated with hemoglobin-adducts and micronuclei frequencies. Furthermore, these studies revealed different transcriptomic responses to environmental carcinogens between the sexes. While exposure levels did not differ significantly between sexes at birth, important gender-specific differences were observed in gene expressions associated with exposure (see Table 1).

Table 1. Numbers of genes correlating with CALUX, Hemoglobin adducts and micronuclei indicating gender-specific differences.

	# of genes	Pos. correlation	Neg. correlation	Gender	# of genes	Pos. correlation	Neg. correlation
DR-CALUX	3	0	3	Boys (n=14)	371	163	208
				Girls (n=29)	39	29	10
ERα-CALUX	20	14	6	Boys (n=17)	493	311	182
				Girls (n=17)	626	459	167
AR-CALUX	83	69	14	Boys (n=16)	1293	946	347
				Girls (n=15)	508	366	142
AA-Hb adducts	0	0	0	Boys (n=34)	23	18	5
				Girls (n=50)	0	0	0
GA-Hb adducts	0	0	0	Boys (n=34)	6	2	4
				Girls (n=50)	0	0	0
MNcb	22	4	18	Boys (n=13)	1397	662	735
				Girls (n=16)	95	28	67

These genes were linked with cell cycle-related processes and general cellular processes such as (post) translation, as well as in immune-related pathways. Moreover, for several cancer genes opposite correlations were identified in relation to the different exposure biomarkers. These differences may be related to the gender-specific differences in the cancer incidence, i.e. of leukaemia and lymphomas.
Novel proteomic effect markers, based on protein expression profiles in different in vitro models indicative of DON and PhIP exposure have been developed. 2-D gel analysis of DON and PhIP treated Jurkat E6.1, RPMI1788 and PBMCs has been conducted. Gel spots of interest have been selected and identification carried out by MALDI-TOF and nano HPLC Ion Trap MSn or MALDI-TOF/TOF. In total, 23 proteins were identified. These proteins were reproducible in three independent experiments composed of three replicates per condition (i.e. 18 gels per cell type). For DON, 5 proteins were common to the *in vitro* and *ex vivo* approach. For PhIP, 2 proteins were common to the in vitro and ex vivo approach.

The BioMark/Fluidigm system was chosen as platform for the high-throughput gene expression analysis of the cohort samples based on quantitative real-time PCR (RT-PCR), and done by ServiceXS BV in Leiden, the Netherlands. The required Taqman assays for the 48 genes were from Applied Biosystems. Initially a pilot run was performed on 88 samples from several cohorts, in order to check performance. All samples were well analysable for almost all genes. Thereafter, all the 1121 samples from newborns of all cohorts were run. All samples were analyzable, resulting in 1121 experiments with 71% (34) – 100% (48) valid RT-PCR values. Correlation analyses for samples analysed twice demonstrated a high technical

reproducibility, with CC from 0.95 to 0.99 (one sample was only 0.67). Inter-platform correlation analyses by Pearson correlation analyses per sample between DNA microarray data and RT-PCR data showed correlation coefficients of 0.62 to 0.22, with 109 out of 110 samples being significant. Significant positive correlations for genes were found for 36 of the 43 non-reference genes, with correlation coefficients ranging from 0.90 to 0.20. Based on these analyses, the RT-PCR method for gene expression analyses was considered well suitable for population based studies, like for all the NewGeneris cohorts. Due to limited correlation between microarrays and RT-PCR for 7 genes, these are proposed to be excluded in further analyses. Statistical data analyses using the NewGeneris database from June 2011 are conducted by GeneData, Basel and National Cancer Research Institute (NCRI), Genoa, and are still ongoing. Some initial data are shown in Table 2-3.

Candidate biomarkers (IMDH2 for DON and AHSA1 and phosphorylated AHSA1 for PhIP) were selected to take forward for analysis in cohort samples. Western blot and flow cytometry techniques successfully confirmed the results for the candidate DON biomarker, IMDH2. For both PhIP candidate biomarkers (AHSA1 and phosphorylated AHSA1) commercial ELISA kits were available and also confirmed initial results. The putative biomarkers were then applied to cohort samples. For the AHSA1 putative biomarkers, commercial ELISA kits were available, for the IMDH2 marker and a rapid high throughput western blot approach was developed. Statistical analysis revealed a significant positive association between AHSA1 levels in maternal and cord blood. A positive, but not statistically significant association was detected between log transformed AHSA1 in cord samples and estimated FFQ derived PhIP intake. A similar relationship was detected for phosphorylated AHSA1. No association was observed with AHSA1 or phosphorylated AHSA-1 in maternal blood samples when compared with FFQ estimated total PhIP intake and chicken consumption. For the putative IMDH2 biomarker, maternal and cord blood levels of IMDH2 were positively correlated but no significant association was observed between IMDH2 levels and FFQ-derived DON intake or maternal urinary DON.

To examine the association between maternal dietary intake of dioxins, PCBs and acrylamide during pregnancy and immune-related health outcomes in the child up to three years of age, a 3-year follow-up study was conducted for the children of the BraMat sub-cohort. Questionnaire-based annual follow-up studies were performed covering topics on the child's common infectious diseases, allergy, asthma, the use of medications and vaccinations. At the 3-year follow-up blood samples were collected from the children to examine child's atopic status (allergic sensitisation) and immune function (response to childhood vaccinations). At the 1-year follow-up, no statistical significantly differences were found with regard to frequency of health outcomes when comparing written questionnaires and telephone interviews. The results suggest, however, that prenatal dietary exposure to dioxins and PCBs may increase the risk of wheeze and infectious diseases during the first year of life (Table 4).
In contrast, prenatal exposure to PCBs and dioxins was no longer significantly associated with wheeze when investigating the third year of life only, i.e. the effect observed in the first year had waned. In multivariate analyses, prenatal dietary exposure to dioxins and dl-PCBs, and ndl-PCBs was associated with an increased number of URTI (upper respiratory tract infections) during the third year of life.

At year three, no statistically significant associations were found between prenatal dietary exposure and the levels of different subpopulations of leukocytes or regulatory T cells in peripheral blood. Furthermore, in multivariate regression analyses, no significant associations were found between exposure to the dietary toxicants and allergic sensitization. Prenatal dietary exposure to ndl-PCBs, and dioxins and dl-PCBs were associated with reduced levels of anti-measles antibodies in multivariate analyses. A number of marginal and not robust associations have not been included in the preliminary results reported above, awaiting the finalization of the statistical analysis.

Table 2: Correlations between DR-CALUX and gene expression showing profound differences between the various cohorts

Calux DR plasma	All cohorts		PCA cluster with cohort 3		PCA cluster without cohort 3		Males		Females		Danish Biobank		BiB		Rhea Crete		INMA Spain	
# of experiments	538		76		462		264		274		131		91		45		186	
Transcripts	P-Value	BH Q-Value	P-Value	BH Q-Value	P-Value	BH Q-Value	P-Value	BH Q-Value	P-Value	BH Q-Value	P-Value	BH Q-Value	P-Value	BH Q-Value	P-Value	BH Q-Value	P-Value	BH Q-Value
HS00152939_M1_TLR4_ITX	1.20E-05	2.16E-04	0.92	1.00	0.04	0.22	6.88E-05	1.24E-03	0.03	0.13	8.03E-06	9.64E-05	0.27	0.52	0.51	0.77	0.30	0.51
HS00167524_M1_ALOX12_GTX	0.45	0.58	0.09	0.42	0.09	0.25	0.41	0.53	0.78	0.88	0.80	0.87	0.22	0.50	0.50	0.77	4.20E-03	0.08
HS00169587_M1_GADD45B_MN	9.65E-03	0.02	0.55	0.84	0.29	0.47	0.02	0.04	0.25	0.41	1.01E-03	3.63E-03	0.27	0.52	0.93	0.99	0.98	0.98
HS00174796_M1_CD28_GTX	4.23E-03	0.01	0.16	0.48	0.23	0.45	7.59E-03	0.02	0.13	0.25	0.06	0.12	0.07	0.36	0.17	0.54	0.43	0.60
HS00177150_M1_MAP2K6_MN	0.03	0.05	4.30E-03	0.15	0.22	0.45	0.30	0.42	0.04	0.13	0.94	0.96	3.78E-04	0.01	4.36E-03	0.16	0.67	0.83
HS00178615_M1_COL4A3BP_ITX	8.75E-04	3.15E-03	0.32	0.63	0.09	0.25	2.30E-03	0.01	0.09	0.20	6.67E-04	3.63E-03	0.34	0.58	0.59	0.79	0.24	0.50
HS00196849_M1_SMC1A_MN	3.55E-05	4.26E-04	0.11	0.42	0.14	0.33	8.04E-03	0.02	7.90E-04	0.01	1.07E-04	9.67E-04	0.07	0.36	0.41	0.77	0.22	0.50
HS00209846_M1_NIPBL_GTX_ITX	0.37	0.49	0.08	0.42	0.90	0.95	0.21	0.34	0.85	0.90	0.01	0.03	0.43	0.64	0.44	0.77	0.70	0.84
HS00210902_M1_POMP_ITX	0.03	0.06	1.00	1.00	0.31	0.47	0.01	0.03	0.61	0.82	0.52	0.66	0.20	0.47	0.99	0.99	0.09	0.33
HS00233544_M1_CD33_FU_ITX	0.03	0.05	0.60	0.84	0.30	0.47	0.22	0.34	0.04	0.13	0.16	0.26	2.69E-03	0.05	0.10	0.54	0.13	0.41
HS00251475_S1_DERL1_ITX	0.91	0.91	0.66	0.86	0.52	0.69	0.76	0.83	0.89	0.92	0.35	0.53	0.65	0.78	0.01	0.18	0.78	0.88
HS00324396_M1_LATS2_GTX	5.35E-04	2.14E-03	0.23	0.58	0.62	0.77	0.02	0.05	0.01	0.11	4.82E-04	3.47E-03	0.71	0.80	0.68	0.84	0.30	0.51
HS00328634_S1_TRIM13_ITX	0.61	0.73	0.24	0.58	0.09	0.25	0.90	0.90	0.70	0.88	0.57	0.66	0.57	0.70	0.77	0.89	0.23	0.50
HS00387062_M1_CDK7_ITX	1.88E-03	6.15E-03	0.58	0.84	0.97	1.00	6.78E-03	0.02	0.08	0.20	6.94E-03	0.02	0.46	0.64	0.96	0.99	0.04	0.33
HS00394890_M1_MAP3K1_GTX	4.63E-04	2.09E-03	0.86	0.97	1.00	1.00	2.65E-03	0.01	0.05	0.14	1.76E-03	5.40E-03	0.52	0.69	0.16	0.54	0.09	0.33
HS00403870_M1_C10ORF46_ITX	1.00E-35	3.60E-34	0.11	0.42	2.74E-06	9.87E-05	1.25E-06	4.51E-05	1.38E-05	4.98E-04	9.19E-09	3.31E-07	0.11	0.45	0.15	0.54	1.01E-03	0.04
HS00427977_M1_ERH_GTX_ITX	3.94E-04	2.02E-03	0.61	0.84	4.34E-04	7.82E-03	1.44E-03	0.01	0.09	0.20	1.80E-03	5.40E-03	0.01	0.09	0.48	0.77	0.34	0.54
HS00429370_M1_STAG3_MN	0.63	0.73	0.28	0.59	0.01	0.08	0.71	0.83	0.24	0.41	0.13	0.23	0.46	0.64	0.88	0.99	0.07	0.33
HS00608272_M1_TSC22D3_GTX	6.51E-05	5.86E-04	0.40	0.68	0.60	0.77	8.03E-04	9.64E-03	0.02	0.12	6.39E-06	9.64E-05	0.53	0.69	0.24	0.60	0.60	0.77
HS00612215_M1_ANAPC13_ITX	1.41E-04	9.06E-04	0.39	0.68	0.30	0.47	1.97E-03	0.01	0.02	0.12	7.89E-04	3.63E-03	0.14	0.45	0.64	0.82	0.35	0.54
HS00758600_M1_FCER1A_FU_ITX	0.01	0.03	0.45	0.74	0.90	0.95	6.41E-03	0.02	0.31	0.49	0.17	0.27	0.24	0.52	0.43	0.77	0.39	0.56
HS00852925_SH_YWHAZ_GTX	0.05	0.08	0.67	0.86	0.38	0.53	0.36	0.48	0.07	0.20	0.54	0.66	8.39E-03	0.09	0.96	0.99	0.54	0.73
HS00902335_M1_IL7R_ITX	0.15	0.23	0.12	0.42	0.36	0.51	0.45	0.56	0.14	0.27	2.88E-03	7.99E-03	0.18	0.47	0.06	0.54	0.04	0.33
HS00958164_M1_PDCD11_GTX	0.03	0.06	0.26	0.58	5.46E-03	0.05	0.04	0.07	0.24	0.41	0.06	0.12	0.10	0.45	0.23	0.60	0.74	0.86
HS00960114_M1_ACIN1_GTX	4.78E-03	0.01	0.10	0.42	0.10	0.26	0.03	0.07	0.03	0.13	9.07E-04	3.63E-03	0.13	0.45	0.13	0.54	0.20	0.50
HS01023087_M1_DHCR7_HBADD	2.40E-03	7.21E-03	0.13	0.42	0.06	0.23	0.03	0.07	0.02	0.12	0.03	0.08	0.68	0.79	0.11	0.54	0.08	0.33
HS01039836_M1_NBN_ITX	0.71	0.77	0.25	0.58	0.04	0.22	0.85	0.89	0.78	0.88	0.40	0.58	0.06	0.36	0.05	0.54	0.05	0.33
HS01550762_G1_NOD2_ITX	0.07	0.11	0.84	0.97	0.06	0.23	0.03	0.06	0.60	0.82	0.04	0.08	0.94	0.96	0.48	0.77	0.36	0.54
HS01553188_M1_TOLLIP_ITX	1.51E-04	9.06E-04	0.03	0.42	0.67	0.81	2.48E-03	0.01	0.01	0.11	8.66E-04	3.63E-03	0.19	0.47	0.32	0.69	0.26	0.51
HS02518862_G1_CKS1B_FU_ITX	0.75	0.77	0.86	0.97	3.77E-03	0.05	0.13	0.24	0.39	0.59	0.92	0.96	0.91	0.96	0.59	0.79	0.23	0.50

Table 3: Correlations for all cohorts together between exposure and effect biomarkers and gene expression showing profound differences between the various biomarkers

All cohorts	Binucleated # of binucleated cells with MN (per 1000 binucleated cells) (Perc_MNCB_VUB)	HB acrylamide adducts (Acrylamide)	HB glycidamide adducts (Glycidamide)	HB ethylene oxide adducts (Ethylen_Oxide)	M1DG adducts (M1DG_adductlevels_ULEIC)	06 Methyl guanine adducts (Adductlevels_NHRF)	CALUX DR fat result (CALUX_TEQ_fat)	CALUX DR plasma result (CALUX_TEQ_plasma)	Plasma ER CALUX result (ER_CALUX)	Plasma AR CALUX result (AR_CALUX)
# of experiments	414	819	819	796	609	582	196	538	527	527
HS00152939_M1_TLR4_ITX	0.22	0.05	0.93	0.35	0.46	0.28	0.98	2.16E-04	0.85	3.86E-05
HS00167524_M1_ALOX12_GTX	0.52	0.38	0.82	0.01	0.02	0.76	0.72	0.58	0.94	0.50
HS00169587_M1_GADD45B_MN	0.41	0.41	0.82	0.01	0.50	0.56	0.98	0.02	0.70	5.96E-03
HS00174796_M1_CD28_GTX	0.01	0.50	0.82	0.08	4.88E-03	0.65	0.72	0.01	0.75	0.29
HS00175195_M1_CTSG_ITX	0.99	0.50	0.63	0.99	0.55	0.93	0.77	0.77	0.18	0.56
HS00175561_M1_LAT_ITX	0.26	0.50	0.82	0.05	0.08	0.73	0.99	0.23	0.70	0.82
HS00177150_M1_MAP2K6_MN	0.26	0.50	0.82	0.94	0.62	0.81	0.72	0.05	0.52	0.70
HS00178615_M1_COL4A3BP_ITX	0.57	0.50	0.82	0.16	0.40	0.81	0.98	3.15E-03	0.56	0.03
HS00196849_M1_SMC1A_MN	2.79E-05	0.50	0.93	0.07	8.91E-03	0.76	0.98	4.26E-04	0.70	0.08
HS00204129_M1_C13ORF15_ITX	0.40	0.50	0.82	0.94	0.02	0.02	0.77	0.48	0.49	0.01
HS00209846_M1_NIPBL_GTX_ITX	0.12	0.50	0.82	0.18	0.86	0.33	0.98	0.49	0.78	0.10
HS00210902_M1_POMP_ITX	0.27	0.50	0.95	0.60	0.72	0.02	0.72	0.06	0.07	0.03
HS00221775_M1_POLD4_GTX	0.85	0.51	0.82	0.37	0.01	0.81	0.98	0.77	0.85	0.13
HS00233544_M1_CD33_FU_ITX	0.88	0.51	0.82	0.86	0.42	0.88	0.80	0.05	0.78	0.02
HS00251475_S1_DERL1_ITX	2.32E-03	0.59	0.82	0.59	0.35	0.92	0.72	0.91	0.85	0.52
HS00324396_M1_LATS2_GTX	0.01	0.59	0.82	0.05	0.08	0.73	0.98	2.14E-03	0.62	0.07
HS00328634_S1_TRIM13_ITX	0.05	0.64	0.95	0.84	0.13	0.99	0.98	0.73	0.49	0.61
HS00387062_M1_CDK7_ITX	2.32E-03	0.64	0.82	0.29	0.79	0.76	0.72	6.15E-03	0.11	0.17
HS00394890_M1_MAP3K1_GTX	0.08	0.64	0.82	0.01	0.06	0.63	0.99	2.09E-03	0.82	0.03
HS00403870_M1_C10ORF46_ITX	0.33	0.64	0.95	0.36	0.83	0.67	0.72	3.60E-34	0.94	4.00E-04
HS00427977_M1_ERH_GTX_ITX	0.09	0.64	0.82	0.07	0.70	0.73	0.72	2.02E-03	0.32	0.02
HS00429370_M1_STAG3_MN	0.57	0.64	0.82	0.60	0.35	0.81	0.72	0.73	0.49	0.72
HS00607830_M1_RAD17_GTX	0.03	0.64	0.82	0.12	0.65	0.79	0.77	0.58	0.54	0.13
HS00608272_M1_TSC22D3_GTX	0.12	0.64	0.82	0.01	0.34	0.27	0.98	5.86E-04	0.73	3.86E-05
HS00612215_M1_ANAPC13_ITX	0.08	0.64	0.82	0.18	0.60	0.92	0.72	9.06E-04	0.25	8.53E-03
HS00758600_M1_FCER1A_FU_ITX	0.23	0.65	0.82	0.28	0.42	0.73	0.72	0.03	0.89	0.39
HS00852925_SH_YWHAZ_GTX	0.08	0.72	0.95	0.19	0.46	0.88	0.72	0.08	0.05	0.02
HS00902335_M1_IL7R_ITX	0.04	0.75	0.82	0.10	0.01	0.73	0.72	0.23	0.85	0.39
HS00925195_M1_PRKCA_GTX	2.32E-03	0.75	0.82	0.01	8.07E-03	0.76	0.72	0.46	0.85	0.99
HS00958164_M1_PDCD11_GTX	0.04	0.85	0.82	0.06	0.02	0.81	0.72	0.06	0.85	0.14
HS00960114_M1_ACIN1_GTX	0.08	0.86	0.82	0.02	0.35	0.67	0.72	0.01	0.49	0.03
HS01023087_M1_DHCR7_HBADD	0.08	0.86	0.82	0.01	0.34	0.76	0.98	7.21E-03	0.49	0.11
HS01039836_M1_NBN_ITX	0.62	0.86	0.63	0.57	0.22	0.73	0.98	0.77	0.49	0.17
HS01550762_G1_NOD2_ITX	0.57	0.89	0.82	0.11	0.66	0.76	0.98	0.11	0.85	0.07
HS01553188_M1_TOLLIP_ITX	0.52	0.93	0.82	0.01	0.06	0.81	0.80	9.06E-04	0.85	0.13
HS02518862_G1_CKS1B_FU_ITX	0.08	0.97	0.82	0.18	0.65	0.85	0.72	0.77	0.01	0.06

Table 4: Significant associations in multivariate analyses[a] ($p < 0.05$) between prenatal exposure to ndl-PCBs, and dioxins and dl-PCBs, and health outcomes for the first year of life.

Health outcome	NdI-PCBs			Dioxins and dl-PCBs		
	Dietary toxicant categories[b]	OR (95% CI)	*p*-Value	Dietary toxicant categories[b]	OR (95% CI)	*p*-Value
Wheeze	≥80th P	2.79 (1.20–6.49)	0.018	≥80th P	3.35 (1.45–7.74)	0.005
Ex. Sub.	Continuous	1.10 (1.02–1.20)	0.018	Continuous	2.38 (1.11–5.13)	0.026
Health outcome	Dietary toxicant categories[b]	**β** (95% CI)	*p*-Value	Dietary toxicant categories[b]	**β** (95% CI)	*p*-Value
URTI	≥80th P	1.16 (0.16–2.16)	0.023[c]	Continuous	1.31 (0.40–2.22)	0.005[c]

P, percentile; Ex. Sub., exanthema subitum; β, linear regression coefficient; URTI, upper respiratory tract infections.

[a] Potential confounding variables initially included in the multivariate analyses were: child's gender, mother's previous breast-feeding, parity, maternal history of atopy, maternal age, maternal smoking, maternal passive smoking, maternal education, maternal BMI, birth season, type of delivery, breast-feeding of the child at six months, Apgar score after 1 min, and day-care attendance at 12 months.
[b] The reference category is the lowest exposure category.
[c] Maternal education remained in the final model.

In summary, the main results from the BraMat cohort at three years is an association between increased prenatal exposure levels to dioxins and PCBs through mother's food and increased numbers of upper respiratory tract infections and wheeze during the first three years of life, with a reduced immune defence against infections as the mechanistic explanation.

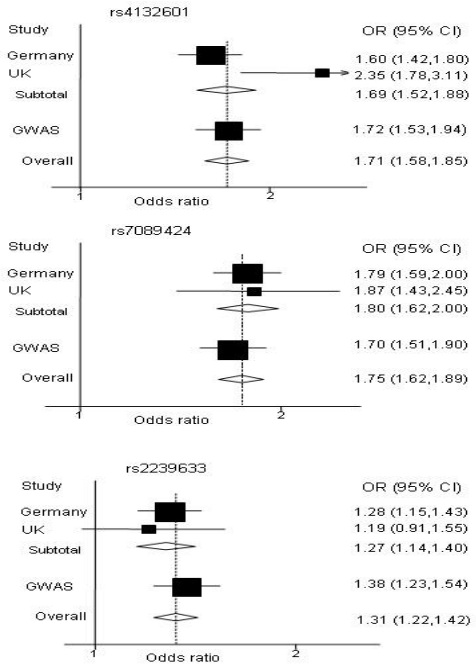
Workpackage 10

Two genome-wide association (GWA) studies have provided evidence that common germline variation influence the risk of ALL. The strongest association was attained at 7p12.2 with the single nucleotide polymorphism (SNP) rs4132601 located 3' to the ikaros family zinc finger 1 (*IKZF1*) gene. Ikaros proteins are master regulators of lymphocyte development and differentiation directing the CD4 versus CD8 T-cell lineage commitment. Mutant mice expressing only non-DNA-binding Ikaros isoforms develop aggressive lymphoblastic leukemia. *IKZF1* somatic deletions are common at diagnosis in high-risk/poor prognosis B-cell precursor ALL and in ALL with BCR-ABL1 fusions. Functional basis for the association between rs4132601 and ALL was provided by the correlation between reduced *IKZF1* expression and risk genotype in lymphocytes. The second locus identified indentified was at 10q21.2 with rs7089424 located in intron 3 of the AT rich interactive domain 5B (*ARIDB5*) gene. ARIDB5 is a member of the AT-rich interaction domain family of transcription factors, important in embryogenesis and growth regulation. The third association seen in only one of the GWA studies was at 14q11.2 with rs2239633 in the vicinity of *CEBPE,* encoding CCAAT/enhancer-binding protein, epsilon a regulator of myelopoiesis.

Recent genome-wide association data has implicated genetic variation at 7p12.2 (*IKZF1*), 10q21.2 (*ARIDB5)* and 14q11.2 (*CEBPE*) in the etiology of B-cell childhood acute lymphoblastic leukemia (ALL). To verify and further examine the relationship between these variants and ALL risk we genotyped 1,384 cases of childhood B-cell ALL and 1,877 controls from Germany and the UK. The combined data provided statistically significant support for an association between genotype at each of these loci and ALL risk; odds ratios, 1.69 ($P = 7.51 \times 10^{-22}$), 1.80 ($P = 5.90 \times 10^{-28}$) and 1.27 ($P = 4.90 \times 10^{-6}$) respectively. Furthermore, the risk of ALL increases with an increasing numbers of variant alleles for the three loci ($OR_{per\text{-}allele}$=1.53, 95% CI:1.44-1.62; $P_{trend} = 3.49 \times 10^{-42}$) consistent with a polygenic model of disease

susceptibility. These data provide unambiguous evidence for the role of these variants in defining ALL risk underscoring ~64% of cases.

Figure 1: Forest plots of odds ratios of ALL for the three SNPs. Boxes denote allelic OR point estimates, their areas being proportional to the inverse variance weight of the estimate. Horizontal lines represent 95% confidence intervals. The diamond (and broken line) represents the summary OR computed under a fixed effects model, with 95% confidence interval given by its width. The unbroken vertical line is at the null value (OR=1.0).

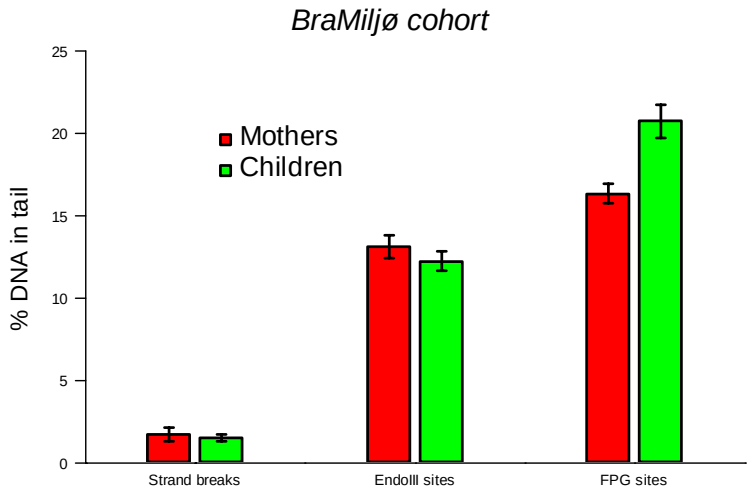


Workpackage 11

The comet assay (single cell gel electrophoresis) was used to measure DNA damage - strand breaks, oxidised pyrimidines and oxidised purines - in lymphocytes from mothers and cord blood. High throughput methods developed in the COMICS project (LSHB CT 2006 037575) were put into practice with the NewGeneris samples.

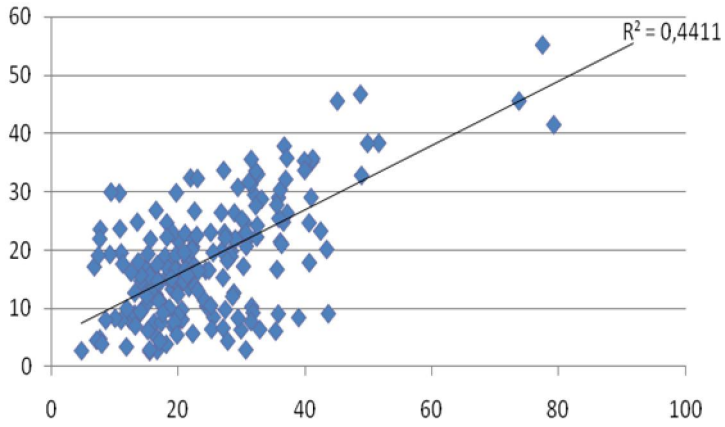
Full statistical analysis has been carried out on our data under WP12, and so only preliminary results (and basic statistics) are presented here. The largest number of samples analysed came from the Oslo BraMiljø cohort. In total, 489 samples (230 maternal, 259 cord) were successfully analysed with the Comet Assay, giving data for DNA strand breaks, net endonuclease III-sensitive sites (oxidised pyrimidines) and net FPG-sensitive sites (oxidised purines, mainly 8-oxoguanine).
Figure 1 shows the mean levels of the different kinds of DNA damage in lymphocytes from maternal and cord blood. In this cohort, there was a significant difference in levels of FPG-sensitive sites, cord blood having about 25% more oxidised purines than maternal blood.

Figure 1: DNA damage analysed with the comet assay, in lymphocytes from maternal and cord blood. Mean values of % tail DNA are shown, with SE of mean. Values of FPG-sensitive sites differ significantly (P=0.0001) between mothers and children.



There is a significant positive relationship between damage levels in maternal and cord lymphocytes, as illustrated for FPG-sensitive sites in figure 2.

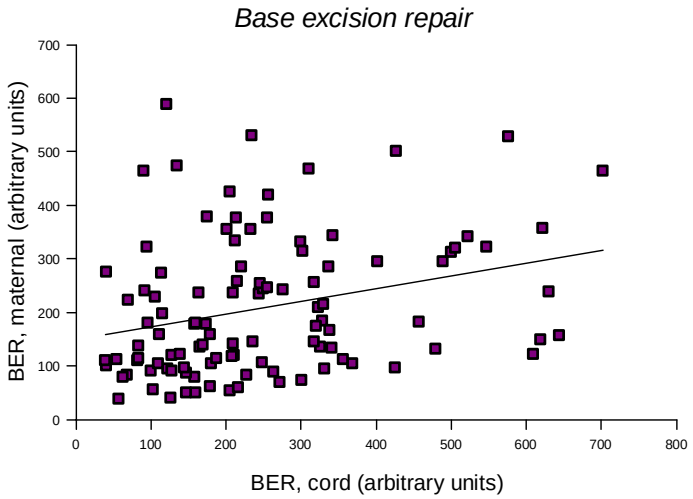
Figure 2: DNA damage (FPG-sensitive sites) measured with the comet assay and expressed as % DNA in tail. Paired samples, from mothers (y-axis) and children (x-axis).



DNA repair was measured with an *in vitro* assay based on the Comet Assay: lymphocyte extracts were incubated with DNA substrate containing 8-oxoguanine and so the activity recorded represents base excision repair (BER) of oxidation damage to DNA. Successful analysis of DNA repair was carried out on the INMA cohort samples. Figure 3 shows results from maternal and cord samples. There is a wide spread of activities (as we have shown previously) and a modest but highly significant correlation between maternal and cord values.

To summarise, the Comet Assay was used to measure DNA damage (strand breaks and oxidised bases), a biomarker of exposure to genotoxic agents in the internal or external environment, in 1151 lymphocyte samples (557 maternal, 594 cord) from different cohorts. A modified version of the assay provided information on the base excision repair capacity of lymphocyte extracts from 181 samples.

Figure 3: Base excision repair activity measured with an in vitro assay on extracts of lymphocytes from mothers and children. Results from paired samples are shown: the correlation is significant (P=0.003).



Workpackage 12

The contribution of WP12 was that of developing the study design required to answer the scientific questions defined within the NewGeneris, to estimate the number of study subjects to enrol in the study and to define the procedures for data collection, including the collection, handling, processing, storage and/or distribution of processed samples to the European laboratories in charge for measuring the array of biomarkers identified as necessary to address the scientific hypotheses. This was done by a tight cooperation with partners from different fields resulting a multidisciplinary effort aimed at optimizing the study conduct.

Biological samples collection as well as data collection and transfer were constantly monitored throughout the five year period: this served to undertake appropriate action whenever the expected accrual rate of pregnant women, the processing and distribution of samples and the transfer of laboratory results to the data repository were lower than the expected. The tools developed to monitor the study were efficient and guaranteed the enrolment of the expected numbers mothers and the measurement of biomarkers of exposure, response and effect in a sufficient number of mother/newborns to evaluate statistically the study hypothesis.

The establishment of a statistical analysis group (SAG) including statisticians from Genoa, Leeds, Barcelona and Geneva as well as experts from the biomarkers and the dietary fields permitted to develop the statistical analysis plan (SAP) an essential instrument for an unbiased statistical analysis. The SAP identified, prior to data freezing, the main hypotheses and the secondary hypotheses avoiding the risk of data mining and data torturing, a risk that is quite high in NewGeneris given the large amount of data collect and the many biomarkers measured in the study population.

All collected data during the project conduct were merged in a unique database (called pooled database), stored in Genoa, IT. This is the database that has been used for all statistical analyses, the production of the statistical reports, and that will be used for the large number of expected sub-analyses in the near future.

Workpackage 13

- Written documentation on data storage, management and security have been written and disseminated to all partners based on input from NewGeneris partners and a review of best practice;
- Creation of the final data-set for statistical analysis including receipt, standardisation and linkage of both cohort and laboratory data;

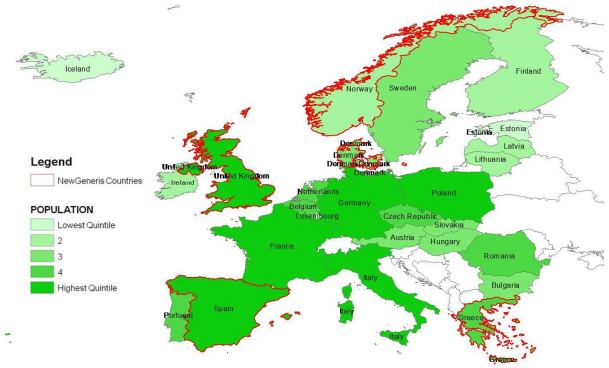
- Data security was considered and guidelines were sent to all researchers emphasising the need to keep data in a secure manner which allowed regular backups to minimise the chance of data loss. Also, the use of PGP encryption for the send and receipt of data using secure keys was encouraged;
- Collaboration with WP12 was achieved allowing the incorporation of data relevant sections in the analysis plan and to ensure the data-set was defined in such a way as to be suitable for the final statistical analysis;
- European legislation and previous good practice have been reviewed to allow the development of guidelines relating to the tracking of biological samples within the NewGeneris project;
- Creation of standardised demographic variables and a report describing their distribution across the different cohorts was produced and disseminated;
- A survey of NewGeneris partners was carried out to investigate the ease of data sends which revealed most of the procedures were easy to follow;
- A data request policy and accompanying form was developed to allow transparent access to the main data.

Public Health implications

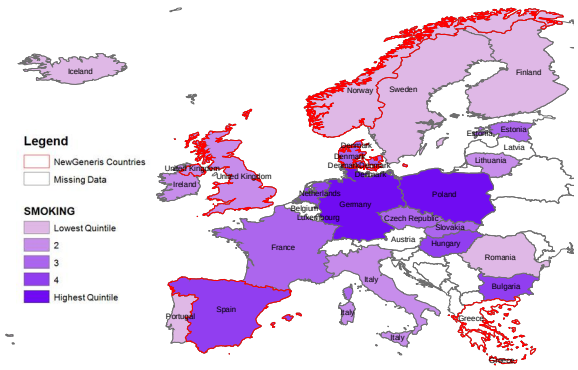
Sources of information and data on cohort countries and individual susceptibility were identified and documented for use in developing descriptive protocols of their effect on exposure assessment and applicability of results to the EU as a whole. These were used to compare the characteristics of NewGeneris cohort countries to the EU as a whole to identify how representative the countries were (Figure 2).

Figure 2: Maps of the European Union representing a) Levels of population density and b) Smoking levels highlighting the countries where the NewGeneris cohorts are based

a)



b)



Two workshops on the public health outcomes of the NewGeneris project were organised and carried out in November 2009 and November 2010. Both have since been written up as a report and several points raised at the meeting will be taken forward to inform the project outcomes.

Publications and dissemination at meetings

- Calculations of estimated exposure to NewGeneris compounds were carried out using data from the UK Women's Cohort Study and several of the other NewGeneris cohorts. This work was presented as a poster at the Integrative Molecular Cancer Epidemiology workshop IARC-EACR-AACR-ECNIS Symposium, Lyon, 3-5 July 2008;
- A poster was presented at the UKEMS meeting under the title 'Recruitment and sample collection as part of a European molecular epidemiology project: Experiences in the Born in Bradford NewGeneris subcohort, UK' in July 2009, Leeds, UK (abstract published in Mutagenesis, November 2009);

- Results from the exploration of dietary intakes of acrylamide in the Born in Bradford cohort were presented as a poster under the title 'Description of dietary intakes of acrylamide in the Born in Bradford birth cohort study' at the Society of Social Medicine meeting Sep 2010, Belfast, UK;
- Dietary intakes calculated from the NewGeneris values were used in the analysis of breast cancer risk using data from the UKWCS; a cohort within the NewGeneris study. The results were published in the British Journal of Cancer in November 2010;
- Results relating measurements of a urinary biomarker for deoxynivalenol intake in the Born in Bradford cohort showing a difference between levels in pregnant women of South Asian and non-South Asian were published online in "Food Additives and Contaminants Part A" in January 2011;
- Dissemination of NewGeneris project results was also carried out locally at research group meetings, cohort progress meetings including the Born in Bradford cohort progress meetings which are attended by midwives, health visitors, children centre staff, paediatricians and researchers and also at NewGeneris annual meetings.

1.4 Addressing ethical and regulatory issues

Alongside the scientific work, NewGeneris systematically addressed the need to identify a series of possible obstacles and/or possible levers to set up and conduct research on developing biomarkers in environmental health, whilst respecting European ethical standards, and to further develop these standards for specific applications in environmental health. This has been done by Workpackage 14: 'Ethics'.

This approach needs to be embedded in firmly rooted EU values and should envisage the facilitation of valuable and ethically correctly conducted research in the field of human biomonitoring.
In particular, the work focuses on communication strategies, on individual as well as on collective level. Good communication of a general description of the research project at the collective level is of overriding importance for a person to consider participation at all. Good accessibility of that information and additional clarification if needed is crucial at this point. If it is too difficult for a potential study participant to get proper information, he may easily give up. This does not only result in the loss of one potential participant for the current research project, but possibly also jeopardizes any future participation of this person. Another crucial issue is communication of results. Research participants have the right to know individual results, a right that is legally embedded in the EU privacy Directive.
Based on the NewGeneris experience, a chapter on communication was included in the ECNIS Volume on *"Ethics and Data Protection in Human Biomarker Studies"*. A film on informed consent *"Informed consent in environmental health research"*. A didactic tool for the improvement of the authenticity of informed consent", was made and disseminated.

A second main objective concerns the perception studies. Perception studies have been performed in four NewGeneris cohorts, all four differing in socio-cultural and political traditions. As a result, many elements came up, which should definitely be taken into account in order to keep the trust and confidence that the mothers on the whole seem to have. Human Biomonitoring has the power to make pollution personal, and provides therefore an excellent opportunity to advice the population on environmental health matters. At the same time one major conclusion of these studies is that the mothers participated because they want to contribute to society. Perception studies make commitment of the participants much stronger. It was only after participation at the perception study that many women really understood concepts such as informed consent and the right to know. All these aspects were investigated and analysed, studying real life situations as well as the legislative and regulatory context. At project level, legal advice is provided in a roadmap. All ethical documents were collected and made available on the NewGeneris website.

In general, the work allowed to increase awareness of ethical aspects and raised many discussions. Of importance was the transnational aspect of the project (including data protection issues), when samples and/or data were collected in one Member State and transported to another for analysis. Other discussions relate on the use of anonymous data and property rights. Furthermore, WP14 always supported and prepared the meetings of the Ethical Advisory Board. A fruitful collaboration was established.
At the same time, the moral framework from which solutions should grow and the needs of both researchers and research participants are reflected upon. The main hypothesis within WP14 is that there is a need for adjustment of the current ethical and legal framework for the area of environmental health research. While safeguarding a pragmatic and casuistic approach, a strong philosophical foundation may be needed. WP14 therefore participated several times to the workshop in Political Theory (Manchester UK) since increasing philosophical knowledge, integration and networking could only be beneficial for our research purposes. The WP14 team is convinced that we should gradually continue to permeate into the area of philosophy by repeatedly attending congresses and presenting cases.
And last but not least, in order to increase awareness of and interest for socio-ethical aspects of human biomonitoring research within as well as outside NewGeneris and with the view on the propagation of the expertise built up within NewGeneris, a close link with policymakers and a close collaboration with the Biomonitoring Implementation Group is done and continued.

1.5 Training and transferring research excellence

The NewGeneris training program included a range of initiatives to transfer expertise and promote excellence in methods for biomonitoring throughout the European Research Area. This has been done within Workpackage 15: 'Training'.

The main aim and approach of the Training were:
- Transfer of knowledge and scientific skills;
- Exchange of researchers between laboratories;
- Networking of existing training and research centres;
- Practical training of the best available technologies.

To achieve this, Training approach was:
- To transfer existing knowledge and scientific skills by training in standardized and already validated methods for biomonitoring to be spread within the consortium including harmonizing procedures and training within and outside the consortium through workshops, short courses and training visits;
- To support exchange of researchers between laboratories with aim to share expertise, to speed up developing of new biomarkers and standardizing new methods; to stimulate mobility especially among young researchers, and integration and team-working across the consortium; An exchange training program with a grant application was used as tool;
- To strength collaboration between all relevant training centres within Europe, especially with HEAR NAS, Marie Curie Training Centre in Molecular Epidemiology; NuGo ECNIS and CASCADE by using existing training infrastructure;
- To organise practical trainings of the best available technologies by producing electronic teaching-training material.

During the period of the project an effective frame for planed training program with flexible scheme was developed. The series of planed thematic scientific workshops and courses were combined with ad hoc training workshops and courses to fulfil project needs. This flexible scheme appeared to be very successful as in this way training reflected to research activities and contributed substantially to the success of the project. Additionally all partners actively participated in the training and thus contributed to the communication and to transfer of knowledge. Enormous activity and mobility among partners was

supported by introducing an exchange training program with a grant application system. Program was successfully implemented and was widely used by all partners within NewGeneris.
While in first period training was focused on basics in knowledge and techniques, training continued with harmonising procedures and training within and across workpackages in available techniques, later adopting new high-throughput methods and approaches. In the last period of the project, training activities continued especially with thematic training courses and exchange visits to support RTD activities. Several hundred students attended specific courses altogether, around 20 students participated in one course. Courses had been organised and supported fully or partially by the project. Several new training courses had been set up additionally to regular every year courses organised in partner's institutions. Thematic courses have been carried out with the aim to learn and share the best available methodologies and skills with focus on harmonising procedures in developed SOPs. For this reasons several ad hoc training in specific techniques have been carried out. An inventory of all courses and practical training organised showed that over 60 short ad hoc training or training courses were organised or co-organised as NewGeneris trainings with altogether several hundred participants. Many students also outside of the project were interested to participate and were trained and together with NewGeneris students contributed to dissemination of the NewGeneris research and methodologies. Vice versa Dissemination WP16 supported extensively each training task with website and active participation in organisation of events.
Altogether in all period over 30 scientific workshops and double amount of courses have been organised or co-organised within approximately 20-60 participants each. Over 100 scientific exchange visits were carried out and mobility of partners contributed substantially to quality of the research. The exchange training program supported all scientific WP's to ensure that both training and RTD tasks will be completed in time. Active mobility had been performed also across several WP's and in last stage of the project supported enormously research effort by giving opportunity to young scientists to improve their skills and knowledge, harmonise procedure and analyse samples and data. At least 12 long (minimum one month) trips had been supported by NewGeneris training program. Results of these mobility actions were immediately applied in research and gained skills were implemented from host institution to home laboratories. Training exchange program substantially contributed to the success of the project.

One of the major achievements is that some of these workshops and practical trainings organized originally as NewGeneris workshops are becoming regular scientific events and will be continuing beyond life of NewGeneris. Networking of NewGeneris with existing training centres within Europe (HEAR NAS, Marie Curie Training Centre in Molecular Epidemiology; NuGo ECNIS, CASCADE) was established and increased the quality and efficiency of training and education in essential issues of NewGeneris and many students benefited from them.

During course of the project young scientists have been involved in all research and training activities. The challenging tasks were stimulating and encourage them to develop their research careers, to communicate and to form their own informal research networks that will strengthen their collaborative activities in the future. Students had been encouraged to represent the consortium at meetings and conferences, and to disseminate the latest NewGeneris results by presenting data orally or in poster format. Young scientists co-organised, chaired and last year fully organised their poster session, they also became more confident in leading discussions, asking questions and chairing scientific sessions. 15 Students obtained price as poster winners.

At least 11 PhD theses started and are being in progress or finished during NewGeneris project and many collaborative papers are being published or are under preparation. This clearly shows that young scientists already created their scientific competence, and built collaboration and network that will continue beyond the NewGeneris project life. Training program supported scientific and networking activities of young scientists and especially accelerated their research productivity.

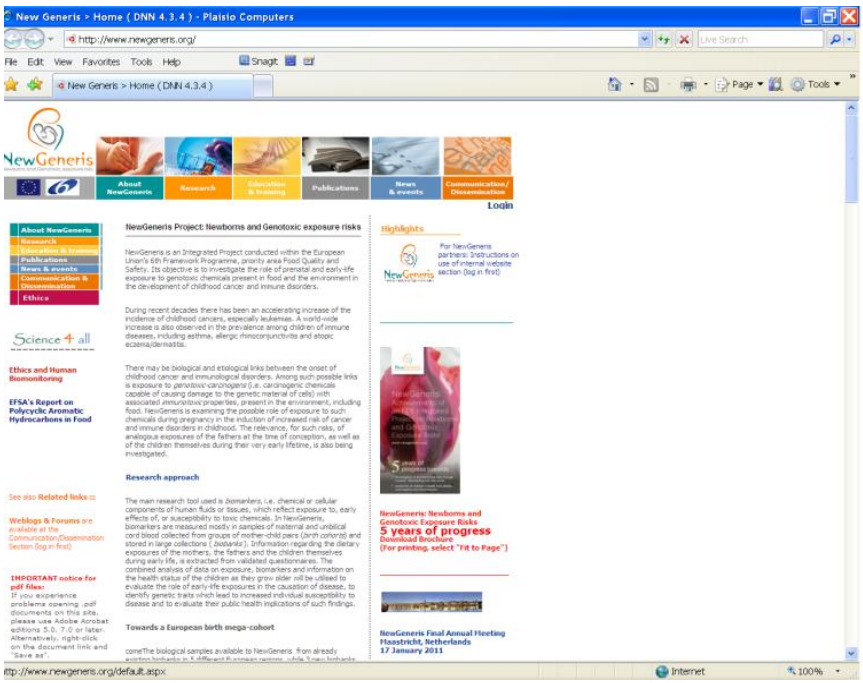
Training media and teaching tools for course were developed: Over 100 copies of the DVD on the DNA damage and repair by the Comet assay was distributed to partners as well as outside of the consortium and because of high demand, it is now placed at NewGeneris website and free for all students and researchers also outside of NewGeneris, Additionally DVD on Ethics for the improvement of the authenticity of informed consent had been partially supported by Training WP as useful educational material for clinical, epidemiologists and medical students.

1.6 Engaging the public

During its 66 months course NewGeneris has implemented a range of innovative outreach initiatives to disseminate information and facilitate public discussion. This has beeen organized within Workpackage 16: 'Dissemination'.

A "Communication and Dissemination Officer" has been appointed from the beginning of the NewGeneris project and provided with appropriate secretarial and other support. A project logo, brochure, poster and Powerpoint Presentation were produced. Furthermore, an "External Dissemination Advisory Board", consisting of key stakeholder representatives (CEFIC, ILSI Europe, WHO, ALSPAC, European Environment Network, Netherlands Ministry of Health) was created. The members of this Board were regularly invited to attend the network's Annual General Meetings and were kept informed and consulted on the project's progress.

- A website (www.newgeneris.org) was established in M2 and was fully developed (including a dynamic internal section) by M9. The website provide basic information on the project and publicises project-related events, which are regularly updated; in addition its "Science4All" section operates as a portal for the enhancement of public awareness on scientific issues related to childrens' environmental health, highlighting documents of basic relevance to the NewGeneris mission as well as recent scientific advances in the area.



The internal, interactive area of the website has served as a dynamic work and document storage area, with sub-sections for each WP. A dedicated Ethics sub-page on the open section of the website was established, with, among other things, documents to facilitate ethical review by local ethics

committees, guidance on ethical and legal aspects in human biomarker research, information on the handling of ethical aspects within NewGeneris, informed consent forms and a list of relevant legislation.

Website statistics:

	2007	2008	2009	2010
Home page	8,786	19,533	21,665	19,588
Total	12,544	22,751	24,614	27,418
Total Unique Visitors	2,795	4,161	5,043	4,091

Country/Territory	Visits	Pages/Visit
United States	528	1.93
Greece	386	6.18
United Kingdom	341	3.58
Netherlands	266	4.66
Spain	234	3.59
Belgium	229	2.83
France	203	3.54
Italy	178	3.82
Norway	161	3.43
Germany	161	2.73

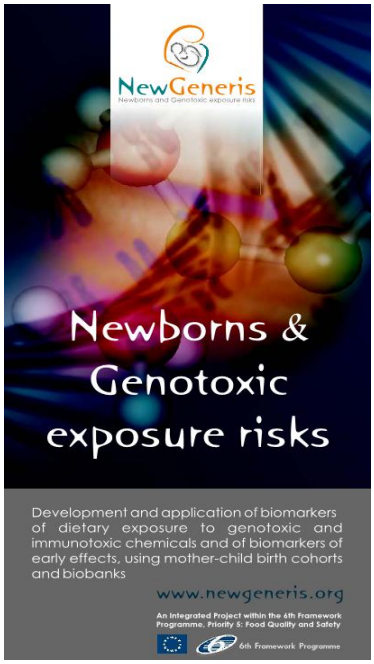
Newsletters and Brochures

Todate, 5 issues (one single and two double) of the Newsletter as well as 2 Brochures have been produced and circulated widely in printed and electronic form.

Contacts with stakeholders

The Communication and Dissemination Office maintained close contact with the project's External Dissemination Advisory Board which represents important stakeholders. The network's dissemination strategy was discussed at working meetings held between the Dissemination Officer and members of the Board at the Annual General meetings. Furthermore, a Dissemination Strategy meeting was held with

the members of the Board in Brussels on 10 July 2008. The strategy for the contacts with all stakeholders was followed giving important results.



Organisation/participation by WP16 of the following scientific or stakeholder's events

- NewGeneris Workshop on "Systems biology approaches to biomarkers of environmental health", Athens - Greece. February 14, 2008;
- NewGeneris Symposium on "Early life exposures to Environmental Chemical and Chronic Disease" at EUROTOX, Rhodes - Greece. October 5-8, 2008;
- NewGeneris Workshop on "Oxidative stress in newborns and consequences for children's health", Stockholm 5 February 2009;
- Joint NewGeneris/ECNIS stakeholders workshop on"Food and Environmental Cancer Risks for Adults and Children", Brussels - Belgium. March 16, 2009;

- NewGeneris Workshop on "Children's cancer risks", ICEM conference, Florence - Italy. 21 August 2009;
- NewGeneris Workshop on "Cancer susceptibility with a focus on children", EEMS 2010, Oslo - Norway. September 17, 2010;
- NewGeneris Workshop on "New approaches in epidemiological research on environment and child health", Heraklion - Greece. February 5, 2010;
- Acrylamide stakeholders' Workshop, Maastricht - the Netherlands. Janauary 18, 2011.

Participation of the NewGeneris Dissemination Officer in the following events, usually with oral presentations on the NewGeneris project:

- EFSA European Food Safety Summit, in Brussels (November 2007);
- Food Chemical Risk Assessment Research Workshop, organised by DG Research, Brussels, 9 September 2008;
- 1st International Conference on Risk Assessment "Global Risk Assessment Dialogue", DG SANCO, Brussels, 13-14 November 2008;
- ICCA-LRI 2009 Workshop "Connecting Innovation in Biological, Exposure and Risk Sciences", 16-17 June 2009, Charleston, South Carolina, USA;
- "European Food Science Day", 18 November 2009, Brussels;
- 1st European "Environment and Public Health" meeting, Brussels 8-10/11/2010;
- Meeting of EU project Communication Managers, organised by DG Research and CommNet, Brussels 23-24 March 2010;
- Workshop "How Human Biomonitoring supports Environment & Health policy" in context of the Fifth WHO Ministerial Conference on environment and health: Protecting children's health in a changing environment, Parma, Italy, 10-12 March 2010.

Other publicity of NewGeneris:

- Presentation of NewGeneris project in "The Parliament Magazine" (Issue 254, October 15, 2007, p. 62);
- Commentary on NewGeneris on DG Research website (European Research Headlines) and on CORDIS website (April 2010).

Section 2 | Dissemination and use

2.1 Exploitable knowledge and its Use

During the project's liftime no exploitable results, defined as knowledge having a potential for industrial or commercila application in research activities or for developing, creating or marketing a product or process or for creating or providing a service, were achieved. Therefor no overview table has been generated as stated in the "Project reporting in FP6 – Appendix 1" guidelines.

2.2 Dissemination of knowledge

The primary dissemination outlet for the results of the project has been through scientific publications.

The Project objective as described in detail within WP16 is to organise the flow of scientific information within the NewGeneris network, to inform all relevant segments of society about NewGeneris and its activities and to promote the dissemination, for the benefit of the European citizen and the European food industry, of results of the work performed in the RTD WP's 1-14, to the scientific community, SMEs, public health officers and medical doctors, policy makers, media and the general public. This activity consists of two task areas:

1. Dissemination of scientific information within the network of NewGeneris partners, for the purpose of supporting the network's smooth operation, exchanging of information and spreading state-of-the-art and new knowledge. This task is closely allied with, and complements, the Training activities, which disseminate new practical skills;
2. Communication of NewGeneris activities, findings and their implications to the general public as well as specific stakeholder groups (including representatives from EU food industry, regulators, health professionals, and consumer organisations).

The main themes of the activity were:

- Internal NewGeneris communications: Tools for internal (secured) NewGeneris communications includes an interactive website (with limited access) for sharing results, procedures; reports, meeting details, and 1-2 day workshops exclusively for consortium members etc.;
- Building relationships with the general public and stakeholder groups EU-wide: Two kinds of activity are envisaged in this context. First, informing society about NewGeneris and its aims and highlighting its activities. Second, disseminating information to various social groups and stakeholders about scientific advances made within and beyond NewGeneris. Various tools are employed for this purpose, including an open website providing information on NewGeneris activities as well as news and alerts related to scientific findings and their significance for different stakeholders, media events (press releases, news conferences, newspaper articles, TV and radio broadcasts), technical reports, health advisories, etc. Leaflets on major scientific achievements are provided for public health officers, policy makers, media and the public. Particular attention has been paid to the establishment of a dialogue with selected, major stakeholders aiming to formulate an efficient framework of interaction with them;
- Organising Thematic Conferences involving both consortium members and key external stakeholders/opinion-formers in order to:
    - Share the appraisal of current scientific knowledge on gene-environment relationships, including new findings emerging from NewGeneris;
    - Strengthen personal contacts and networks with the stakeholders, and within NewGeneris;
    - Build commitment to NewGeneris among stakeholders.

It should be noted that many NewGeneris partners are also participants in already established Networks in the areas of children health and biomarker research, e.g. the CHILDRENGENONETWORK, ECNIS and EnviroGenomarkers. This will further facilitate dissemination of results, as information on the outcome of the projects can be shared and disseminated even more effectively to the stakeholder groups.

The focus of dissemination activities of Year 5 has been on discussing its goals and results to stakeholders and establishing tools needed for ongoing dissemination. Tools developed include:

- Updating and expansion of the NewGeneris website: (www.newgeneris.org);
- Announcement of NewGeneris news/events as well as other events (conferences, meetings etc) that are in the NewGeneris area of interest (e.g. the NAS Exposome meeting, Washington, 25-26/02/2010; the "*Food for the 21st Century: The Impact of EU Research upon European Food and Safety*" conference, Brussels, 8 July 2010; the 40th EEMS Meeting, Oslo, 15-18 September 2010; the EFSA Report "*Overview of food acrylamide levels*"; the Parma Declaration on Environment and Health);
- Reports of NewGeneris events:
  - ◊ 4th Annual General Meeting of NewGeneris, Heraklion, 3-4 February, 2010;
  - ◊ NewGeneris Workshop on "*New approaches in epidemiological research on environment and child health*", Heraklion, 5 February 2010;
  - ◊ Publication of NewGeneris Newsletter Issue 4-5;
  - ◊ Commentary on NewGeneris on DG Research website (European Research Headlines) and on CORDIS website (April 2010);
  - ◊ Participation of NewGeneris at the Fifth Ministrial Conference on Environment and Health, Parma, 10-12 March, 2010;
  - ◊ Participation of NewGeneris at DG Research meeting for Communication Managers, Brussels, 23-24 March 2010;
  - ◊ NewGeneris Final Annual Meeting, and NewGeneris Final Workshop, Maastricht, Netherlands, 17-18 January 2011.
- Updating of "*Science for All*" section with new information: scientific issues related to children's environmental health, and recent scientific advances in the area (documents, reports, books etc, produced by major international organizations) and in general all events and information in highlights;
- Links to running FP6 /FP7 EC projects and other projects, relevant to NewGeneris, were added:
  - ◊ HENVINET Health and Environment Networking Portal (in Highlights);
  - ◊ CommNet (participation of NewGeneris in this net which brings together the communication managers of 30 EU projects);
  - ◊ EnviroGenomarkers Genomics Biomarkers of Environmental Risk;
  - ◊ ENRIECO Environmental Health Risks in European Birth Cohorts;
  - ◊ COPHES Human Biomonitoring for Europe;
  - ◊ WHO Children's Environmental Health Website;
  - ◊ ATHON: Assessing the Toxicity and Hazard of Non-dioxin-like PCBs present in food;
  - ◊ CASCADE: Chemicals as contaminants in the food chain: a Network of Excellence for research, risk assessment and education;
  - ◊ EUROPREVALL: The prevalence, cost and basis of food allergy across Europe;
  - ◊ NUGO: European nutrigenomics organisation-linking genomics, nutrition and health research;
  - ◊ SAFE FOODS: Promoting food safety through a new integrated risk analysis approach for foods;
  - ◊ EUROPEAN LEUKEMIA NETWORK;
  - ◊ GABRIEL: A multi disciplinary study to identify the genetic and environmental causes of asthma in European Community;
  - ◊ GA^{2}LEN: Global Allergy and Asthma European Network;

- ◊ UFIPOLNET: Ultrafine particle size distributions in air pollution monitoring networks;
- ◊ ESBIO: Eurean Human Biomonitoring;
- ◊ PHIME: Public health impact of long-term, low level mixed element exposure in susceptible population strata;
- ◊ ECNIS: Environmental Cancer Risk, Nutrition and Individual Susceptibility;
- ◊ CARCINOGENOMICS;
- ◊ EPIC: European Prospective Investigation into Cancer and Nutrition;
- ◊ EUROPEAN BIRTH COHORTS.

- Participation of the Dissemination officer at the Fifth WHO Ministerial Conference on environment and health in Parma, March 2010 and presentation on "Trends in biomarkers research and their potential for biomonitoring strategies: A NewGeneris and ECNIS overview" in the context of a workshop on biomonitoring coorganized with the COPHES project;
- Organisation of a "*Workshop on Acrylamide*" in the context of the final NewGeneris Meeting (Maastricht, January 18, 2011), focusing on the overall project's findings on acrylamide;
- Participation of the Dissemination officer in the Environment & Public Health conference, Brussels, 8-10 November 2010; exhibition of a NewGeneris poster and oral presentation on "*Cancer and the environment: The EU-funded Newgeneris, ECNIS and Envirogenomarkers projects and their links and interaction*" in session on "Translating science into policy development";
- NewGeneris highlighted in EC Research and CORDIS sites ("*EU investigates impact of toxic exposures on babies*", 16-4-2010).

NewGeneris partners - Dissemination of knowledge overview table

Actual dates	Year	Type	Title of event	Type of audience	Countrie addressed	Size of audience	Partner responsible / involved
December	2006	Symposium	International symposium Environment and Health, December 15 2006, Brussels, by Ludwine Casteleyn	Research	Belgian	100	KUL: Casteleyn L, Dumez B, van Damme K.
December	2006	Symposium	Towards consistent social, ethical and legal approaches in the use of human biomarkers in environmental health. Ethiek in wetenschapsbeleid. Middagdebat 19 december 2006 Vlaams Parlement; Brussels	General public	Belgian	100	KUL: Casteleyn L, Dumez B, van Damme K.
June	2006	Symposium	Rethinking Informed Consent: The limits of autonomy? Sandhamm, Sweden	Research	EU	200	KUL: Casteleyn L, Dumez B, van Damme K.
March	2006	Conference	Research on Ethics and Communication within NewGeneris (Newborns and Genotoxic exposure risks). Lisbon	Research	EU	150	KUL: Casteleyn L, Dumez B, van Damme K.
May	2006	Conference	Communication of results of the Flemish Biomonitoring Campaign - Adolescents	Research	Belgian	100	KUL: Casteleyn L, Dumez B, van Damme K.
November	2006	Conference	High-Level Roundtable on Environmental Health aspects of the Lisbon Agenda and the Sustainable Development Strategy. November 27 2006, Brussels	Research	EU	100	KUL: Casteleyn L, Dumez B, van Damme K.
October	2006	Workshop	Ethical issues associated with Human Biomonitoring studies	Research	EU	250	KUL: Casteleyn L,

			illustrated by (hypothetical) case studies. Workshop on human biomonitoring "Ethics and Data Interpretation", organised on behalf of the European Commission; Brussels				Dumez B, van Damme K.
Septembe r	2006	Conference	Towards consistent social, ethical and legal approaches in the use of human biomarkers in environmental health. Conference of INES (Institutionalisation of Ethics in Science & Technology Policy). Brussels	Research	EU	300	KUL: Casteleyn L, Dumez B, van Damme K.
aug-01	2007	Workshop	Workshop Ethical Issues raised by Personalized Nutrition, Lund University	Research	EU	100	KUL: Casteleyn L, Dumez B, van Damme K.
January	2007	Symposium	Stay Healthy, Stop Mercury: Halting the child brain drain"; Health & Environment Alliance and Health Care Without Harm Europe, European Parliament	General public	EU	60	KUL: Casteleyn L, Dumez B, van Damme K.
June	2007	Symposium	Integration of genome-based knowledge and technologies into Environmental health practices in Belgium, Belgian PHGen meeting	Research	EU	150	KUL: Casteleyn L, Dumez B, van Damme K.
June	2007	Workshop	Integration of genome-based knowledge and technologies into Occupational health practices, K Van Damme, Presentation at the Belgian PHGEN task force meeting	Research	EU	150	KUL: Casteleyn L, Dumez B, van Damme K.
June	2007	Symposium	Green Week 2007, Health Sessions, 12 June 2007, Brussels	Research	EU	200	KUL: Casteleyn L, Dumez B, van Damme K.
June	2007	Course	International Course on Molecular Epidemiology and Ethics, June 18-19. 2007, University of Kuopio	Research	EU	50	KUL: Casteleyn L, Dumez B, van Damme K.
march	2007	Workshop	"Research on ethics in ECNIS and NewGeneris: A bottom up approach" by Birgit Dumez, ESBIO workshop on ethics and communication	Research	EU	150	KUL: Casteleyn L, Dumez B, van Damme K.
February	2008	Newsletter	Ethiek en biomonitoring: hoe zit dat eigenlijk? (deel 2); De Biomonitor - De digitale nieuwsbrief van het Medisch Milieukundig netwerk in Vlaanderen; February 2008	General public	Belgian	300	KUL: Casteleyn L, Dumez B, van Damme K.
February 11-14	2008	Conference	Second NewGeneris Annual Meeting, Athens, Greece. Detection of white blood cell bulky DNA adducts in mother - newborn child pairs from the DKbiobank	Research	Europe	150	NIEH: Schoket B., Anna L., Kovács K., Schoket B., Győrffy E./ DKFZ; UC
January	2008	Newsletter	Ethiek en biomonitoring: hoe zit dat eigenlijk? (deel 1); De Biomonitor - De digitale nieuwsbrief van het	General public	Belgian	300	KUL: Casteleyn L, Dumez B, van

			Medisch Milieukundig netwerk in Vlaanderen; januari 2008				Damme K.
March	2008	Symposium	Presentation on 'A coordinated approach to HBM' European Parliament, Brussels	General public	EU	50	KUL: Casteleyn L, Dumez B, van Damme K.
March	2008	Workshop	WP1 Practical considerations for use of biomarker methodology in collaborative human studies (including plans for pilot studies), Barcelona	Research	EU	200	KUL: Casteleyn L, Dumez B, van Damme K.
May 29-31	2008	Conference	4th National Conference of the Young Hungarian Hygienists, Győr, Hungary. i) The importance of sample treatment of biological specimens in molecular epidemiologycal studies ii) The Impact of the DNA isolation method on the measurement of polycyclic aromatic hydrocarbons (PAH)-DNA adduct biomarkers	Research	Hungary	70	NIEH: Anna L., Kovács K., Schoket B.
September	2008	Conference	38th Annual Meeting of the Hungarian Hygienists, Balatonvilágos, Hungary. The impact of DNA isolation method on the measurement of polycyclic aromatic hydrocarbon (PAH) type aromatic DNA adducts	Research	Hungary	150	NIEH: Anna L., Kovács K., Schoket B.
September 21-25	2008	Conference	38th Annual Meeting of the European Environmental Mutagen Society, Cavtat, Croatia. The Impact of the DNA isolation method on the measurement of bulky DNA adduct levels by 32P-postlabelling	Research	Europe	350	NIEH: Anna L., Kovács K.
dec-01	2009	Collaboration meeting	COPHES kick-off meeting, Brussels	Research	EU	100	KUL: Casteleyn L, Dumez B, van Damme K.
May, 14-16	2009	Conference	5th National Conference of the Young Hungarian Hygienists, Eger, Hungary. i) Increase of through-put of 32P-postlabelling for polycyclic aromatic hydrocarbon (PAH)-type bulky DNA adducts to determine aromatic DNA adducts. PAH type aromatic DNA adducts from Spanish, Danish and Crete mother-child sample pairs deriving from European cohorts - overview of our result on the ongoing NewGeneris EU project	Research	Hungary	70	NIEH: L. Anna, K. Kovács

October, 6-8	2009	Conference	39th Annual Meeting of the Hungarian Hygienists, Balatonvilágos, Hungary. i) Increase of through-put of the 32P-postlabelling method to determine polycyclic aromatic hydrocarbon (PAH) type aromatic DNA adducts. PAH type aromatic DNA adducts from Spanish, Danish and Crete mother-child sample pairs deriving from European cohorts - overview of our result on the ongoing NewGeneris EU project	Research	Hungary	150	NIEH: Anna L., Kovács K., Rudnai P., Schoket B.
apr-10	2010	Collaboration meeting	ECNIS Final Annual Meeting, Lodz, Poland	Research	EU	100	KUL: Casteleyn L, Dumez B, van Damme K.
apr-10	2010	Collaboration meeting	Working meeting COPHES, Madrid, Spain	Research	EU	30	KUL: Casteleyn L, Dumez B, van Damme K.
sep-10	2010	Symposium	'Nutzen für die Politik - Herausforderung für die Wissenschaft / Political benefits – scientific challenges' 26. - 28. September 2010, Ludwig Erhard Haus, Berlin organised by the Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit and the Umweltbundesamt. LC invited speaker on 'HBM as a link between health and environment in Europe'	Research	EU	500	KUL: Casteleyn L, Dumez B, van Damme K.
January	2010	Collaboration meeting	Coordination team meeting COPHES, Luxembourg	Research	EU	30	KUL: Casteleyn L, Dumez B, van Damme K.
January	2010	Collaboration meeting	Participation at the first meeting of the Scientific Board of the planned HBM survey in France, January 2010, Paris - France	Research	EU	50	KUL: Casteleyn L, Dumez B, van Damme K.
July 2010	2010	Collaboration meeting	Coordination team meeting COPHES, London, UK	Research	EU	30	KUL: Casteleyn L, Dumez B, van Damme K.
March 10-12	2010	Conference	5th WHO inter-ministerial Conference on Environment and Health, Parma, 10-12 March 2010. LC; Workshop on 'How HBM supports Environment and Health policy: European feasibility study on mothers and children'.	Research	EU	500	KUL: Casteleyn L, Dumez B, van Damme K.

May, 27-29	2010	Conference	6th National Conference of the Young Hungarian Hygienists, Debrecen, Hungary. Comparative observations to detect DNA adducts of polycyclic aromatic hydrocarbon model compounds with immuno-assay and 32P-postlabelling method. Assessment of maternal and fetal polycaclic aromatic hydrocarbon exposure in European mother-newborn cohorts - overview of our result on the ongoing NewGeneris EU project	Research	Hungary	80	NIEH: Anna L., Kovács K., Lukács V., Rudnai P., Schoket B./ NHRF
October	2010	Collaboration meeting	Annual General Assembly Health and Environment Alliance (HEAL)	Research	EU	100	KUL: Casteleyn L, Dumez B, van Damme K.
October	2010	Collaboration meeting	DEMOCOPHES kick-off meeting, Brussels, Belgium	Research	EU	150	KUL: Casteleyn L, Dumez B, van Damme K.
October	2010	Symposium	INCHES, Children's Health and the Environment: Measuring exposure of children and their mothers in the European Union	Research	EU	200	KUL: Casteleyn L, Dumez B, van Damme K.
October	2010	Workshop	From human Biomonitoring to policy: a sustainable 'marriage' between health and environment, Brussels	General public	EU	200	KUL: Casteleyn L, Dumez B, van Damme K.
October 13-15	2010	Conference	TOX'2010 - Annual Conference of the Hungarian Toxicologists: Assessment of exposure to polycyclic aromatic hydrocarbons in European mother-newborn cohorts with determination of DNA adducts	Research	Hungary	150	NIEH: Lukács V., Anna L., Kovács K., Rudnai P., Schoket B.
October 22-24	2010	Symposium	RAMAZZINI DAYS 2010, New-Age: Measuring Exposure Of Children And Their Mothers In Europe'	Research	EU	150	KUL: Casteleyn L, Dumez B, van Damme K.
October, 5-7	2010	Conference	9th National Congress of the Hungarian Hygienists, Balatonvilágos, Hungary. Assessment of maternal and fetal polycyclic aromatic hydrocarbon exposure in European mother and newborn child cohorts - overview of our result on the ongoing NewGeneris EU project	Research	Hungary	150	NIEH: Anna L., Lukács V., Kovács K., Rudnai P., Schoket B.
September 15-18	2010	Conference	40th Annual Meeting of the European Environmental Mutagen Society, Oslo, Norway. Assessment of environmental exposure to polycyclic aromatic hydrocarbons during pregnancy with bulky DNA adduct biomarker in european mother and newborn child cohorts	Research	Europe	400	NIEH: Anna L., Kovács K., Lukács V., Rudnai P., Schoket B.

September, 6-8	2010	Conference	Eighth International Symposium on Biological Monitoring in Occupational and Environmental Health, Espoo, Finland. Assessment of polycyclic aromatic hydrocarbon (PAH) exposure during pregnancy by measurement of levels of bulky DNA adducts in European mother and newborn child cohorts	Research	Wide international	200	NIEH: Lukács V., Anna L., Kovács K., Rudnai P., Schoket B.
apr-11	2011	Collaboration meeting	COPHES/DEMOCOPHES inception meeting, Budapest	Research	EU	100	KUL: Casteleyn L, Dumez B, van Damme K.
February	2011	Collaboration meeting	COPHES/DEMOCOPHES meeting, Brussels, Belgium	Research	EU	80	KUL: Casteleyn L, Dumez B, van Damme K.
January 17-18	2011	Conference	NewGeneris Final Annual Consortium Meeting and Final Workshop, Maastricht, the Netherlands. Bulky DNA adduct measurements in NewGeneris mother-newborn cohorts – overview of the results	Research	Europe	150	NIEH: Lukács V., Schoket B., Anna L., Kovács K., Rudnai P., Győrffy E.
March 28-29	2011	Conference	UKEMS / Dutch EMS-sponsored Workshop on Biomarker of Exposure and Oxidative DNA Damage & 7th GUM 32P-Postlabelling Workshop, Münster, Germany: Assessment of exposure to polycyclic aromatic hydrocarbons during pregnancy with bulky DNA adduct biomarker in European mother - child cohorts	Research	Europe/ USA/overseas	150	NIEH: Anna L., Kovács K., Lukács V., Schoket B., Rudnai P., Győrffy E.

2.3 NewGeneris publications[1]

- Ilse Decordier, Kelly De Bont, Kirsten De Bock, Raluca Mateuca, Roberta Ciardelli, Dominique Haumont, Lisbeth E. Knudsen and Micheline Kirsch-Volders. *Genetic susceptibility of newborn daughters to oxidative stress.* Toxicol Lett., 2007, 172(1-2):68-84
- Gast A, Lorenzo Bermejo J, Flohr T, Stanulla M, Burwinkel B, Schrappe M, Bartram CR, Hemminki K, Kumar R. *Folate metabolic gene polymorphisms and childhood acute lymphoblastic leukaemia: a case-control study*. Feb-07. Reference: Leukemia 2007
- Hemminki K, Lorenzo Bermejo J, Försti A. *Balance between heritable and environmental aetiology of human disease.* Dec-06. Reference: Nature Reviews Genetics 2007
- van Leeuwen DM, van Herwijnen MH, Pedersen M, Knudsen LE, Kirsch-Volders M, Sram RJ, Staal YC, Bajak E, van Delft JH, Kleinjans JC. *Genome-wide differential gene expression in children exposed to air pollution in the Czech Republic.* Aug-06. Reference: Mutation Research
- van Leeuwen DM, van Agen E, Gottschalk RW, Vlietinck R, Gielen M, van Herwijnen MH, Maas LM, Kleinjans JC, van Delft JH. *Cigarette smoke-induced differential gene expression in blood cells from monozygotic twin pairs.* Oct-06. Reference: Carcinogenesis
- Stefano Moretti. *Minimum cost spanning tree situations and gene expression data analysis.* Oct-06. Reference: ACM International Conference Proceeding Series; Vol. 199. INRCA
- Vito Fragnelli, Stefano Moretti. *A game theoretical approach to the classification problem in gene expression data analysis*. Reference: Computers & Mathematics with Applications. INRCA

[1] NewGeneris members's publications relevant to NewGeneris field of study or directly related to the project.

- Paolo Fardin, Stefano Moretti, Barbara Biasotti, Annamaria Ricciardi, Stefano Bonassi, Luigi Varesio. *Low-density microarray normalization based on a spike-in approach: analysis of macrophage cell lines expression profile/* Sep-06. Reference: submitted to BMC Genetics. INRCA
- Stefano Moretti. Fioravante Patrone, Stefano Bonassi. *The class of Microarray games and the relevance index for genes.* Nov-06. Reference: submitted to TOP. INRCA
- Merlo DF, Ceppi M, Stagi E, Bocchini V, Sram R, Rossner P. *Baseline chromosome aberrations in children./* Sep-06. Reference: submitted to Tox. Letter. INRCA
- Fucic, A., Merlo, D.F., Ceppi, M., Lucas, J.N. *Spontaneous abortions in female populations occupationally exposed to ionizing radiation.* Jan-07. Reference: submitted Int Arch Occup Environ Health. IMROH
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- Marie Pedersen, Franco Merlo, Lisbeth E. Knudsen. (2007) Ethical issues related to biomonitoring studies on children. Int J Hyg Environ Health. 2007 May; 210(3-4):479-82
- Lisbeth E. Knudsen, Åse Marie Hansen (2007). Biomarkers of intermediate endpoints in environmental and occupational health Int J Hyg Environ Health. 2007 May; 210(3-4):461-70
- Maja Myren, Tina Mose, Line Mathiesen, and Lisbeth Ehlert Knudsen (2007). The human placenta – an alternative for studying foetal exposure. Toxicol In Vitro
- Uffe Lind, Lisbeth E.Knudsen and Tina Mose Participation in environmental health research by placenta donation - a perception study Environmental Health
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- Anderson D, Cemeli E, Schmid TE, Baumgartner A, Brinkworth M and Wood JM. *Oestrogenic Compounds and Oxidative Stress*. In: Anderson D and Brinkworth MH (Eds) (2007) Male-mediated Developmental Toxicity. Royal Society of Chemistry, Cambridge pp.259-272
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- Baumgartner A, Cemeli E, Anderson D (2008). *The Comet assay in male reproductive - toxicology* Cell Biology and Toxicology (e-pub)
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