NewGeneris
Newborns and Genotoxic exposure risks

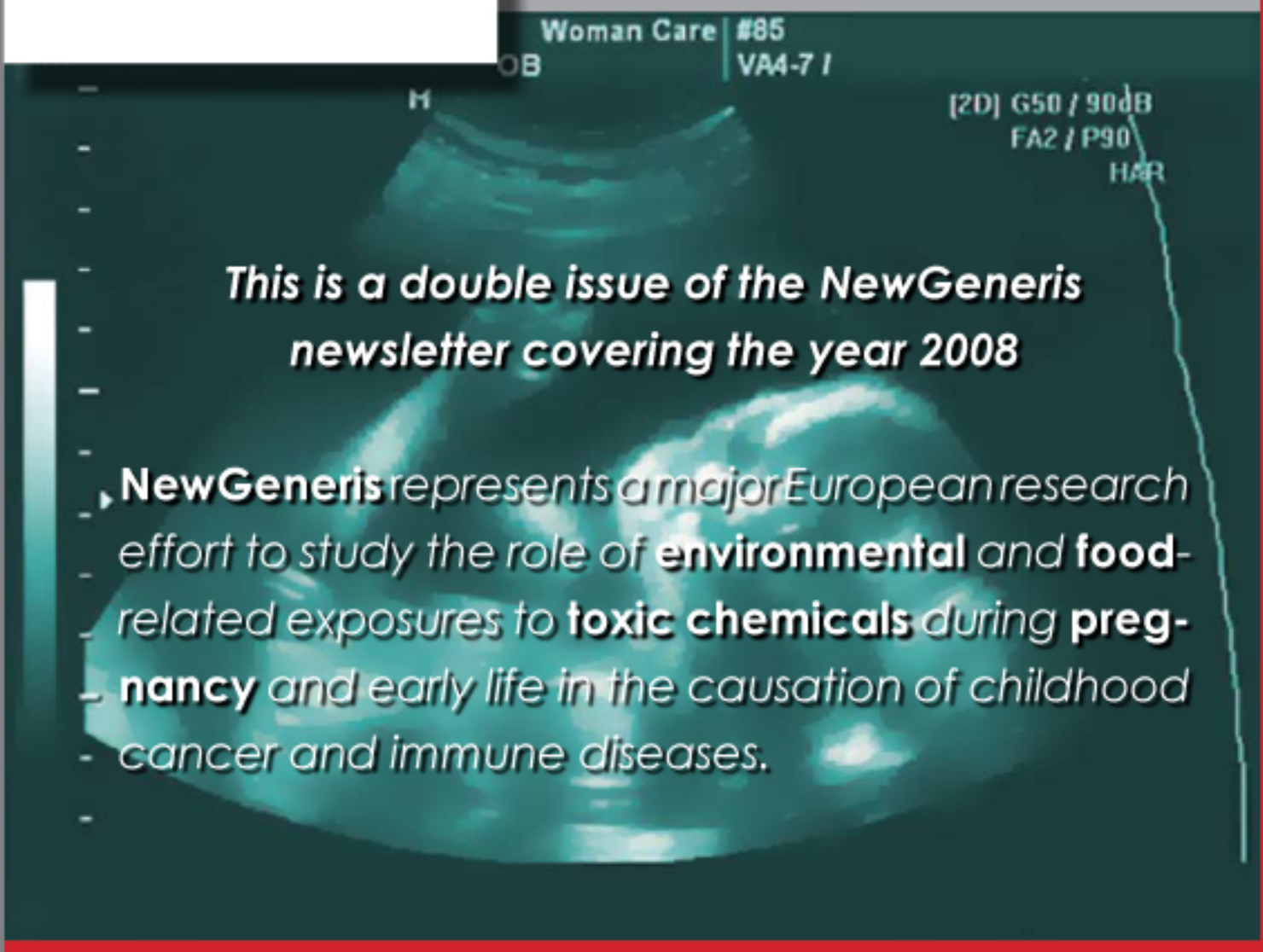
6th Framework Programme

An Integrated Project within the 6th Framework Programme, Priority 5: Food Quality and Safety

Development and application of biomarkers of dietary exposure to genotoxic and immunotoxic chemicals and of biomarkers of early effects, using mother-child birth cohorts and biobanks

NEWSLETTER

December 2008
2-3
Issue



This is a double issue of the NewGeneris newsletter covering the year 2008

***NewGeneris** represents a major European research effort to study the role of **environmental** and **food-related exposures** to **toxic chemicals** during **pregnancy** and early life in the causation of childhood cancer and immune diseases.*

During its third year of life, NewGeneris has made significant progress towards its objectives, including the assessment of dietary exposure of pregnant mothers and the fetus to toxic chemicals of interest, and the development of new biomarkers of exposure and early biological effects of genotoxins and immunotoxins. With the first data already in hand, the network looks forward to continuing at an increasing pace the generation of new data and their utilization to study the health significance of exposure to dietary chemicals.

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News from Science (16-24):

Message from the Coordinator

To learn how the knight moves

In the early days of biomarker research, we were happy to find some exposed people with their matched non-exposed controls, to take their blood samples, and to study some kind of cytogenetic damage through laborious microscopic analysis, thereby ending up, after weeks till months, with a single quantitated parameter, calculated on a piece of paper.

Nowdays, genomics analysis of the same blood sample, could easily bring us thousands and thousands of data, on whole genome gene expression, on the proteome, on the metabonome, more or less within a split second, and we need elaborate hard and soft ware to be able to manage these data. We are convinced that by taking the genomics approach, we are studying gene-environment interactions in a much more relevant way, than by counting, say, sister chromatid exchanges. But we are not completely certain that we understand better. Obviously, we are hampered by the fact that for many, many genes, the expression of which we now can routinely measure by means of microarray technology, we do not know their functionalities, let alone how they interplay in genetic networks, and how disruptions of such networks by adverse environmental factors predispose to disease. And where we can see numerous peaks pouring out of our mass spectrometers, we cannot identify all proteins they are representing, nor do we understand what these proteins may do.

So, it is arguable that we understand less about gene-environment interactions than we did, or we thought we did, in those early days, although we are capable of measuring a hell of a lot more.

In interrogating the huge and multi-layered genomics data bases, it is very difficult to face our lack of knowledge on molecular mechanisms of gene-environment interactions.

A while ago, this started to annoy me, because obviously, I wanted the beauty of these novel technologies to bring us new insights, rather than to point out to us again what we could have read in the toxicological literature. So, how to interrogate the huge data bases without any a priori knowledge, how to grasp hitherto unknown mechanisms?

I posed this question to my colleague at Maastricht University, who holds the chair on Informatics, but in real life, is a professional chess player. “Yes” he said after listening to my explanation, “I understand. You want to learn about unknown patterns in your microarray data which predict disease. That resembles our need in chess where we want to create positions on the board which accurately predict winning the game. So, we fed a lot of chess data into an enormous computer, and we did some pattern analysis. And we found out that the pattern with the highest predictability of winning is the distance in centimeters between the knight and the king !!!“ I must have looked bewildered as he continued: “This undoubtedly is a pattern, but it has no meaning because it does not comply with the rules of the game. Consequently, if you want to retrieve unknown but relevant patterns from your microarray data, you must know the rules of the genetic games”. And that concluded it all.

Where the 19th century is considered the era of chemistry, and the 20th century that of physics, the 21st century may be called the era of biology. For the moment, we do not yet fully know all rules of gene-environment interactions. But because of the genomics boost we are now experiencing, our understanding in how toxicants disturb genetic networks, and how this leads to environmental disease, will dramatically increase. So, in order to be prepared for that, we must analyze, analyze, analyze!! But, okay, that is what being part of a scientific revolution is all about. And that also is what makes the NewGeneris project that challenging.

Jos Kleinjans

Summary

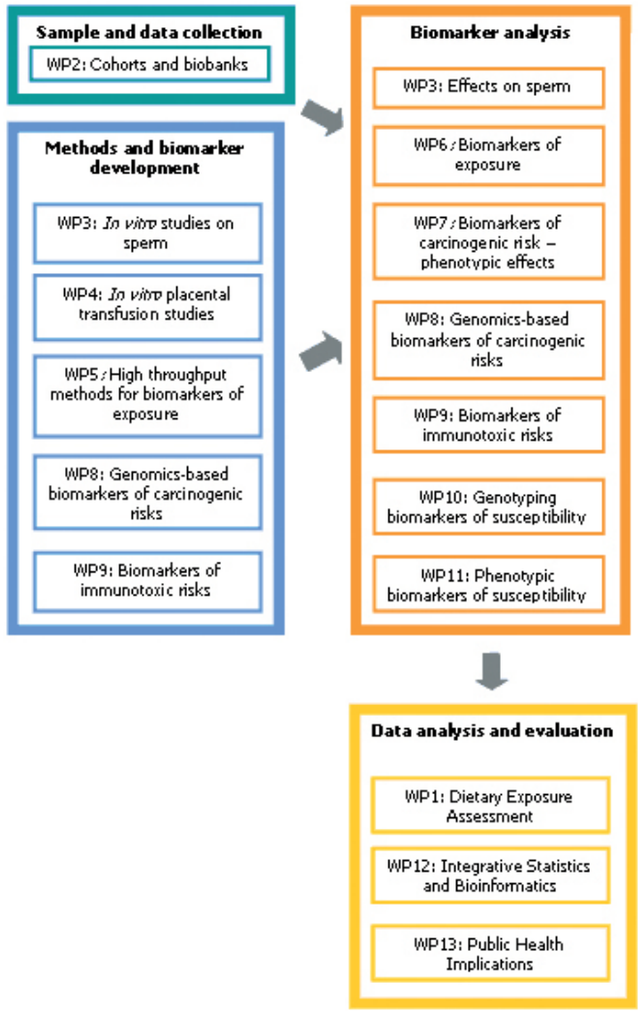
NewGeneris Project Summary

NewGeneris is an Integrated Project conducted within the European Union's 6th Framework Programme, priority area Food Quality and Safety, and is based on the collaboration of 25 institutions from 16 European countries. The project was launched on 1st February 2006 and will run for 5 years.

Objective
Investigate the role of prenatal and early-life exposure to genotoxic chemicals present in food and the environment in the development of childhood cancer and immune disorders (asthma, rhinitis, eczema/ dermatitis)

Chemicals
- polycyclic aromatic hydrocarbons (food, smoking, ambient air)
- heterocyclic amines (food)
- nitrosamines (food, water, endogenous formation)
- acrylamides (food)
- mycotoxins (food)
- organochlorinated compounds as Dioxin, PCBs (food, environment)
- DNA-reactive aldehydes (lipid peroxidation)
- alcohol

Cohorts and Biobanks
Apart from the use of existing ones (Norwegian MoBa, Danish DNBC, UK UKWC, Menorca Spain and German Childhood Cancer Registry) new ones were established:
- The Bradford Multi-ethnic Longitudinal Birth Cohort, UK
- The Spanish general population mother-child cohort (INMA study)
- The Crete cohort (Rhea study)
- The Danish Biobank
- The MoBa Bramat cohort, Norway



Exposure assessment tools
Food Frequency Questionnaires
Database construction

Main tool
Biomarkers of exposure and early effects

Technology
Genomics, transcriptomics, proteomics, DNA and protein adducts, micronuclei

Detailed information on project is found on its webpage

www.newgeneris.org

NewGeneris 2nd Annual Meeting

Athens, 11-14 February 2008

Closing its 2nd year of life, NewGeneris held its Annual meeting in Athens, at the National Hellenic Research Foundation (NHRF), on 11-14 February 2008. The meeting brought together more than 130 collaborating senior and junior scientists coming from the 25 institutions of 16 European countries which participate in the project consortium, as well as members of the external advisory bodies (Scientific, Ethical and Dissemination Advisory Board).

A special Workshop was held during this meeting, under the title of "Systems Biology Approaches to Biomarkers of Environmental Health".

The meeting was opened by the co-ordinator of NewGeneris, Prof. Jos Kleinjans followed by a welcome by Dr. Soterios Kyrtopoulos. Important invited lectures followed and the Workpackages presented their progress, while, in addition, they organised parallel meetings of their members for solving ongoing issues and problems. A number of posters of young scientists was exhibited and a special poster session was organised.

During the General Assembly meeting an overall evaluation of the progress was made and final decisions were taken. The members of the External Advisory Bodies (Dr.Alberto Mantovani, Prof. Riccardo Haupt, Prof. Marja Sorsa, Prof. Elisabeth Rynning and Dr. Marie-Noel Brune) made important remarks and proposals for the further development of the project.

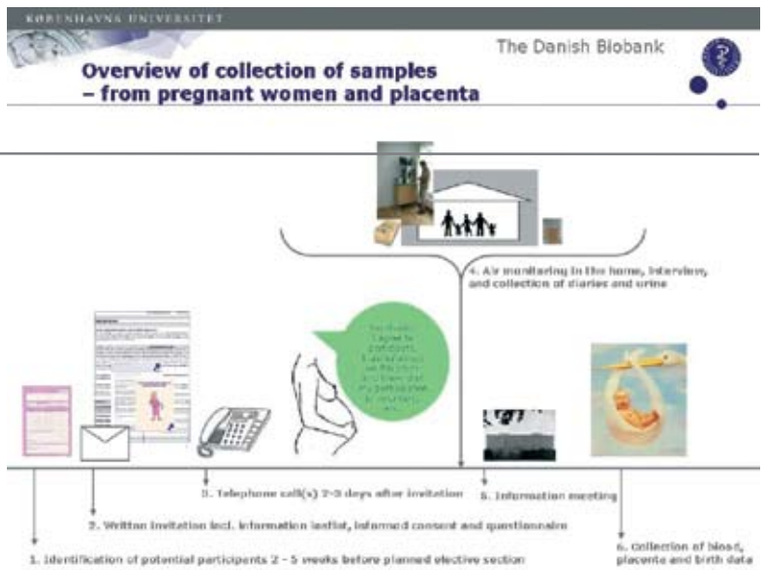
After the intense organizational and preparatory activities of the first year, NewGeneris during its second year of life achieved a very substantial progress on all fronts. Some of the developments include:

- The setting up of chemical content databases for all the chemicals, the exposure database, the Food Frequency Questionnaires Cohort Data and the establishment of exposure assessment methodology based on questionnaires
- The complete establishment of all the cohorts and rapid production of samples
- The substantial progress with the validation of biomarkers for all the substances with analysis of a number of samples (mother and cord blood) coming from several cohorts
- A first approach of relating biomarkers with exposure assessment
- The development of biomarkers/bioassay techniques with improved sensitivity

Following the evaluation of progress and the new plans made during the meeting, NewGeneris moves with confidence towards its third year with a well-designed program, so as to achieve its goal, which is the examination of exposure of the foetus to genotoxic and immunotoxic chemicals and the role of this exposure in the development of childhood cancer and immune diseases.

Some pictures, extracted from the presentations of the workpackages follow, to highlight the work accomplished:

HIGHLIGHTS
of the 2nd annual meeting



WP2 **(M. Kogevinas)**

WP4 **(L. E. Knudsen/ K.Vahakangas)**

Human placental perfusion enables studies on transplacental kinetics of carcinogens including transfer, transfer mechanisms, xenobiotic metabolism and DNA-binding. Food carcinogens reach fetal circulation through full term human placenta.

The results of placental perfusion are related to the substances: Benzo(a)pyrene, PhIP(heterocyclic amines), IQ(heterocyclic amines), acrylamide, TCDD, aflatoxin B1, ethanol.

Placental perfusion system

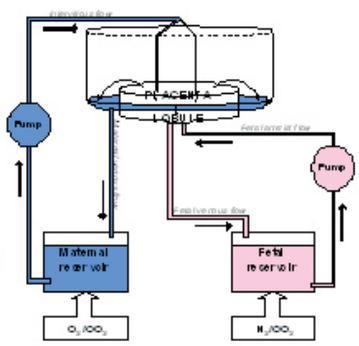
(Pienimäki et al. 1995, 1997)

Krebs-Ringer-phosphate-bicarbonate buffer with heparin, glucose and Dextran

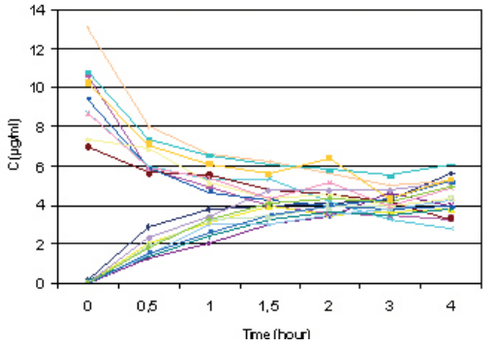
Both maternal and fetal sides perfused

Perfusates recirculated

Criteria for successful perfusions
- leak from fetal to maternal circulation less than 2 ml/h
- fetal pressure less than 70
- blood gas analysis



Transfer of acrylamide from maternal to fetal circulation in human placental perfusion

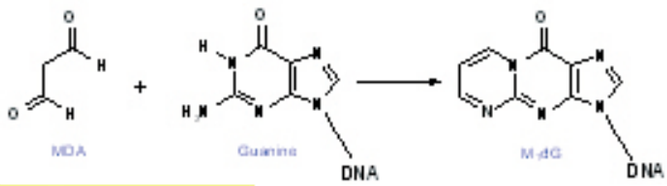


Immunoslot Blot Analysis of M1dG Adducts

N. Brady, University of Leicester, UK (ULEIC, Partner 6).

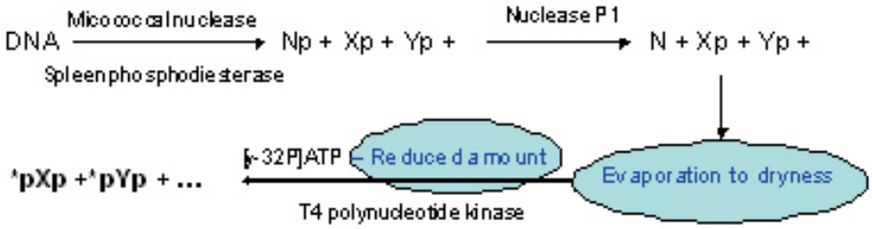
WP5 (P. Farmer)

Examples of methods' development:



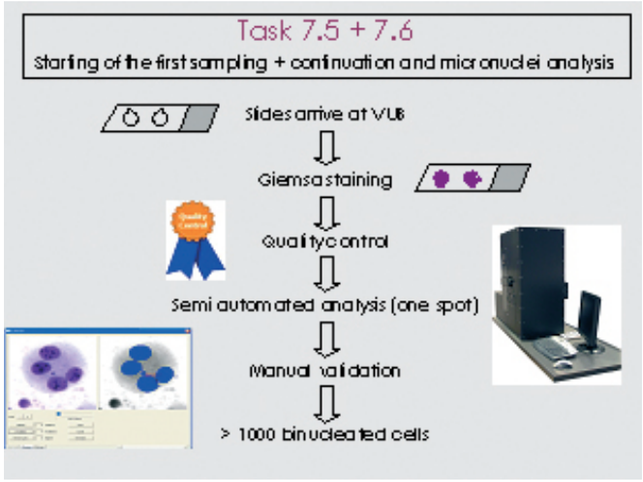
Modification of ^{32}P- postlabelling for determination of PAH-type bulky DNA adducts

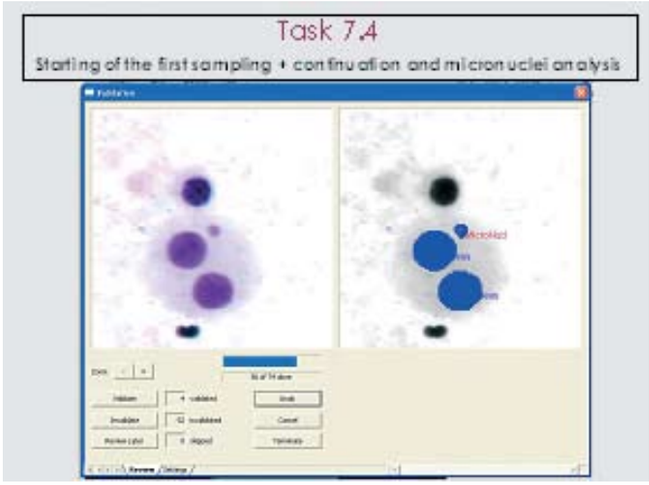
B Schoket, National Institute of Environmental Health, Hungary (NIEH, partner 26, former FJ)



WP7 (M. Kirsch-Volders)

A high throughput automated facility (image analysis) was developed for scoring of the in vitro cytokinesis-block micronucleus assay. The genotoxicity of potential food carcinogens was characterized. Samples of maternal and cord blood obtained from the biobanks of Denmark, Crete and Norway were so analysed for the assessment of DON, BaP, PhIP and TCDD.

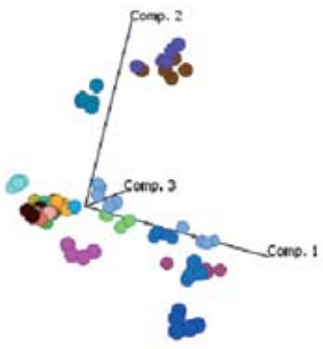


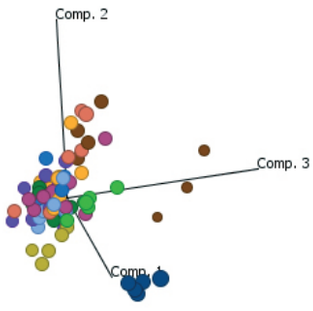


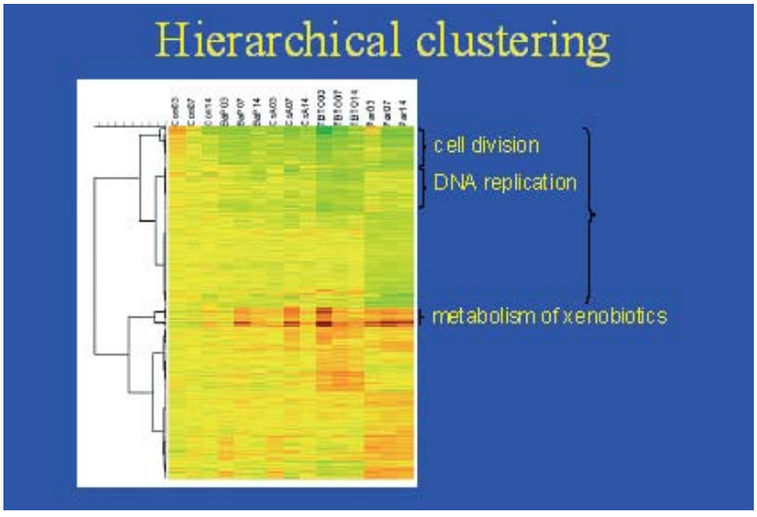
WP8 (J. van Delft)

Development of effect biomarkers based on transcriptome and proteome profiles that are specific for exposure to genotoxic or to non-genotoxic carcinogens. Micronuclei (with WP7) will also be investigated as validated early biomarkers for cancer risk.

PCA: Principle Component Analysis showing some outstanding experiment groups for some compounds (1, 2, 3) of the project:







WP9 (H. van Loveren)

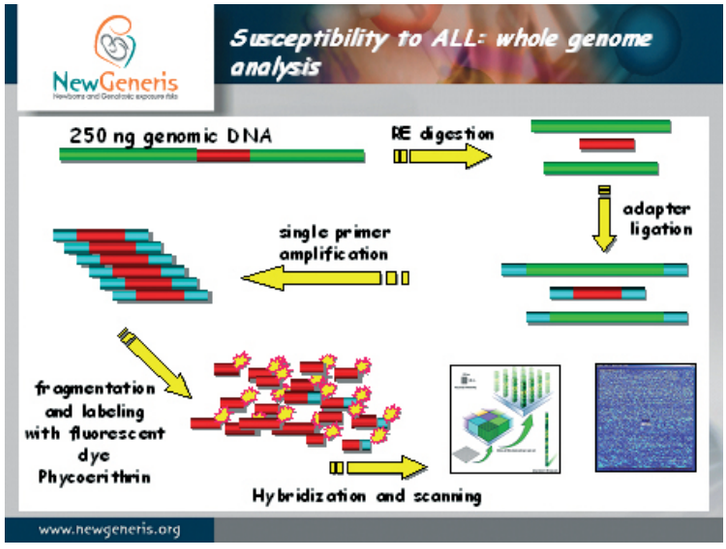
Immunotoxicity
Studies using gene expression profiling

WP10 (Rajiv Kumar)

Genotyping markers of susceptibility

- childhood leukemia
- NewGeneris populations

Acute lymphoblastic leukemia (ALL): most common malignancies afflicting children world wide



NewGeneris 2nd Annual Meeting Poster Session

During the 2nd Annual Meeting of NewGeneris, a special session was organised for the oral presentation by the young scientists of posters with the aim of giving them the relevant experience. The session was presided over by the last year's poster award winners, present at the meeting, Marie Pedersen and Kristine Gutzkow. Twenty- six posters were presented and four of them were selected to receive a prize. The awarding Committee consisted of Maria Dusinska, Alberto Mantovani and Maria Botsivali. The prize winners were:

- Hans von Stedingk (Stockholm University, Sweden) for the poster "Biomarkers of exposure - Analysis of haemoglobin adducts formed from acrylamide, glycidamide and ethylene oxide"
- Halldorsson T. I. (Statens Serum Institut, Copenhagen) for the poster "Maternal Exposure to Dioxin and Dioxin like Compounds: Dietary predictors and infants birth weight"
- K. Hochstenbach (Maastricht University, The Netherlands) for the poster "Genomics-based biomarkers for genotoxic and immunotoxic risks of newborns exposed to chemicals during pregnancy"
- Joost O. Linschooten (Maastricht University, The Netherlands) for the poster "Use of spermatozoal mRNA profiles as exposure biomarker for human germ cells"

The titles of all posters along with their presentations and abstracts are available at News& Events internal section of NewGeneris website

www.newgeneris.org

Workshop

Workshop on Systems Biology








National Hellenic Research Foundation
Athens, 14 February 2008

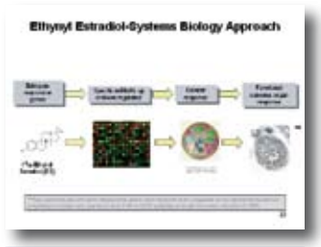
The last day of NewGeneris 2nd Annual Meeting was devoted to a Workshop on "Systems biology approaches to biomarkers of environmental health", organized by S. Kyrtopoulos.

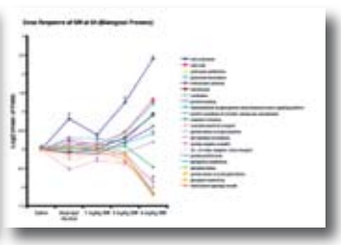
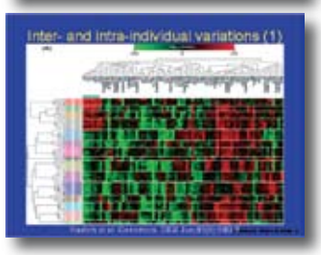
Distinguished scientists in this area were invited and gave very interesting and up-to-date lectures, which were followed by fruitful discussions.

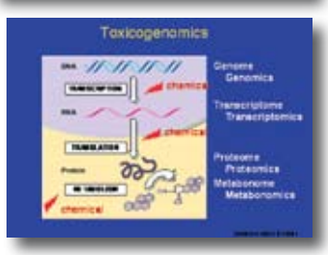
Some of the lectures given were the following:

- "Systems toxicology and its relevance for biomarkers of environmental health" by Dr. C. J. Portier, National Institutes of Environmental Health Sciences, Research Triangle Part, NC, US
- "Transcriptomics as a biomarker for molecular epidemiology on environmental carcinogen exposure, by Dr. J. van Delft, Univ. of Maastricht
- "Epigenomics for biomarker discovery in cancer research", Z. Herceg, International Agency for Research in Cancer, Lyon
- "Genome-wide scanning" by Dr. G. Thomas, US National Cancer Institute, Bethesda, MA, USA
- "Metabonomics" by Dr. Muireann Coen, Imperial College, London
- "The Future of Genomic in Dose Response Modeling: Useful Lessons from Systems Based Approaches", by Prof. Elaine Faustman, University of Washington, US

A dinner was also organised for the invited speakers at the Benaki Museum.





Jagdish Nair Obituary

Dr. Jagdish Nair, one of NewGeneris key members and a formerly researcher at the German Cancer Research Centre (DKFZ), died unexpectedly on August 2007. The gap which his demise leaves will not be easy to fill.

Jagdish Nair will be fondly remembered by his collaborators in the NewGeneris network not only for his important scientific contribution but also for his kind and cheerful character and his willingness to support younger scientists.

He was born in 1953 in India, where he completed his University studies, obtaining his PhD in 1981 from the Cancer Research Institute, of Bombay. He came to Europe in 1983, joining the International Agency for Research in Cancer (IARC), Lyon, were he worked under the leadership of Prof. H. Bartsch. His early studies at IARC focused on the formation of carcinogenic nitrosamines during tobacco chewing. Later, his research switched to lipid peroxidation and its importance in carcinogenesis, an activity which he expanded further after moving to DKFZ in 1994. He became one of the leading figures worldwide in the area of lipid oxidation-induced DNA damage, making major contributions to the demonstration of the accumulation in DNA of etheno adducts and other related types of damage under conditions of oxidative stress. This activity formed the basis of his participation in the NewGeneris project.

During the NewGeneris 2nd Annual Meeting, Dr. Roger Godschalk gave a Memorial Lecture dedicated to Dr. Jagdish Nair, presenting his scientific work in the field of etheno-DNA adducts in degenerative diseases.

Roger Godschalk ended his speech referring to a story that Jagdish Nair had told him during their collaboration: A wise man sits near the bank of a river giving advice to people intending to cross the river. A young man comes and asks for his advice. He tells him that he cannot cross the river. The young man thinks a little, decides to cross the river and succeeds. Dr. Nair used to say the story as an advice to young researchers for surpassing their supervisors and crossing the difficult rivers of research.

A recent article by members of NewGeneris, concerning etheno adducts (p.19), was dedicated to him.

NewGeneris

2nd Annual Review Meeting

Brussels, 23-24 April 2008

The 2nd Annual Review Meeting of NewGeneris was held in Brussels, on 23-24 April 2008 and was attended by the project's Workpackage leaders, the 2 reviewers, Prof. H. Autrup and Prof. Francesca Pacchierotti, and the Project Officer Domenico De Martinis.

The general conclusion was that "the project, despite its complexity, has been fairly successful, well-structured and managed", with satisfactory progress concerning its first results (questionnaire validation and biomarker analysis).
Some constructive recommendations were made by the reviewers.

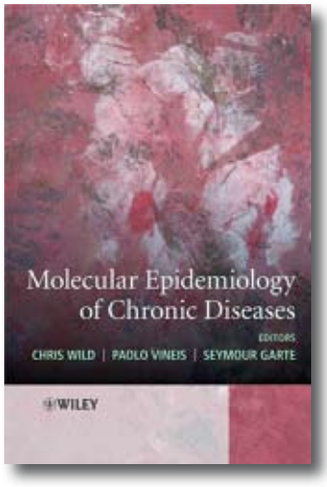
News News

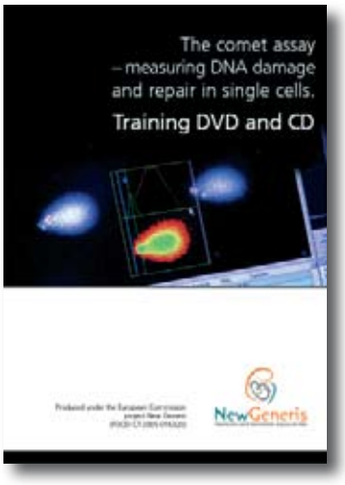
New IARC Director: Prof. Christopher Wild, currently PI for the University of Leeds in the NewGeneris network, was recently elected as the new Director of the International Agency of Cancer (IARC) in Lyon. Prof. Wild has a long-standing interest in research on the use of biomarkers to study the interaction of environmental and genetic risk factors in cancer.

Following his election, Prof. Wild has declared that he wishes to see IARC develop into a center for collaborative research efforts and to further integrate laboratory science and population-based research in order to achieve the goal of cancer prevention. NewGeneris looks forward to contributing to these efforts and wishes him success in his new position.

A new book: "The Molecular Epidemiology of Chronic Diseases"

A new book on Molecular Epidemiology edited by two NewGeneris members, C.Wild and P. Vineis, has been recently published.
The book covers categories biomarkers of exposure, susceptibility and disease, novel technologies such as genomics, transcriptomics, proteomics and metabonomics as well as new statistical and bioinformatics approaches. It also explores, among others, practical elements of conducting population studies, including biological repositories and ethics.





Training by DVD/CD: COMET ASSAY

A training DVD was produced at the University of Oslo by Prof A. Collins and his team in the framework of NewGeneris for the benefit of scientific community. More information can be found on the NewGeneris webpage.

2nd Congress of the European Academy of Paediatrics Meeting, Nice, France, 24-28 October 2008 NewGeneris was presented at the above Congress by a poster of Prof Jos Kleinjans and DF Merlo

NewGeneris

Statistical Analysis Meeting

University of Leeds, 13 June 2008

In the meeting participated: Diana Anderson, Adi Baumgartner, Maria Botsivali, Victoria Burley, Eduardo Cemeli, Sarah Hepworth, Patricia McKinney, Franco Merlo, Renee Mijal, Marie Pedersen, Pauline Raynor, Catherine Reynolds and Chris Wild.

A major challenge for NewGeneris concerns the handling and analysis of very large amount of data coming from different cohorts. Some of the topics discussed were the sharing of these data, the operation of the database, the strategies to ensure high quality results from across all the cohorts, the validation studies concerning the correlation of biomarkers (laboratory results) with the exposures calculated through food frequency questionnaires as well as the need for reviewing the exposure data (cooking methods, mixed dishes, added fats, scenarios etc).

The statistical approaches for handling the data were discussed, as well as factors needed to address susceptible groups and how the results extrapolated back to the population level (e.g. European population).

Data so far available on exposure assessment and biomarker levels were discussed, as well as the use of game theory analysis to look at the interaction of different genes and its application to NewGeneris experimental data.

The whole statistical plan was discussed in depth.

NewGeneris Symposium at *EUROTOX 2008*

Rhodes, Greece, 5-8 October 2008

In the context, of the 45th Congress of the European Societies of Toxicology (EUROTOX 2008), held in Rhodes on October 5-8, 2008, NewGeneris organized a Symposium on **"Early life exposures to Environmental Chemical and Chronic Disease"**.
The symposium was co-chaired by Soterios A. Kyrtopoulos and Jos Kleinjans, and the topics presented were the following:

Biomonitoring at the beginning of life to assess environmental factors influencing children's health (Gurdrun Koppen Technological Research, Mol, Belgium)

Children's exposure to environmental chemicals (Rudolf Rull, Northern California Cancer Center, Berkeley, USA)

Fetal exposure to environmental toxicants (Lisbeth E. Knudsen, Denmark Center for Health and Society, Institute of Public Health, Denmark)

Application of biomarkers to investigate the role of environmental exposures in childhood disease (Jos Kleinjans, Maastricht University, The Netherlands)

Early life exposures and subsequent disease: Results from the Avon Longitudinal Study of Parents and Children (ALSPAC) (Andy Ness, Department of Oral and Dental Science and Department of Social Medicine, Bristol Dental School, UK)

NewGeneris Dissemination Meeting (*WP16*)

Brussels, ILSI, 10 July 2008

The meeting was organized by the dissemination officer M. Botsivali, aiming at a special collaboration with the members of the external advisory board and was attended too by the members of Workpackage 16 and the project manager Rene Reijnders

The members of this External Advisory Board are:
- Dr. Ursula Mathar, CEFIC
- Dr. Christian Farrar-Hockley, EPHA/EEN
- Dr. Nico van Belzen, ILSI Europe:
- Prof. Jean Golding, ALSPAC
- Dr. Marie-Noel Brune, WHO
- Dr. J. W. Wieke Tas, VWS

Topics that were discussed concerned the publicity of NewGeneris objectives, the promotion of contacts with different stakeholders and collaboration with other research projects, the organization of workshops and further development of the website (www.newgeneris.org)

Food Chemical Risk Assessment Research Workshop

EC, DG Research, Brussels, 9 September 2008

The workshop was organised by DG Research, Unit E3, Food-Health-Well-being, (Scientific Officer Dr. Ebba Barany), in Brussels. Its aim was to discuss the risk assessment related activities of various ongoing research projects.
In the Workshop participated the EU Projects SAFE FOODS, FACET, CASCADE, ATHON, NEWGENERIS, PHIME, ECNIS, and HI-WATE.
It was attended by the Head of the Unit Antonio Di Giulio and other EC officials from the DG Research, DG SANCO and Environment.
The presentations given were:

Risk of combined exposure of chemical contaminants and natural toxins (Jacob Van Klaveren, SAFE FOODS)

Food chemical surveillance (Mike Gibney, FACET)

Risk assessment of different categories of chemicals in food (Helen Håkansson, CASCADE and ATHON)

Risk assessment approaches of the NewGeneris Project (Maria Botsivali, NEWGENERIS)

Long-term low-level cadmium exposure and health effects (Agneta Åkesson, PHIME)

Carcinogenic mechanisms and their impact on hazard and risk assessment with special emphasis on low levels and on the action of mixtures (Franz Oesch, ECNIS)

Risk-benefit assessment of disinfection by-products and microbes in drinking water (Päivi Meriläinen, HIWATE)

A general discussion and conclusion followed concerning "Future aspects of food safety research: Research on hazards or risks".

Meeting

NewGeneris Exposure Assessment Review Meeting *(WP1)*

Athens, 30-31 October 2008

A review meeting was organized in Athens by M. Botsivali with the participation of all the WP1 members: Soterios Kyrtopoulos, Maria Botsivali, Nektaria Polychronaki, Sarah Hepworth, Victoria Burley, Franco Merlo, Theo de Kok, Simone van Breda, Margaretha Haugen, Marie Pedersen, Thorhallur Halldorsson , Michelle Mendez, Talita Duarte, Katerina Sarri, Lilian Papadopoulou.

The aim of this meeting was to finalize the content of the database kept in Leeds and the methodology employed for exposure calculations. Among the items on which discussions focused were included:

- Food Lists (Food grouping/subgrouping)
- Mixed dishes
- Added fats and oils
- Cooking methods and doneness
- Scenarios for the calculation of exposures
- Correlation of biomarkers with exposures

Special attention was given to the updating of the data related to the levels of chemicals in foods, through re-examination of the literature and revising of their code numbers. For example, for PAHs, the current EFSA's Report (June 2008) was decided to be utilized.

	Units	ug/kg
PAHs		
Food description	Codes	
	PAH - main analysis	PAH - Sensitivity
Meat - Smoked and Barbecue meat (any kind)	1	1
Meat - Meat excluding Smoked, Barbecued; Sausages; Chicken and Poultry	1	2
Meat - Sausages	1	3
Meat - Chicken + Poultry (not smoked)	1	4
Fish - All types Smoked	2	5
Fish - Mussels	2	6
Fish - All types excluding Smoked and Mussels	2	7
Cereal products - Breakfast cereals	3	8
Cereal products - All types of Bread but whole	3	9
Cereal products - Whole bread	3	10
Cereal products unspecified	3	18
Fruits - Dried coconuts and dried fruits	4	11
Fruits - All, excluding dried coconuts and dried fruits	4	12
POTATOES AND TUBERS	5	14
VEGETABLES	6	15
MILK and DAIRY Products	7	17
EGGS	8	21
FATS ADDED	9	22
SWEET AND DESSERTS	10	23
ALCOHOLIC BEVERAGES	11	24

1st International Conference on Risk Assessment

"Global Risk Assessment Dialogue" EC, DG SANCO

Brussels, 13-14 November 2008

The Conference was organised by the Directorate-General for Health and Consumers of the European Commission, in close collaboration with the Directorates for General Industry and Enterprise, Environment, Employment, Research and the Commission Joint Research Centre.

The purpose of this Conference was to facilitate a global dialogue on risk assessment between EU and its main international partners as US and Canada.

The participants exchanged views on the issues of risk assessment, communication and emerging risks, and gave information on risk management, risk analysis principles, and regulations in their countries.

The ultimate goal was to enhance understanding of different methodologies, tools, uncertainties and needs and, eventually, make progress towards improving use of exposure assessment to inform risk assessments and regulatory decision making.

The Conference was addressed by the Commissioner for Health, Androulla Vassiliou, who expressed her full support for this Global Dialogue. The Conference presentations proceeded as follows:

In the first place presentations were given, concerning the Regulatory Risk Assessment process in EU, US, Canada, Japan, China, Australia, and Russia.

Additional presentations addressed Policy issues, such as the relationship between Risk Assessment and Risk Management, while more specific topics were explored through presentations of the following content:

Expression of uncertainties
New and emerging risks related to chemical physical and biological agents
Assessment of carcinogens and mutagens

Parallel sessions were organised for more in depth discussion of:

Terminology
Emerging issues and challenges
Non-Threshold Carcinogens
Exposure Assessment

Dr. Maria Botsivali, the NewGeneris Dissemination Officer, participated in this Conference.

Smoking pregnant women and vulnerable neonates

The impact of maternal exposure to carcinogens during pregnancy, on childhood cancer risk, may be especially relevant for infants that are genetically susceptible and therefore sensitive, although there is sufficient indication that there is profound concern for genetic risks in neonates generally of mothers exposed to mutagens during pregnancy.

A molecular epidemiological approach, which has the potential to characterise processes between exposure and subsequent health effects in newborns by using biomarkers, is expected to provide valuable information for identifying such vulnerable neonates.

Several kind of biomarkers have been studied such as biomarkers of exposure (e.g. cotinine and metals in cord blood), biomarkers of the biologically relevant dose (e.g. DNA and protein adducts) and biomarkers of early effects (e.g. occurrence of somatic mutations in cord blood). The application of similar biomarkers to cohorts of mothers and their newborn children is one of the major goals of NewGeneris project.

In a recent Mini Review(1) the most important data concerning such biomarkers studies, related to neonates, were summarized and compared to a study (59 mother-child pairs) in which a wide variety of these biomarkers were assessed simultaneously. This group consisted of mothers smoking through pregnancy, mothers having stopped smoking through the first pregnancy semester, and some who were never smoking.

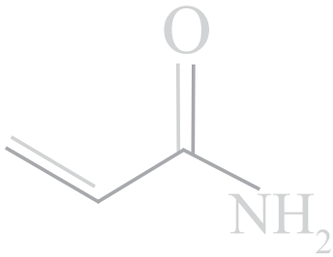
From this study it is concluded that plasma cotinine levels, carcinogen –adduct levels and the frequencies of mutant hypoxanthine phosphoribosyltransferase (HPRT) are increased in cord blood of neonates of exposed mothers and were related to reduced birth weight. By this study was also shown that it is possible to identify a susceptible group of newborns and that the adverse effects of maternal cigarette smoking may be more relevant in subsets of susceptible newborns. Overall, it seems that some neonates may be more susceptible than others and an individualised approach could identify these susceptible subgroups.

Relatively few studies have examined the genotoxic effects of maternal smoking on the foetus and its health implications. Nonetheless, new methods and end-points, especially genomic methods and the establishment of biobanks, as the ones established by the NewGeneris project, offer hope for progress.

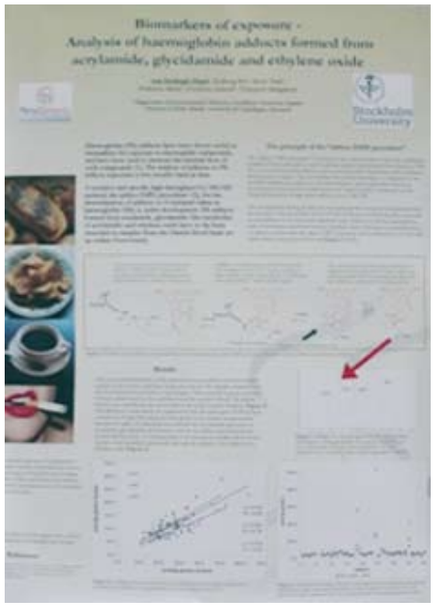
Reference (1): Godschalk RW, Kleinjans JC. "Characterization of the exposure-disease continuum in neonates of mothers exposed to carcinogens during pregnancy." Basic Clin Pharmacol Toxicol. 2008; 102(2):109-17.

(Article on www.newgeneris.org / publications of members)

Presented by M.B., NHRF



Acrylamide in food



Acrylamide is a large-scale industrial chemical commercially available since 1950s. The compound has shown a spectrum of toxic effects in animal experiments. In humans with occupational exposures to acrylamide, neurotoxic effects have been observed, while the compound is classified as probable human carcinogen by IARC.

It was an unexpected finding that acrylamide is formed during cooking at high temperatures in foods, such as potato crisps, bread, coffee, etc. The precursor is the amino acid asparagine, which forms acrylamide after reaction with reducing sugar. This occurs in the complex Maillard reaction, which is the major route to flavour and browning in cooked foods. By now, the average daily intake of acrylamide has been estimated to be about 0.5 µg/kg body weight in several countries.

The finding, that food is a general exposure source of acrylamide, initiated research in many fields, for instance biomarkers and epidemiology. Epidemiological studies have not shown any association between increased cancer risk and intake of acrylamide, except the two most recent studies where increases of cancer risks have been found. One of these studies measured the exposure to acrylamide through haemoglobin adducts in addition to food frequency questionnaires. It is intriguing that the observed cancer risks are high compared to the risk estimate for acrylamide from animal cancer tests.

It is considered that the mutagenic metabolite, glycidamide, is the cancer risk factor in acrylamide exposure. There is also an ongoing debate whether a non-genotoxic mechanism contributes to the outcome in published cancer tests. Cancer tests within the US National Toxicology Program, soon completed, will most probably give a conclusive answer to this question.

Acrylamide in food and health risks is an interesting issue. For instance, could it ever be possible to prove by epidemiological methods, a cancer risk from a compound like acrylamide in food? The exposure yields a relatively limited range and probably the compound occurs simultaneously as other potential risk factors. At the present stage of knowledge, it could not be ruled out that acrylamide in food constitutes a cancer risk of non-acceptable magnitude, though it will be difficult to prove by epidemiology.

Acrylamide is one of the chemicals studied in NewGeneris by measuring haemoglobin adducts in mother's and umbilical cord blood.

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References: Dybing, E. et al.: "Human exposure and internal dose assessments of acrylamide in food." Food Chem. Toxicol. 43 (2005) 365-410.

Thonning Olesen, P. et al.: "Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health Study." Int. J. Cancer (2008)
(Article on www.newgeneris.org / publications of members)

Measuring folate stores in mothers and their newborn babies

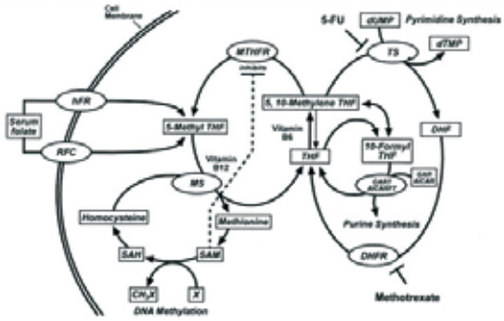
Folate, a B-vitamin, fulfils many important roles in cell function, in particular in the maintenance of DNA stability. A growing body of evidence indicates that deficient supplies of folate may contribute to the development of several human diseases.

The increased maternal metabolic rate and foetal demands during pregnancy require high levels of B vitamins such as folate. An adequate maternal supply of folate is highly important for the health of the foetus, both in the preconception period and later during pregnancy.

Deficiencies of micronutrients as folate causing genome damage may induce developmental defects in the foetus or increased risk of cancer in the child. Folate plays a major role in the prevention of neural tube effects, Down syndrome, other congenital malformations, low birth weight as well as early pregnancy loss. It was shown that neural-tube defects in folate deficient human foetuses coincide with increased genome damage and an increased risk of childhood leukaemia and brain tumours in children of mothers with folate deficiencies.

Furthermore it is know that folate deficiencies in adults may induce Alzheimer disease, cardiovascular diseases and cancer. These deficiencies in folate can lead to i) elevated DNA damage rate and altered DNA methylation, both important risk factors for cancer and ii) an increased level in homocysteine (HC) status, an important risk factor for increased risk of cardiovascular disease. Folate levels can affect MN (micronuclei) frequencies, which are known to be predictive for cancer risk.

Since the major objective of NewGeneris is to investigate the effects of maternal diet on newborn children and the relation with increased risk of cancer, folate levels will be analysed in both mothers and children. Therefore folate stores will be measured in



erythrocytes of mothers and their newborn children from the different European cohorts that are studied within NewGeneris.

Some of the references used:

Leopardi P., Marcon F., Caiola S., Cafolla A., Siniscalchi E., Zijno A., Crebelli R. (2006) "Effects of folic acid deficiency and MTHFR C677T polymorphism on spontaneous and radiation-induced micronuclei in human lymphocytes." Mutagenesis, 21, 327-33.

Thompson J.R., Gerald P.F., Willoughby M.L., Armstrong B.K. (2001) "Maternal folate supplementation in pregnancy and protection against acute lymphoblastic leukaemia in childhood: a case-control study. "Lancet, 358, 1935-40.

Bonassi S. et.al. (2007) "An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans." Carcinogenesis, 28, 625-31. *(Article on www.newgeneris.org / publications of members)*

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DNA oxidative damage and etheno adducts in the blood of mothers and newborns

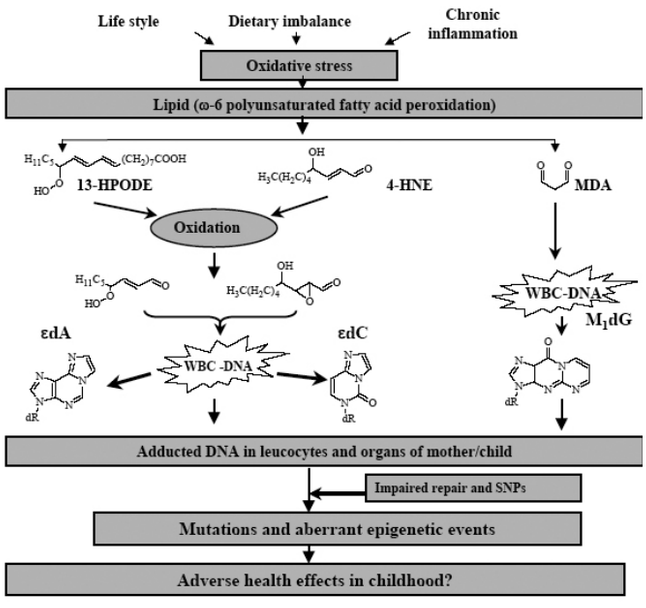
The impact of DNA damage, commonly thought to be involved in the causation of chronic degenerative diseases, is particular detrimental during fetal development. It has been shown that for some kind of DNA damage the fetus may be even 10 times more susceptible to DNA damage.

A typical signature of DNA damage, particularly related to oxidative stress, involves etheno-DNA adducts (εdA, εdC), which are generated by the reaction of lipid peroxidation (omega-6 polyunsaturated fatty acids) products, as the 4-hydroxy-2-nonenal (4HNE), with the DNA bases. Oxidative stress may be caused by the life style, dietary imbalance or chronic inflammation.

A relevant pilot study (1) has been recently conducted in the context of the NewGeneris project. The above mentioned DNA-adducts were measured with a very specific and sensitive method in the blood (white blood cells) of 77 Danish pregnant women and in the cord blood (related to the newborn).

In this study, the DNA-adducts of the cord blood were about two times lower than those of mothers, showing in the first place a protective role of placenta. On the other hand, it is for first time shown that the lipid-peroxidation induction of DNA damage occurs in the WBCs of both mother and newborn while a standard relationship of two kind of adducts (εdA, εdC) could serve as a special signature for this type of damage and these two adducts could serve as reliable biomarkers.

Further studies within NewGeneris project will seek to relate these findings to diet and lifestyles while prospective cohort studies are needed to reveal whether these two adducts could serve as risk indication for developing hematopoietic cancers and other disorders later in life.



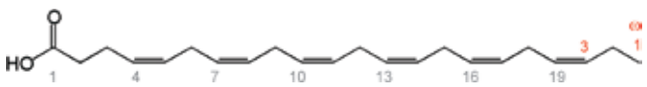
Reference: Arab K, Pedersen M, Nair J, Meerang M, Knudsen LE, Bartsch H. "Typical signature of DNA damage in white blood cells: A pilot study on etheno adducts in Danish mother-newborn child pairs." Carcinogenesis Advance Access published November 26, 2008

(Article on www.newgeneris.org / publications of members)

Presented by M.B, NHRF

Maternal fish and other seafood intakes during pregnancy and child's neurodevelopment

Although generally pregnant women are advised to limit seafood intakes because of possible neurotoxin contamination (as by methyl mercury), there are studies suggesting that overall maternal seafood intakes are also associated with improved child neurodevelopment, perhaps because of higher intake of DHA (Docosahexaenoic acid), an omega-3 fatty acid.



It is obvious that there is need for weighting the potential risks as well as the benefits of fetal exposure to substances found in food.

Last year, US and UK government agencies have issued advice that pregnant women limit seafood intakes to 340 g or about 3 servings per week. In a recently published study (1) involving 482 pregnant women constructed in the Spanish Mediterranean island of Minorca, a setting where seafood consumption is common, it was examined the relationship between consumption of fish and other seafood in pregnancy and children's performance in cognitive and motor skills tests (relevant to neurodevelopment) at age of 4 years.

The results of this study, suggest that moderately high maternal fish intakes in pregnancy are associated with enhanced intellectual development in children, although it is uncertain whether intakes exceeding current recommendations are beneficial.

Future studies in larger samples are needed to further explore relationships between neurodevelopment and maternal fish intake and elucidate the pathways involved relevant to neurotoxic (risk) and non-neurotoxic (beneficial) compounds.

Reference (1): Mendez MA, Torrent M, Julvez J, Ribas-Fitó N, Kogevinas M, Sunyer J. "Maternal fish and other seafood intakes during pregnancy and child neurodevelopment at age 4 years." Public Health Nutr. 2008:1-9.

(Article on www.newgeneris.org / publications of members)

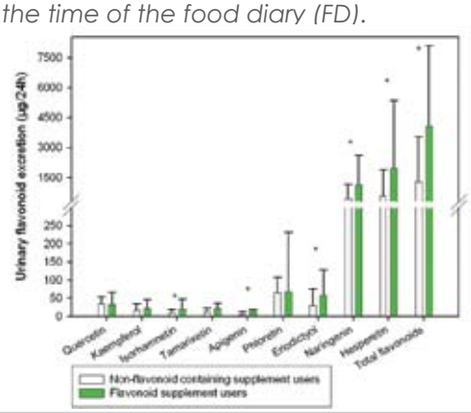
Presented by M.B, NHRF

Intake of fruit, juice, vegetables and tea by pregnant women and biological markers

NewGeneris has toxic substances in food as a main focus, but attention is also given to protective compounds in the diet. Antioxidants are supposed to be among the most important of these, including several vitamins and non-nutrients such as a number of flavonoids, which are polyphenolic substances. As fruit and vegetables are important carriers of many antioxidants, it is important to clarify by validation studies if reported consumption data can be trusted in the different cohorts, as it is MoBa that participates in NewGeneris.

A validation study with 119 women in MoBa was carried out. In the study, the intake of fruits, vegetables and tea estimated by FFQs (Food Frequency Questionnaires) was compared with urinary flavonoid excretion, plasma carotenoid concentration and the intake measured by a four-day weighed food diary (FD).

Figure: Twenty-four-hour urinary excretion of 9 flavonoids in nonflavonoid and flavonoid supplement users at the time of the food diary (FD).



In this study, nine flavonoids were determined in 24-hour urine samples and six carotenoids were determined in plasma samples. The amounts of excreted flavonoids as well as the concentration of carotenoids in the pregnant women were significantly higher in women who reported intake of a dietary supplement containing either flavonoids or carotenoids. However, independently of dietary supplement use, the study demonstrated an association of fruit, juice, vegetable and tea intake with the biomarkers in both urine and plasma. The strongest correlations were found between citrus fruits/juices and citrus flavonoids, and between all fruits, phloretin, a flavonoid found in apple. Fruit and juice intake was more strongly correlated with flavonoids, and vegetable intake with carotenoids.

Overall, urinary flavonoids were more closely correlated with the sum of all fruit and vegetables than were plasma carotenoids. The results also showed good agreement between the FFQ and the food diary for self-reported intakes of fruit, juice, vegetables and tea. FFQs, so, proved to be a reliable instrument for this case of food intakes.

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Some of references used:
1. Brantsæter AL, Haugen M, Rasmussen SE, Alexander J, Samuelsen SO & Meltzer HM (2007) Urine flavonoids and plasma carotenoids in the validation of fruit, vegetable and tea intake during pregnancy in the Norwegian Mother and Child Cohort Study (MoBa). Public Health Nutr. 10, 274-283.
2. Brantsæter AL, Haugen M, Alexander J & Meltzer HM (2008) Validity of a new food frequency questionnaire for pregnant women in the Norwegian Mother and Child Cohort Study (MoBa). Matern Child Nutr. 4, 28-43.

(Articles on www.newgeneris.org / publications of members)

Dioxin-like activity in plasma of pregnant women and infant development

Dioxins (PCDDs, PCDFs, TCDD) are formed as byproduct in various industrial processes, one of them being the waste incineration. There are also in the environment some other compounds as PCBs, with dioxin-like properties.

Maternal exposure to dioxins and dioxin-like compounds has been related to a number of adverse health outcomes in infants. These adverse outcomes may be related to fetal growth retardation, delayed psychomotor development, poorer cognitive development or even impaired immune functions.

All these compounds because of their lipophilic nature and stability accumulate through the food chain and WHO has set for them a tolerable daily intake. It has been estimated that diet, particularly foods of animal origin, contributes to around 90% of human exposure. In some exposure studies a positive correlation has often been observed between fish intake and concentrations in blood (plasma) or even human milk.

A recent study(1), supported partly by NewGeneris, involves a number of pregnant women coming from the Danish National Birth Cohort and explores the relationship between food intake through Food Frequency Questionnaires and analysis of their blood for dioxin and dioxin-like compounds, and birth weight as well as infant's development at 6 months.

This study suggests that fat intake through red meat, fats and oils is associated with higher dioxin-like activity in plasma compared to a diet that is low in fat but high in fatty fish, fruits and vegetables.

A positive association between plasma dioxin-like activity and fatty fish was not detected, partly due to lower fat intake among high-fatty fish consumers. It was obvious that when estimating this kind of exposure it is necessary to take into account the whole dietary pattern.

In this population the dioxin-like activity was not related to birth weight but might be related to children's early motor development. Further work is needed and could be of value.

Reference (1): Halldorsson TI, Thorsdottir I, Meltzer HM, Strøm M, Olsen SF. "Dioxin-like activity in plasma among Danish pregnant women: Dietary predictors, birth weight and infant development." Environ Res. (2008)
(Article on www.newgeneris.org / publications of members)

Presented by M.B, NHRF

Polycyclic aromatic hydrocarbons and detection of carcinogen DNA adducts

PAHs are ubiquitous environmental pollutants which are formed in incomplete pyrolysis of organic materials. Sources include industrial activities, residential heating with coal and wood, vehicle exhausts and tobacco smoke. PAHs from environmental pollution can contaminate cereals, vegetables and fruits. PAHs are produced in meat and fish that are grilled or barbecued over a high-temperature direct flame. Several PAHs are potential carcinogens. It is important to measure the exposure of humans and to intervene where possible to decrease PAHs exposure and related health risk.

Consumption of charbroiled red meat and meat-derived PAHs has been associated with risk of colorectal adenoma, a precursor of colorectal cancer. White blood cell PAH–DNA adduct levels have been demonstrated to increase in response to charbroiled red meat intake. In a recent study, the relationship between white blood cell PAH-DNA adduct formation and colorectal adenoma, was investigated in a clinic-based case–control study by using competitive chemiluminescence immunoassay. There was a positive association between PAH–DNA adduct level and adenoma prevalence.

PAH-DNA adducts are formed by binding of PAHs' metabolic activation products to DNA. DNA adduct formation is a key event in the initiation of adverse cellular processes that may lead to carcinogenesis if such DNA damages are not repaired by protective mechanisms of the cells. DNA adducts serve as biomarkers of exposure and their levels are an integrated measure of both exposure and metabolism, reflect intake of the parent compound as well as biotransformation, DNA repair capacity and cell turnover; whereas assessment of exposure by the dietary questionnaire reflects only exposure.

Methodology of carcinogen-DNA adduct determinations has developed substantially during the recent two decades, particularly for the applications in human biomonitoring. Immunoassays are very sensitive DNA adduct measurements which are used in the methodological arsenal of NewGeneris. Mother's and umbilical cord blood are examined by such assays for the presence of PAH-DNA adducts.

References:
Biomarkers of carcinogen exposure and early effects: Eds. Peter B. Farmer and Jean M. Emery, ECNIS, 2006, (www.ecnis.org).

World Cancer Research Fund/American Institute for Cancer Research: Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington DC, AICR, 2007.

Marc J. Gunter, Rao L. Divi, Martin Kulldorff, Roel Vermeulen, Kathryn J. Haverkos, Maryanne M. Kuo, Paul Strickland, Miriam C. Poirier, Nathaniel Rothman and Rashmi Sinha: Leukocyte polycyclic aromatic hydrocarbon–DNA adduct formation and colorectal adenoma. Carcinogenesis 28:1426–1429, 2007.

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Handling ethics within NewGeneris

Within NewGeneris personal data and biological samples from several European mother-child birth cohorts are analyzed and biobanks are being set up in different European regions.

In this perspective, transfer of personal data and biological samples from one Member State to another is inevitable common practice. From an ethico-legal perspective however, the conditions for transfer of data and/or samples need to be clear and straightforward. Domestic legislation and rules are not always easily applicable in international research. There seems to be considerable difference in ethical assessments of similar research activities between different countries and even within countries.

In the context of the complex handling of general ethical aspects in international research, one of the aims is to assist to facilitate these practices at project level. To this end, a central ethics dossier is provided that should support the application to the local ethics committees by researchers using personal data and samples from foreign cohorts. For each cohort, the dossier has copies of the ethical permits, the information sheets, the informed consent forms and the notification to the privacy authorities. A covering letter explains in more general terms the ethics management within NewGeneris.

Participation in these kind of studies is always on a voluntary basis. To allow further research in this field and a wide participation, whilst preserving rights and addressing concerns of every study partcipant, more insight is sought for expectations of study participants and for reasons why potential study participants decide either to participate or to refuse participation.

In general, the objective of the "Ethics" work is to identify possible obstacles and levers to 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 research using mother child cohorts and biobanks.

The organization and implementation of these procedures have become effectual within NewGeneris. In view of striving for transparency and openness, information concerned is also available to the general public, via the website.

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NewGeneris

3rd Annual Consortium Meeting

Stockholm University, Stockholm, Sweden February 2-5, 2009

The 3rd Annual Consortium Meeting will take place at the Stockholm University, Sweden, and it is scheduled to proceed in six main thematic parts:

Part 1: "Exposure assessment in NewGeneris"
Part 2: "Childhood disease cohorts"
Part 3: "Gene-environment interactions"
Part 4: "From samples collection to scientific conclusions: what might we learn from NewGeneris and how do we get there?"
Part 5: "Training and Dissemination"
Part 6: "Ethical implications within NewGeneris"

A Poster Session is also included.

The Meeting will close with a Workshop on "Oxidative stress in newborns and consequences for children's health", organised by Prof Micheline Kirsch-Volders and Prof. Ola D. Saugstad.

More information is available on the website

www.newgeneris.org

December 2008
Issue 23

NewGeneris
Newborns and Genotoxic exposure risks

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Project title: NewGeneris: Newborns and Genotoxic exposure risks: Development and application of biomarkers of dietary exposure to genotoxic and immunotoxic chemicals and of biomarkers of early effects, using mother-child birth cohorts and biobanks • Integrated Project, 6th Framework Programme, Priority 5: Food Quality and Safety • Contract no. FOOD-CT-2005 016320 • Duration: 1.2.2006 – 31.1.2011 •Total budget: 15,665,436 Euro, European Union contribution: 13,594,976 Euro. • Project co-ordinator: Prof. Dr. J.C.S. Kleinjans, Maastricht University, Dept. ofHealth Risk Analysis and Toxicology, P.O. Box 616, 6200MD Maastricht, Netherlands • Tel.: 31-43-3881097, Fax: 31-43-3884146, email: j.kleinjans@grat.unimaas.nl. • Project Manager: R.J.H.M. Reijnders M.Sc., Maastricht University, GRAT, P.O. Box 616, 6200MD Maastricht Tel.: 31-43-3881845, Fax: 31-43-3884146, email: r.reijnders@grat.unimaas.nl • Communication and Dissemination Officer: Dr. Maria Botsivali, National Hellenic Research Foundation, 48 Vassileos Constantinou Avenue, Athens 11635, Greece, Tel: 30-210-7273740, Fax: 30-210-7273677, email: mbots@eie.gr. • EC Project Officer: Ebba Barany, European Commission, DG RTD, Unit E3: Food - Health - Well-being, SDME 8/89, B-1049 Brussels, Tel : +32 2 29 88 068, Fax. + 32 2 29 64 322, e-mail: ebba.barany@ec.europa.eu

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