4th Informal Consultation on Long-term Studies on Environmental Threats to the Health of Children in Developing and Industrialized Countries

Considering the environment and health measurements required for internationally harmonized studies

Chulabhorn Research Institute (CRI)
Bangkok, Thailand

18-20 August 2005

Report W/comment P. Farmer, Xibiao Ye, Karen Birmingham,

1. The Fourth Informal Consultation on Long Term Studies (LTS) on Environmental Threats to the Health of Children in Developing Countries, convened by the World Health Organization (WHO) was hosted by the Chulabhorn Research Institute in Bangkok, Thailand, 18-20 August 2005. The meeting was organized by WHO with the support of the United States Environmental Protection Agency (USEPA) jointly with the National Institute for Child Health and Development (NICHD) of the National Institutes of Health (NIH) and the Centers for Disease Control (CDC) in the USA. The local organizer was Dr Mathuros Ruchirawat, Vice-President for Research of the CRI. The meeting took place at the CRI, which provided excellent meeting facilities and organizational support.

2. Participants were professionals from Australia, China, Denmark, Guatemala, Mexico, Thailand, UK and USA, and representatives of The Wellcome Trust and the host organization, who are planning, promoting, conducting or supporting LTS on children's health and the environment. (See Annex I, Participants list, and Annex II, Agenda of the consultation).

3. The objectives of the 4th consultation were to:
    a) Review action taken since the 3rd consultation (Cuernavaca, Mexico, Nov 2004): on-going activities, challenges and achievements, future plans.
    b) Present the core set of draft hypotheses on: pregnancy outcome, growth and development, respiratory disease, neurodevelopment and reproduction, and cancer.
    c) Present and discuss the biological and environmental measurements required in the context of internationally harmonized LTS.
    d) For participants that were taking part at the XVII World Congress of the International Epidemiological Association, to brief colleagues about the LTCS Session.
    e) To plan the next steps.

4. Dr M. Ruchirawat, host of the event, opened the meeting welcoming everyone to CRI and to Bangkok. Drs Krotoski (NICHD/USA) and Pronczuk (WHO) welcomed participants on behalf of their respective organizations and proposed to co-chair the meeting in the absence of Dr Correa. The apologies of colleagues who were unable to participate: A. Correa (CDC), T. Damstra (WHO), J. Golding (UK), P. Landrigan (USA), J. Olsen (Denmark) and C. Santos Burgoa (Mexico) were conveyed. It was mentioned that other colleagues involved in the international initiative on LTCS will be participating at sessions organized by Dr Thea de

Wet (South Africa) at the coming international environmental epidemiology event in Johannesburg, South Africa.

5. Dr Jenny Pronczuk gave an overview on the technical activities developed by WHO in the areas of (i) national profiles to assess the state of children and the environment; (ii) collaborative research between countries - that encompasses the LTCS-; and (iii) training package and workshops for health care providers. The WHO promotion of the Paediatric Environmental History taking and the "green page" (an environmental record to include in pediatric case files) were mentioned as activities that may contribute to the implementation of LTCS.

6. Dr Danuta Krotoski (NICHD/NIH) briefed participants on progress made by the National Children's Study (NCS) in the USA, where the vanguard centers will be selected at the end of September. The NIH and EPA are organizing a meeting September 28-29 to discuss the establishment of a consortium of longitudinal cohort studies to address the causes of childhood cancers. The next Study Assembly of the NCS is 29-30 November 2005 and plans are in hand to organize meetings of the International Interest Group (IIG) at that time.

7. Ms Felicity Flack (Australia) presented progress made on the collaborative research established between her institute and a health centre in Pune, India, that looks at environmental factors and respiratory disease in children. Asthma incidence is increasing in India in the cities, and remains relatively low in rural areas suggesting that biomass burning is not as strong a factor if compared to traffic. Gene-environment interactions and many other factors will be addressed in order to increase knowledge and also help develop more adequate preventative measures. The Indian birth cohort (2.500 children in 5 centres) is running a feasibility study in 10 pregnant women and 10 children at 6 weeks and 6 months, with ethics committee approval. Some practical difficulties arise, such as: filling in questionnaires (e.g. time of visits should be shorter, questions should be culturally appropriate); finding the homes (no addresses!) and the lack of electricity. Main needs identified are: better history-taking, indoor air monitoring, study of aspirates for viral load and monitoring of water for heavy metals and infectious agents (both community source and home storage). A standard protocol used by different people in different areas could allow a comparison of results among communities. This protocol could include instructions on how to plan and set up the study, measure outcome, address confounders, and could include a set of harmonized modules and extra modules for specific pollutants of interest.

8. Ms Karen Birmingham (ALSPAC, UK) described the study of 15.000 mothers enrolled during pregnancy, and their children who are now 12 and 13 years old. The study is currently addressing puberty issues (including adolescent pregnancy with tentative plans to enroll the children's children). The fact that the cohort was set up to collect a broad range of detailed data rather than driven by specific hypotheses had led to some criticism. Some of the main challenges were: a need for longer and longer questionnaires due to the many collaborators' interests; funding requirement for a large cohort including core support required for the sustainability of effective and frequent follow-up. The study has a wealth of samples and other data that are available to collaborators. Development of cell lines is underway. Although a non-interventionist study, the parents are informed of certain results which are decided by the ALSPAC Law & Ethics Committee, (high blood pressure, anemia,

zygocity of twins, deficient sight and deficient hearing). The ethics committee has been rigorous in its consideration of all aspects of the study. The environmental factors - such as ambient air and water measures - could be obtained from other sources. The importance of not restricting data collection to specific hypotheses was underscored, as it is important in LTCS which aim to test future unknown hypotheses. Long-term studies require a good infrastructure, capacity building and sustainability.

9. Dr Robin Braithwaite (UK) described the activities of his Regional Laboratory for Toxicology in Birmingham, that provides analytical support to health practitioners and for legal cases. His laboratory has large expertise on drugs of abuse and on how to tackle ethical issues – what are the implications and what about treatment – what's in it for the individual child and family. Ethical issues concerning the provision of health care or the use of resource data banks differ and will be determined by cultural and other factors.

10. Dr Ruben Grajeda (Guatemala) described the study that followed a group of children over the past 35 years. The cohort is based on a community trial of protein supplementation on cognitive development and function addressing human capital formation and productivity. Ethical issues regarding informing authorities are particularly important in developing countries since children may be exposed to levels of pollutants not previously known – especially when very high.

11. Dr Lilia Rivero Rodriguez (Mexico) mentioned the joint declaration of ministers of health and environment of the Americas that has stated the need to promote research on CEH issues and the recent Mar del Plata high-level conference that mentions these long-term studies. Mexico is in the initial planning phases of a LTCS and the first meeting will taking place in November 2005. Dr C. Santos Burgoa is leading this initiative from the Ministry of Health and is calling for the participation of other Mexican organizations and agencies.

12. Dr Xibiao Ye (China) described a project on environmental exposure and pregnancy outcome focusing on children's neural development. It is being led by the local CDC in coordination with community hospitals. Recruitment of 400 mothers (12 to 16 weeks of gestation) has started in July 2005 and will continue until 2006. Children will be followed up to 12 months of age. Funding is available only for 3 years. Studies are done on food, soil dust, water and air and in urine, blood cord and meconium. The main challenges faced are technological, for example, on the analyses of mercury species and pesticides. Dr Ye stated that parameters have to be validated in China, as the international ones may not be valid. Biological sample collection is difficult, as finding mothers that will go to a given hospital for delivery is challenging. In addition, the mothers recruited do not like to sign any forms (ethical issues related to the informed consent).

13. Dr Bandit Thinkhamrop (Thailand) presented the Thai study that focuses on a holistic approach to development. Exposures are assessed through observation and interviews and the only samples collected are breast milk and mothers' blood at the time of delivery (for maternal haematocrit). The questionnaire records maternal medications during pregnancy. Information about garbage, sewage and perception of air pollution is recorded. Growth parameters have been recorded. New factors to include are: pychosocial (in the children that

are now 4 years of age) and five areas: allergy and parasitic infections; growth and social adaptation, language and cognitive development.

14. Dr Alan Doyle (Wellcome Trust, UK) gave an overview of the LTS that his organization is supporting, some of them with the Gates/Grand Challenges. He stated the importance of clear case definition, use of experienced laboratories to deal with samples and involving centers of excellence serving the different countries. The protocol should include quality assurance and use the same reference ranges. The Wellcome Trust is supporting the UK Biobank initiative that has an ethics and governance council overseeing its activities to ensure ethical use and best resource utilization. International activities on longitudinal and demographic studies are being supported in Thailand, sub-Saharan Africa and South Africa (a birth to twenty study that provides 60m US dollars over the next 5 years). They include genomics research, as new technologies are available to look at biomarkers of exposure (for which they need to store samples under specific conditions). This requires looking at the practical conditions of data collection and storage (e.g. 40 ml of blood per individual, that will be handled by modern robotics). The UK Biobank protocols that have been validated will soon be on the website.

15. The plenary discussion addressed the following issues:

- Good collection and storage protocols are required - looking at what will be measured and the costs of storage and analyzing the samples (e.g. Europe is working on a new integrated project involving established EU cohorts).

- Pilot work to be done on the collection and storage of samples under field conditions in different countries, in order to see how the different conditions may have an impact on specific biomarkers (there may be dramatic differences among centers due to the ways the specimens are collected).

- The great potential offered by measuring protein adducts (e.g. in stored samples of red blood cells), finding the metabolites of different chemicals in urine, doing genomic studies (e.g. genetics of individuals, tumors and host genotype, profile expression of genes across time and patients).

- Need to consider what is available in commercial laboratories and not only in the academic or government laboratories, as well as what may be coordinated with agencies that are doing work on specific issues (e.g. UNEP and the Global Environmental Fund are supporting projects related to persistent organic pollutants -POPs- whose analyses require sophisticated methodologies). It is important to consider the advantage of using agencies providing high level of support of quality assurance.

- Importance of interacting with European and other cancer consortiums.

16. Dr Krotoski introduced the core hypotheses on: *respiratory effects, pregnancy outcome and birth defects, neurodevelopment, growth and development, and cancer.* The hypotheses on *Injuries* and *Child apnea* would not be addressed at this opportunity. The hypothesis on *Cancer* will be considered separately in view of the international initiatives on the subject.

17. Dr Braithwaite gave a presentation on the challenges posed by issues such as: selecting the right specimen according to the target chemical or its metabolite, the problems posed by speciation, finding the adequate biomarkers of exposure/effect and the validation analysis required. He mentioned that some techniques may have to be done relatively quickly as long-term storage may effect the stability of markers. The interpretation of results is crucial, as the reference ranges may vary in different laboratories and countries. In the context of harmonized studies it is crucial to agree on a list of key analytes and their priority, on the biomarkers of exposure and on measurements of markers of nutritional status. It is also important to consider what is being administered to the mother, such as oxytocin, that may alter some results. Protocols should be prepared for specimen collection (choice of specimen, volumes and containers, avoiding contamination and mismatches), for ensuring their stability and good storage and transportation. The tests and methodologies to be used should be agreed upon. Laboratories should have accreditation status (e.g. adoption of ISO 17025 quality guidance), work with reference experienced labs; use common quality control materials, use internal standards and external quality assurance schemes, and certified reference materials. The techniques that are in use include: gas chromatography (GC), mass spectrography (MS), liquid chromatography (LC) and Inductively Coupled Plasma (ICP), that may be combined (e.g. GC-ICP-MS or LC-ICP-MS). The sample size can determine the approach. These methods may allow the screening and measurement of about 20 to 40 samples at the same time. In addition to these, there are DNA techniques or immunological techniques. (See Annex III)

18. The measurements commonly available in toxicological laboratories include:

- Dimethylarsonic acid (DMAA), used as general marker of arsenic exposure
- Drugs of abuse in urine
- Nicotine (cotinine)
- Ethanol (ethyl glucuronide) particularly sensitive used in adults
- Cannabis (THC-COOH
- Cocaine (benzoylecgonine)
- Opiates (morphine, codeine, 6-MAM)
- Amphetamines (amphetamine, methamphetamine)
- Ecstasy (MDMA, MDA, MDEA)
- Others: Ketamine,GHB
- Pesticide metabolites (should be pre-defined)
- Other

19. The biological samples to be considered in the context of LTCS include: mother's blood and urine; cord blood; child urine; hair and teeth. The value of using blood from heel prick is low (as it is almost no longer done). Amniotic fluid, meconium and faecal samples are of limited interest. Placenta tissue and descriptions of shape and photographic recording may be valuable (as discussed at the 3rd consultation). The basic environmental samples to study would include: water, outdoor air quality and soil, but the most important issue is to keep a good environmental questionnaire.

20. The discussions that followed addressed the following issues:

- Need to define the community of users, those that will be profiting from the research, and also those who may use the resources.

- The importance of Biobanks, that are being set up in European countries, North America and Japan. For the time being substantial communication among biobanks and coordination is very patchy.

- The potential for involving environmental agencies for setting up bioenvironmental banks using human data as indicators of environmental degradation.

- Hardware and consumables are expensive.

- Stability of biomarkers is an issue - room temperature may be crucial, and also the type of preservative added.

- Dr Braithwaite prepared a summary list of key issues concerning analytical techniques in the context of LTCS (see Annex III).

21. Dr Peter Farmer (UK) gave a presentation on biomarkers of exposure and effects of genotoxic carcinogens. The importance of quality assurance and standardization of procedures, their validation and quality assurance were stressed. The ethical considerations – particularly working with DNA and with different countries - are of relevance. It is important to know the period of exposure and how it relates to the biomarker and its half-life. Some examples of analytical methods were mentioned: styrene metabolites in urine; benzene in blood, urine and breath; chloroform in breath and blood; dioxins in blood and fat; polycyclic aromatic hydrocarbon metabolites in urine. Newer techniques are nowadays available for measuring biologically effective doses, e.g. for DNA adducts (that may be measured within hours to several weeks after exposures). The DNA samples can be obtained from blood, placenta, bladder epithelium and buccal mucosa. DNA repair products can be detected in urine. The analysis of DNA adducts may be complex. It is done through immunoassay, postlabelling, mass spectrometry and HPLC. Protein adducts are also good exposure monitors but of lesser significance with regard to carcinogenic risk. Dr Farmer mentioned the susceptibility factors, the genes involved in activation, detoxification and DNA repair when looking at organic carcinogens and the important effects of diet (e.g. vitamins). For long-term studies it may be better to measure DNA adducts because they are better predictors of risk. Urine may be used (up to 100 mL), blood and also placenta. Meconium is difficult to work with and there is not a lot of experience with amniotic fluid.

22. Dr Herman Autrup (Denmark) mentioned the EPIC project that to detects cancer in non-smokers and the planning of a biobank (Genair) that will look at markers of susceptibility. His advice was to concentrate on the preparation of standard protocols. In his country the government and cancer societies are paying to support the cohort. Dr Autrup described the way samples are collected, processed and stored in different sites and the logistical problems related to shipment, storage and quality control. The accessibility to results, ethical issues were discussed. Lymphocytes and erythrocytes are commonly used, and serum and urine to

study markers of oxidative damage. Two centres in Denmark are able to do RNA expression on lymphocytes DNA within 4 hours of collection.

23. Dr. Alan Boobis (UK) briefed participants about new technologies, especially toxicogenomics. Good exposure measures will be important in deciding which are the really important environmental chemicals. For example, placental CYP1A1 is highly elevated after smoking. Looking at profiles of genes or proteins reflecting negative exposures may be of interest, but difficult to validate. The current procedures are too long and complex. A good informatics infrastructure is critical for interpreting reams of data. International standards have been developed for the various "omics": Transcriptomics (microarrays), proteomics (chromatographies, 2D gel E, mass spectrograph, protein arrays) and metabolomics (chromatography, MRN, mass spectrometry). Brief reference was made to the expression platforms, transcriptomics and microarrays, proteomics and metabolomics – (metabolite profile) to be used on biofluids or tissue.

24. The discussion that followed focused on the needs and challenges faced in developing countries, such as:

- Specimen collection – requires good and sustainable collaboration with the clinic that helps in collecting the samples and the clinical and other data.
- Infrastructure required for processing and facilities for equipment and storage.
- Shipment is possible with the use of dry ice (e.g. how vaccines are shipped).
- Analysis may require international collaboration, centralized facilities in developing countries or contract laboratories.
- Epidemiological data difficult may be difficult to retrieve.
- The issue of labeling is important for samples – bar coding reduces problem of tracking.
- May the Global Positioning Systems be useful in the context of LTCS?

25. Three working groups discussed the measurements that may be required in the context of the different hypotheses, their timing and relevance (essential or optional). The materials provided include: (i) list of environmental factors (chemical, physical, biological, psychosocial, built-in environments, hygiene, diet, media, physical activities, social network and other), (ii) list of outcomes, (iii) publications of the EHP on "lessons learned" from the NCS study in relation to air pollution, asthma, pesticide exposure and community based participatory research (EHP online 24 June 2005 - http://dx.doi.org/). The working groups presented their recommendations in plenary session. Ms Flack presented a matrix that summarizes the clinical assessments, biological samples and environmental questionnaires and measurements to be done during pregnancy (mother's first visit to the clinic), at the time of delivery and weekly or monthly, up to age 5. (See Annex IV). At the first visit of the mother urine and blood samples are taken for routine analysis. At this time, extra amounts

could be taken for cell lines, DNA studies or other, and samples could be stored under liquid nitrogen. Lymphocytes can also be obtained from the cord at the time of delivery.

26. Dr E. Aagaard (Denmark) gave an overview on the 'Better Health for Mother and Child' study, a Danish nationwide prospective study of 100,000 pregnant women and their children that aims at studying the long- and short-term consequences of fetal development and the factors that may influence fetal growth (e.g. infections, diet, medicine, lifestyle/environment). Data on exposure is self-reported, followed by telephone interviews. Biological samples are collected (blood, cord blood, other…). They are looking into the possibility of collecting more environmental data and samples.

27. Dr A. Doyle (Wellcome Trust) made a presentation on Challenges and Constraints and led the discussions that followed. The main points raised by the speaker and the participants were:

- Dealing with the community - the role the community plays, benefits they may gain and provide from enrolling in the study - as well as the constraints - should be made clear right from the beginning. Incentives should be considered together with the ethical issues involved. Better health care provision is one of the incentives, but this may be debatable.

- Involving the local/national government - importance of having the authorities "on board" from the very beginning. WHO may play a role here, providing technical support, coordination and advice through the regional and country offices (some of which play a key role in promoting health sector activities) and ensuring a certain degree of harmonization (that can start regionally and then integrate countries).

- Harmonization issues among longitudinal cohorts – within the country and internationally

- People/team in charge - defining who is responsible, who manages the study, who is part of the secretariat or steering committee.

- People involved - important to recruit individuals who can stay in the study over a long term.

- Technical committees - need to be set up to audit and visit laboratories, to keep up the response rate, deal with specific issues.

- Ownership and access to data - to be discussed and agreed, and set up through formal agreements. Steering committees may have access and ownership, but there should always be a strong scientific leadership (as this makes greater impact).

- Providing feedback - as soon as results and observations are made, to provide immediate feedback to the community and authorities. For example, part exposure assessments and markers that will be useful for planning and taking protective actions.

- Funding - a flexible approach is required: different partners may bring in resources. LTS are an opportunity for mentoring and training. Non traditional donors may be involved (e.g. UNDP, UNEP and World Bank).

- Building on existing infrastructures - this may enhance the possibilities of success, especially when LTCS are planned in developing countries (where a LTCS may actually strengthen the health system).

- Power calculation - an important scientific issue, requires the best advice and may need to address issues of capacity building in this area.

- Benefit from technology developments - these are increasing and may provide important benefits to studies (this may be tested through pilot studies, as the ones done by the NCS).

- Complexity of dealing with genetic materials and studies - there may be some fear in misinformed communities in developing countries as there is a distrust of genomics. Dealing with this involves ethical and political issues.

- Sampling and storage - where should the emphasis be? How to start preparing for and using the genomic technologies. Bar coding is considered essential to ensure the identification of samples.

- Use of existing samples - the filter spots (in biobanks, blood is stored on filter paper) or the Guthrie tests could be used for genetic studies. A possibility is to relate early deaths and Guthrie's studies, as this may provide useful information.

- Quality assurance and control - important to write into quality assurance schemes, especially when looking at certain exposures that need international assurance. Some of these schemes already exist and may assure harmonization (e.g. POPs). If the LTS initiative becomes an international resource (e.g. in terms of protocols) they will need an audit process.

- Communication strategy - important for managing the expectations of the community, the scientific and other sectors (e.g. involving the media, selecting the right language). It is a challenge to deal with the community and how the community perceives the LTCS. E.g. ALSPAC published a newsletter that keeps people informed.

28. Dr Mathuros gave an overview on the type of studies that CRI is performing on the impact of air pollution on children's health. In a city with traffic problems such as Bangkok exposure to genotoxic compounds in air represents a concern. CRI is studying particle associated PAHs and benzene in five traffic-congested areas, measuring the compounds and also biomarkers of exposure, such as urinary 1-hydroxypyrene for PAH and t,t-muconic acid for benzene. School children from the city present higher levels than those who live outside. It was noted that pollution falls about 90% only 500 meters away from high traffic areas. This study is an excellent example of research whose results may be used to initiate preventive or corrective

measures, so that risks form exposure to traffic-related pollution can be reduced. This experience is very valuable in the context of LTCS. CRI is exploring the possibility of studying atmospheric carcinogens and their impact on childhood cancer.

29. Drs Pronczuk and Choprapawon informed participants about the Symposia on Long-term Children Studies as a tool to enhance the achievement of the Millennium Development Goals, to be held Monday 22 August, at the XVII World Congress of Epidemiology. The colleagues who have kindly agreed to present their experience were: Drs Vorasith Sornsrivichai (Thailand), Karen Birmingham (ALSPAC; UK), Ruben Grajeda (Guatemala) and J. Pronczuk (WHO).

30. Participants and organizers expressed their thanks to CRI, especially to Dr Mathuros and her staff for the excellent facilities and logistical support provided. Drs Pronczuk and Krotoski thanked all participants for their excellent contribution this meeting where for the first time the measurements required for LTCS were considered. The experience gained through this and the previous three consultations should be disseminated to provide the basic information that countries need in order to include environmental considerations into their on-going or planned LTCS.

31. The next steps proposed and agreed by participants were:
    a. prepare the report of the meeting and circulate to participants (D. Krotoski; J. Pronczuk)
    b. provide annex on basic laboratory considerations (R. Braithwaite)
    c. provide matrix on biological measurement (F. Flack)
    d. organize work on cancer hypothesis (D. Krotoski)
    e. plan publication of the work done (reports, recommendations, hypotheses, biological measurement, and other section (J. Pronczuk, A. Correa, D. Krotoski)
    f. plan a new working group on "strategy of pilots" (D. Krotoski)
    g. circulate the environmental questionnaire (J. Pronczuk)
    h. plan a session at the next Study Assembly of the NCS (D. Krotoski)

Annex I - List of Participants

1. **AAGAARD NOHR, Ellen**
   Danish Epidemiology Science Centre
   Department of Epidemiology and Social Medicine
   University of Aarhus
   Vennelyst Boulevard 6
   Building 260
   8000 Aarhus C
   Denmark
   Tel: +45 8942 6087 (work)
   Tel: +45 9888 9025 (home)
   Fax: +45 8613 1580
   Email: ean@soci.au.dk

2. **BIRMINGHAM, Karen**
   ALSPAC - Avon Longitudinal Study of Parents and Children
   Department of Community Based Medicine
   University of Bristol
   24 Tyndall Avenue
   Bristol
   BS8 1TQ
   United Kingdom
   Tel: +44 117 331 1707
   Email: Karen.Birmingham@bristol.ac.uk

3. **BRAITHWAITE, Robin**
   Director
   Regional Laboratory for Toxicology
   City Hospital
   Birmingham B18 7QH
   United Kingdom
   Tel: +44 121 507 4134
   Fax: +44 121507 6021
   Email: robin.braithwaite@swbh.nhs.uk

4. **DOYLE, Alan**
   Science Programme Manager
   Populations & Public Health
   The Wellcome Trust
   215 Euston Road
   London NW1 2BE
   UK
   Tel: +44 20 7611 8248
   Fax: +44 20 7611 8352
   Email:a.doyle@wellcome.ac.uk

5. **FLACK, Felicity**
Executive Officer, Division of Clinical Sciences
TVW Telethon Institute for Child Health Research
Roberts Rd
Subiaco 6008
PO Box 855
West Perth 6872
Australia
Tel: +61 8 9489 7816
Fax: +61 8 9489 7700
Email: felicity@ichr.uwa.edu.au

6. **GRAJEDA TOLEDO, Rubén**
Instituto de Nutrición de Centro América y Panamá (INCAP)
Carretera Roosevelt, Zona 11
Apartado Postal 1188
01011
Guatemala
Tel: +502 2440 9742
Fax: +502 2473 6529
Email: rgrajeda@incap.ops-oms.org

7. **RIVERO RODRIGUEZ, Lilia**
Subdirección de Manejo de Riesgos en Unidades de Salud
Dirección General de Planeación y Desarrollo en Salud
Av. José Vasconcelos No. 221 6° piso
Col. San Miguel Chapultepec Deleg. Miguel
Hidalgo D.F.
C.P. 11850
Mexico
Tel: +52 55 5553 0167
Fax: +52 55 5256 0119
lrivero@salud.gob.mx

8. **YE, Xibiao**
School of Public Health
Fudan University
138,Yixueyuan Road
Shanghai,200032
China
Tel: +86 21 5423 7358
Fax: +86 21 6422 3464
Email: xbye@shmu.edu.cn

Internal/CRI/Thailand

1. **AUTRUP, Herman**
   Institute of Public Health
   Department of Environmental and Occupational Medicine
   University of Aarhus
   Vennelyst Boulevard 6
   8000 Aarhus
   Denmark
   Fax: +45 8942 6199
   Tel: +45 8942 6180
   Email: HA@MIL.AU.DK

2. **BOOBIS, Alan R.**
   Professor of Biochemical Pharmacology
   Director of Department of Health Toxicology Unit Experimental Medicine and Toxicology Division of Medicine Imperial College London
   Room 9S10B
   CWB Hammersmith Campus
   Ducane Road
   London W12 0NN
   United Kingdom
   Fax: +44 20 8383 2066
   Tel: +44 20 8383 3221
   Email: a.boobis@imperial.ac.uk

3. **FARMER, Peter**
   Cancer Biomarkers and Prevention Group Biocentre
   University of Leicester
   University Road
   Leicester LE1 7RH
   United Kingdom
   Fax: +44 116 223 1840
   Tel: +44 116 223 1823
   Email: pbf1@le.ac.uk

4. **CHOPRAPAWON, Chanpen**
   104/1 Mu 13
   Soi Saneha, Krungtepkreeta Road
   Sapansoong
   Bangkok 10250
   Thailand
   Tel: +66 1 868 3634
   Fax: +66 2 736 2105.
   Email: cchopra2004@yahoo.com

5. **PHUPAIBOOL, Ruja**
Faculty of Nursing
Ramatiborde Hospital
Mahidol University 270
Rama 6
Ratave Bangkok
10400
Thailand
Tel: +66 2 201 2130
Mobile: +66 1279 8528
Fax: +66 2 201 1698
Email: rarph@mucc.mahidol.ac.th

6. **RUANGDARAGANON, Nichara**
Faculty of Medicine
Rama Hospital
Mahidol University
Rama 6
Ratave Bangkok
10400
Thailand
Tel: +66 2 201 1772-3
Fax: +66 2 201 2266 or 1850
Email: ranrd@mahidol.ac.th

7. **SORNSRIVICHAI, Vorasith**
Epidemiology Unit
Faculty of Medicine
Prince of Songkla University
Hat Yai
Songkhla 90110
Thailand
Tel: +66 7442 9754
Fax: +66 7421 2900
Mobile: +66 1303 1376
Email: vorasith@psu.ac.th

8. **THINKHAMROP, Bandit**
Associate Professor
Department of Biostatistics and Demography
Faculty of Public Health
Khon Kaen University
123 Mitraparb Highway
Muang District
Khon Kaen 40002
Thailand
Tel: +66 43 203 453

Mobile: +66 1975 4582
Fax: +66 43 203 453
Email: karawa@gmail.com

9. **TUNTISIRIVIT, Kanit**
Nan Hospital
Nan Province
55000
Thailand
Tel: +66 5 471 0138/9 Ext. 3132
Fax: +66 5 477 1620/1
Email: kanit_tantisirivit@hotmail.com

10. **WITTAYASOOPORN, Jariya**
Faculty of Rama Nursing
Rama Hospital
Mahidol University
Rama 6
Ratave Bangkok
10400
Thailand
Tel: +66 2 201 1698
Fax: +66 2 570 0231
Email: rajwt@mahidol.ac.th

Secretariat

1. **KROTOSKI, Danuta**
Acting Associate Director
Office of Prevention Research and International Programs
NICHD/NIH
6100 Executive Blvd.
Room 2A-01 MSC 7510
Bethesda, MD 20892-7510
USA
Tel: +1 301 435 7566
Fax: +1 301 435 0009
Email: krotoskd@mail.nih.gov

2. **PRONCZUK, Jenny**
Medical Officer
International Programme on Chemical Safety
Protection of the Human Environment
World Health Organization
20 avenue Appia

1211 Geneva 27
Switzerland
Tel: +41 22 791 3602
Fax: +41 22 791 4848
Email: pronczukj@who.int

3. **RUCHIRAWAT, Mathuros**
Vice President for Research
Chulabhorn Research Institute (CRI)
Vibhavadee-rangsit Highway, Lak Si
Bangkok 10210
Thailand
Tel: +66 2 574 0615
Fax: +66 2 574 0616
Email: mathuros@Tubtim.cri.or.th

4. **SATAYAVIVAD, Jutamaad**
Associate Vice President for Scientific Affairs
Chulabhorn Research Institute (CRI)
Vibhavadee-rangsit Highway, Lak Si
Bangkok 10210
Thailand
Tel: +66 2 574 0615
Fax: +66 2 574 0616
Email: jutamaad@cri.or.th

Annex II - Agenda

Fourth Informal Consultation on Long Term Studies (LTS) on Environmental Threats to the Health of Children in Developing and Industrialized Countries

Considering the environment and health measurement required for internationally harmonized studies

Thursday 18 August

09:00-09:30 Informal opening and welcome by representatives of:
CRI, NIH, WHO
Introductions

09:30-10:00 Objectives of the consultation and proposed work plan:
Summary of previous meetings
Specific Suggestions for Variables to be measured in the context of harmonization and developing countries

J. Pronczuk

Session I:

10:00-10:45 Short review of activities undertaken since the 3rd Consultation *Participants to provide a very brief report (5' to 8') on their activities in the last year: progress made since the 2nd (PAHO) and/or 3rd (Cuernavaca) consultations, challenges encountered, expectations and plans.*

Activities of the Wellcome Trust – *A. Doyle*

D. Krotoski/ All participants

10:45 -11:00 Coffee Break

Session II

11:00 -11:30 Presentation of the draft hypotheses for internationally harmonized LTS
D. Krotoski
(a) Respiratory
(b) Pregnancy outcome
(c) Neurodevelopment
(d) Growth and development (need to go over this one!)
--(f) Injuries--

(g) Birth Defects
(h) Cancer (pending)

13:00-14:00 Lunch

Session III

14:00-14:30 Prioritization of hypotheses and variables to be measured.

Discussion facilitator: *C. Choprapawon*

14:30-16:30 Panel discussion on issues for consideration by the working groups in the context of LTCS in developing countries:

- A core set of biological and environmental measurements required in the context of LTCS
- Assessment of Exposure and Expression
- Biomarkers of Expression

A. Boobis and P. Farmer

New technologies and analytical toxicology methods available: their applicability into internationally harmonized LTCS

R. Braithwaite

16:30-17:00 Preparation for Working Groups

Respiratory Group: *F. Flack, J. Pronczuk, R. Braithwaite, Thai participants*

Pregnancy Outcomes, Growth and Neurodevelopment:

R. Grajeda, A. Doyle, E. Aagaard Nohr, K. Birmingham, L. Rivero, C. Choprapawon, Thai Participants

Cancer and other rare outcomes: exposure assessment issues

M. Ruchirawat, X. Ye, P. Farmer, D. Krotoski, Thai Participants

Working Documents: Table of Hypotheses, Environmental Exposures, Outcomes and Methods of Measures.

Objectives: review of the document and proposals for revision or refinement. What studies, measurements and markers that may be proposed for each hypothesis/outcome?

Friday 19 August

Session IV

9:00-10:00 Preparation for Working Groups: issues for consideration

H. Autrup and P. Farmer

10:00-13:00 Working Groups (Coffee Break around 10:45)

13:00-14:00 Lunch

Session V

14:00-15:30 Working Groups

15:30-15:45 Tea break

Challenges and Constraints

E. Aagaard Nohr/A. Doyle

15:45-16:30 Report of Working Groups

16:30-17:00 Plenary discussion

Saturday 20 August

Session VI

9:00 -12:30 Plenary discussion

Approval of the meeting recommendations

Planning the next steps

Annex III - Analytical considerations

General Guidance on Biological Specimen Collection and Investigations for the Assessment of Exposure and Effect

Robin Braithwaite - robin.braithwaite@swbh.nhs.uk

1. Specimens are best collected at the same time as those that are indicated for clinical purposes during pregnancy, birth and child development. This is ideally achieved with consent by taking additional volumes of blood (venepuncture) or collection of non-invasive specimens (e.g. urine, breast milk, cord blood, hair etc).

2. Protocols should be developed for specimen collection, including volume, specimen containers, date and time of collection, unequivocal subject identification (e.g. use of bar code labelling) and handling.

3. Protocols should be developed for understanding the ideal conditions for specimen transport and storage (e.g. -20 ^{o}C, liquid nitrogen). This is particularly important if some specimens are to be stored for long periods of time (e.g. several years or decades). It is important to consider dividing all specimens collected into several aliquots and storing these in different refrigerators or locations. Transport of samples overseas would require ethical approval and conform to international guidelines.

4. It will be important to undertake preliminary work to decide the sample volume (or mass) requirements for measurements to be carried out on individual stored aliquots of specimens. This will include limit of detection, optimum specimen type, volume and analytical performance (reliability).

5. It will be important to undertake studies at an early stage to determine the stability of all analytes under different storage conditions (e.g. time and temperature). Whereas some analytes may be relatively stable on long-term storage (e.g. metallic elements) others (e.g. DNA-adducts, proteins or enzymes) may be relatively unstable over a long period of storage.

6. Detailed protocols will be required for separation of certain blood specimens following collection, particularly for the separation of red-cells (washed or unwashed) plasma, or white cells etc. A refrigerated centrifuge may be required for some types of specimen collection technique.

7. Great care is required in the selection of appropriate specimen containers. Ideally these should come from the same batch. Studies should always be undertaken before their general use in order to check the quality of the storage container, including tight closure of cap with low temperature storage, contamination due to manufacture of plastic container materials. It can be useful to undertaken extensive studies to determine the level of contamination that might be found in collection tubes, particularly if relatively low levels of exposure may be expected in some study populations. This particularly applies to “standard” blood collection tubes used in routine medical practice, some of which may contain separating gels or beads that often contain volatile chemicals. Many containers also contain anticoagulants that can be a source of contamination or interfere with some tests.

8. It may be very helpful to consult other protocols established for long-term storage of biological materials (e.g. Biobank).

9. Before starting any studies if will be important to consider the availability and possible future development of validated analytical procedures for the measurement of various analytes, their speciation and metabolic, or biomarkers of exposure and effect.

10. It is very important to consider quality assurance aspects of all analytical measurements carried out on collected biological specimens. Generally this should consider important aspects, such as accuracy and reproducibility as covered in international guidelines (e.g. ISO 17025). This is particularly important when longitudinal studies are carried out or findings from different studies are to be compared over a long period of time (years or decades).

11. Analytical measurements are best carried out using well described (published peer reviewed) methods ideally using established analytical techniques, according to written Standard Operating Procedures (SOP's).

12. Use of appropriate certified or other appropriate reference materials, common Internal Quality Controls (IQC's) and participation in an External Quality Assurance Scheme (QAS) to monitor laboratory performance and ensure adequate standards are maintained is highly recommended wherever possible.

13. Routine biochemical, haematological and immunological techniques used for patient investigation (blood and urine) should be well described, including agreed reference ranges. Information on QA performance for all analytes should where possible be collected during all studies.

14. It may be helpful to carry out collaborative studies between different centres within a Region and between developed and underdeveloped Regions. This is particularly important for the long term international harmonisation of findings concerning exposure and outcome.

15. It can be important to consider feeding back measurements carried out on local populations as soon as possible, also to carry out regular clinical audit and feedback of findings. This has the benefit of helping the recruitment of new study subjects and capacity building of local health services. It can also achieve useful short term health gains for the local population, particularly those living in poor areas where there may be significant exposure to hazardous chemicals or other agents.

16. It is important to consider the ethical issues of biological specimen collection at an early stage. This includes use of invasive techniques such as venepuncture in children, use of sensitive material (e.g. placenta), volume of blood collected, use of particular tests (e.g. DNA testing, drugs of abuse). There may also be ethical issues concerning the recording of information, access to and ownership of genetic information and other test results. Ethical issues also include feedback of results to individual family members, advice on possible health consequences, and possible intervention for active treatment (e.g. high blood levels).

17. Nutrition during pregnancy and early childhood may be an important confounding factor in some study populations and it may be important to carry out the investigation of nutritional status using available biological specimens. Consultation of other nutritional study protocols should be carried out to determine the most appropriate investigations to carry out a nutritional assessment.

This may include blood vitamins (A, C, D E?) and essential trace elements, plasma protein and albumin, ferritin, folate, red-cell zinc protoprophyrin (ZPP) "anti-oxidant status".

18. Protocols should have some flexibility to include the collection of other biological specimens, particularly if this includes the clinical assessment and management of particular disorders in children (e.g. asthma and allergy, respiratory illness, cancer, birth abnormalities etc).

19. Separate protocols will need to be developed to deal with the deaths of children. This will include perinatal deaths and Sudden Infant Death Syndrome (SIDS). Close collaboration with local pathologists is important to have access to and storage of post-mortem blood and tissues. The ethical issues surrounding collection and storage of post-mortem material should be considered with close regard to both local and national guidelines.

20. It can be helpful to review the range of key analytes to be measured in biological specimens. This includes "markers" of exposure and effect also genomic information on all subjects to study the interaction between genes and environment. Some investigations will be easily available whereas others (particularly effect markers) may only be available at specialist centres within a Region or other countries.

21. Assessment of exposure and effect may be quite complex. Some substances and their metabolites, or effect markers, may only give information on current or relatively recent exposure. Some markers may give information on historical exposure, covering several months. It is, therefore, important to understand the toxicokinetics of target pollutants and their metabolites, also "markers" or effect on toxicity as part of the study protocol.

22. It is thought not be appropriate to take any blood specimen from the child at birth by capillary sampling (heel or hand "prick"). However, use of left-over material from blood spots from the routine Guthrie Test may be appropriate to consider, particularly for DNA analysis.

23. Urine collection from children is non-invasive and preferable to blood collection. However, it is recognised that collection of urine is difficult, particularly in very young children and babies. Use of left-over blood, when taken for clinical purposes, may be used as an addition to the study protocol. However, it may be possible to undertake blood specimen collection in a sub-population of children for the investigation of particular types of exposure (e.g. lead) if this is considered to be a significant problem in such a population, and ethical approval has been agreed.

24. It may be useful to carry out investigation of genetic polymorphism of Cytochrome P450 isoenzymes involved in the metabolism of various pollutants. This particularly includes genotoxic compounds and carcinogens.

25. It will be helpful to consider the collection of good sized urine specimens (50-100 ml) at each clinical assessment (mother and child) as indicated on the study protocol. These should be aliquoted and stored according to standardised protocols. Measurement of urine specific gravity or creatinine may also be useful.

26. During pregnancy, at each clinical assessment, it may be helpful whenever blood is taken for routine clinical purposes, to take an additional volume (e.g. 20-30 ml?) for the purposes of the study. This should be divided into aliquots according to standardised protocols and may require separation.

27. Collection of cord blood is non-invasive and is regarded as the most suitable sample for collection during birth for the assessment of exposure. It may also be possible to collect the placenta for further examination and tissue sampling, subject to ethical approval.

Annex IV - Matrix of protocol, sample collection and timing

OBJECTIVES

Primary Objective
Secondary Objective

HYPOTHESES

MODULE 1 – LONGITUDINAL BIRTH COHORT

Module 1 describes the protocol and outcome measures for a longitudinal birth cohort to describe the impact of environmental exposures in the first 5 years of life.

STUDY DESIGN

Description

This is a longitudinal birth cohort study. Children will be followed from birth until the age of 5 years.

Study Definitions

In this protocol, the following definitions will apply:

Selection and Withdrawal of Participants

Inclusion Criteria

- Patients who meet all of the following criteria are eligible for enrolment as study participants:
- Written informed consent signed and dated by parents or legally acceptable representatives according to local regulations.
- Mothers in the 2nd trimester of pregnancy.
- Families willing to remain in the study until the 5-year end point.

Exclusion Criteria

- Patients who meet *any* of these criteria are *not* eligible for enrolment as study participants:
- Children born prematurely (gestation < 33 weeks).
- Major birth defects such as spina bifida or biliary atresia.

- Families unlikely to remain in the region for the duration of the study.

Participant Withdrawal Criteria

Withdrawal by Parents or Legal Guardians

Parents/ or legal guardians will be free to withdraw their children at any time;

Premature Termination from the Study

In addition to being withdrawn from the study by their parents or legal guardians, participants may be prematurely withdrawn from the study because of failure to return (i.e., they are lost to follow-up) or death.

Replacement of Participants

Participants who withdraw consent or who are prematurely terminated from the study for any other reason will not be replaced.

Study Procedures

Enrolment

This research study will be explained in lay terms to each potential research participant's parent(s) or legal guardian(s). The potential participant's parent(s) or legal guardian(s) will sign an informed consent form before the participant undergoes any screening study procedures. Participants who are deemed eligible for the study will be enrolled and assigned a unique participant number.

Study Visits

For a schedule of study visits see Appendix 1.

Screening Visit −1 (Study Day −1)

At the first antenatal clinic visit. Adherence to the inclusion criteria will be verified at the screening visit. The following assessments will be done:

- Informed consent **Essential**
- Demographic history (address, socioeconomic status, ethnicity) **Essential**
- Maternal health and nutrition **Essential**
    - Physical examination
    - Medical history
    - Blood sample (if being taken for clinical purposes)

        - Cryopreserve lymphocytes
    - Urine
    - Diet questionnaire
    - Pre-conceptional history of exposure to toxic substances
- Ultrasound **Optional**
- Family health and lifestyle **Essential**
- Environmental conditions at home, work & community, (eg. animals, type of dwelling, cooking fuel etc, waste sites, occupational exposure etc) Repeat every time the child changes address. **Essential**
- Outdoor air quality in local area **Optional**
- Water quality **Optional**

Baseline (Birth) Visit 1 (Study Day 0)

Participants must meet all eligibility criteria before entering the 5-year study period.

- Pregnancy, delivery and neonatal record **Essential**
- Physical Examination **Essential**
- Cord Blood **Essential**
- Meconium **Optional**
- Placenta (weigh, section, photograph, store tissue) **Optional**
- Urine **Optional**
- Hair **Optional**

Study Follow-Up Visits

The children participating in the study will be brought to the investigational site for assessment three times in the first year of life to coincide with the routine vaccination schedule. There will be

an annual (birthday) visit in years 2-5. If the child requires a blood or urine sample for health purposes, take an additional aliquot for the LTS.

Visit 2, (Week 6)

- Physical examination **Essential**
- General medical history (record of all diseases including infections) **Essential**
- Nutritional status (height, weight, BMI, anthropometrics, food intake) **Essential**
- Developmental assessment **Essential**
- Record of vaccination **Essential**
- Faecal sample **Optional**
- Environmental conditions at home, work & community, (eg. animals, type of dwelling, cooking fuel etc, waste sites, occupational exposure etc) Repeat every time the child changes address. **Essential**

Visit 3 (Month 6-9)

- Physical examination **Essential**
- General medical history (record of all diseases including infections) **Essential**
- Nutritional status (height, weight, BMI, anthropometrics, food intake) **Essential**
- Record of vaccination **Essential**
- Faecal sample **Essential**
- Environmental conditions at home, work & community, (eg. animals, type of dwelling, cooking fuel etc, waste sites, occupational exposure etc) Repeat every time the child changes address. **Essential**

Visit 4 (Month 12)

- Physical examination **Essential**

- General medical history (record of all diseases including infections) **Essential**
- Nutritional status (height, weight, BMI, anthropometrics, food intake) **Essential**
- Developmental assessment **Essential**
- Record of vaccination **Essential**
- Faecal sample **Essential**
- Environmental conditions at home, work & community, (eg. animals, type of dwelling, cooking fuel etc, waste sites, occupational exposure etc) Repeat every time the child changes address. **Essential**

Visit 5, 6, 7 and 8 (Month 24, 36, 48 and 60)

- Physical examination **Essential**
- General medical history (record of all diseases including infections) **Essential**
- Nutritional status (height, weight, BMI, anthropometrics, food intake) **Essential**
- Developmental assessment **Essential**
- Record of vaccination **Essential**
- Faecal sample **Optional**
- Urine sample **Essential**
- Environmental conditions at home, work & community, (eg. animals, type of dwelling, cooking fuel etc, waste sites, occupational exposure etc) Repeat every time the child changes address. **Essential**

Visit Windows

Study visits should take place within the time limits below:

- Visit 1-4: within ± 7 days of the scheduled appointment
- Visit 5–8: within ± 14 days of the scheduled appointment

	Day		Week	Month					
Time Point	**-1**	**0**	**6**	**6-9**	**12**	**24**	**36**	**48**	**60**
Visit	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Clinical Assessments and Questionnaires									
Inclusion/exclusion criteria, informed consent									
Demographic data									
Socioeconomic & cultural status scale									
Family & lifestyle questionnaire									
Maternal medical history									
Paternal medical history									
Maternal physical examination									
Pregnancy, delivery & neonatal record									
Food frequency & diet questionnaire									
Developmental assessment									
Child medical history									
Child physical examination									
Record of vaccination									
Biological Samples									
Cord blood									
Placenta									
Meconium									
Breast milk									
Blood sample									
Faecal sample									
Urine									
Hair									
Teeth									
Environmental Questionnaires & Measures									
Environmental questionnaire									
Outdoor air quality									
Water quality									