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INTERNATIONAL CENTRE FOR ENVIRONMENTAL AND INDUSTRIAL TOXICOLOGY (ICEIT)

CRI's ICEIT has been designated as a
"UNEP Centre of Excellence for Environmental and Industrial Toxicology".

CRI Receives SAICM Award for Most Outstanding Quick Start Programme Project in the Asia-Pacific Region for the Development of Distance Learning Tool in Risk Assessment of Chemicals

SAICM Award for the Most Outstanding Quick Start Programme (QSP) Project in the Asia-Pacific Region and SAICM Certificate of Appreciation for CRI, in its role as implementing institution for the project.

The Chulabhorn Research Institute (CRI) received an award for the **Most Outstanding Quick Start Programme (QSP) Project in the Asia-Pacific Region** from Mr. Luay Almukhtar, Asia-Pacific Strategic Approach to International Chemicals Management (SAICM) Regional Focal Point, and Mr. Nassereddin Heidari, Asia-Pacific Representative of the ICCM4 Bureau on behalf of SAICM. The awards ceremony was held during a gala dinner on March 24, 2014, organized by the SAICM Secretariat to honor successfully completed SAICM QSP projects from the region. This was held as part of the 4th Asia Pacific Regional Meeting on the Strategic Approach to International Chemicals Management (SAICM) and related workshops, held in Kuala Lumpur, Malaysia, between March 23-27, 2014.

CRI received the award as the implementing institution and on behalf of all collaborating institutions on a project entitled, **"Development of course materials and a distance learning tool for the assessment of risk from the use of chemicals to support SAICM's capacity building efforts in developing countries",** which aimed to build up a needed critical mass of qualified professionals with an appropriate level of understanding of the basic sciences that form the foundation of risk assessment through 2 key project components: (a) development of distance learning course materials on risk assessment that is tailored to the needs of developing countries, while also being relevant globally, and (b) development of an interactive electronic distance learning tool (eDLT) based on the developed distance learning material.

The eDLT is a web-based self-learning tool that makes use of narration, animation,

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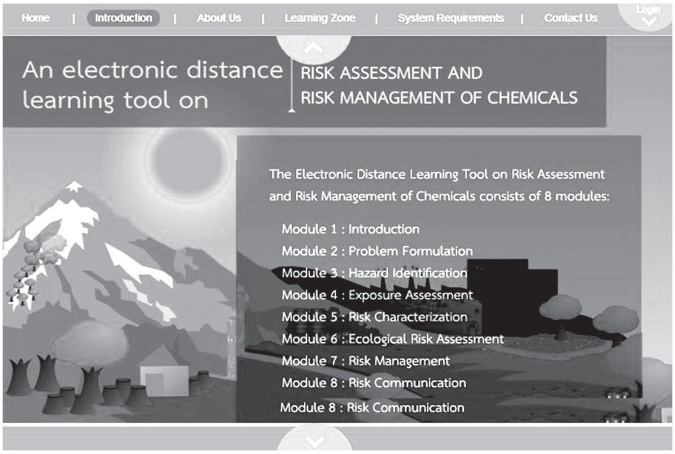

and interactive exercises to explain the fundamentals of risk assessment and risk management through 8 modules: (1) Introduction, (2) Problem Formulation, (3) Hazard Assessment, (4) Exposure Assessment, (5) Risk Characterization – Humans, (6) Risk Characterization – Ecological, (7) Risk Management, and (8) Risk Communication. The collaborating institutions/ organizations on the project are CRI, as the implementing institution, the World Health Organization's International Programme on Chemical Safety (Geneva, Switzerland), University of Ottawa (Canada), and Utrecht University (the Netherlands). Development of the eDLT has been completed, and it was officially launched on February 14th, 2013 at the WHO South-East Asia Regional Office (WHO SEARO) in New Delhi, India, by Her Royal Highness Princess Chulabhorn Mahidol, President of CRI. CRI is WHO Collaborating Centre for Capacity Building and Research in Environmental Health Science and Toxicology.

Interested individuals and institutions should send an e-mail to envtox@cri.or.th to request registration, and include in that e-mail the full names and affiliations of all individuals who will be using the eDLT. A nominal fee of $100 USD per person is charged to cover the costs of maintaining the eDLT and Learning Management System, and a username and password will be generated and sent for each individual user for access to the eDLT through the website. For more information on the eDLT, please visit *http://www.chemDLT.com*.

The SAICM QSP is a voluntary, time-limited trust fund administered by the United Nations Environment Programme (UNEP), with the main objective to support initial enabling capacity building and SAICM implementation activities in developing countries, least developed countries, small island developing states and countries with economies in transition. SAICM is a policy framework to foster the sound management of chemicals.

HEALTH EFFECTS OF MAN-MADE CHEMICALS

The number of man-made chemicals used in the environment has increased dramatically during the last decades. Although the toxicity of certain pollutants, like lead and arsenic, has been known for years, potential deleterious health effects of most other substances are largely unknown.

One area of major concern is that several of the high-volume produced chemicals could interfere with the basal hormonal systems governing fundamental homeostatic systems in humans. Therefore, the term "endocrine disruption" has been coined to describe this general action of some environmental contaminants.

Most of the compounds regarded as endocrine disrupters are highly lipophilic chemicals that accumulate in adipose tissues resulting in long half-lives and slow elimination. They are collectively named persistent organic pollutants (POPs), and a number of these chemicals have been identified as deleterious for health and have been listed at the Stockholm convention. Amongst these are organochlorine pesticides, such as hexachlorobenzene (HCB) and DDT, chlordane, PCBs, dioxins, brominated flame retardants and fluorinated compounds.

Exposure to dioxin and dioxin-like PCBs has been shown to induce a rise in blood pressure in rodents. The association between exposure to POPs and hypertension has also been investigated in a number of epidemiological studies. For example, higher levels of circulating polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans were related to prevalence of newly diagnosed hypertension in women. Elevated PCB levels have been associated with increased odds for hypertension in men and women and a tendency for increased hypertension among men only. For dioxin-like PCBs an association was reported for 18-39 year olds whereas a borderline protective effect was seen for > 40 year olds. Organochlorine (OC) pesticides such as DDT have been associated with an increased risk of hypertension although no clear association has been reported.

These epidemiological studies included rather small sample sizes in different geographical regions. To test the hypothesis that there might be an association between POP levels and hypertension in a rather large sample of men and women in Sweden, a recent study used data from almost 1000 elderly individuals included in the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study, in which circulating levels of 23 different POPs were measured together with blood pressure.

The results indicated that 732 subjects (72%) showed hypertension. When the POPs were treated as continuous variables and adjusted for gender only, two PCBs with a low number of chlorine atoms (PCB 105 and 118) were related to prevalent hypertension. Also the OC pesticide p,p'-DDE was related to hypertension with the strongest association. Following further adjustment also for BMI, smoking status, education level and exercise habits, only p,p'-DDE was still significantly related to hypertension.

Source: Environmental Research, Vol. 129, Pages 27-31, February 2014

EFFECT OF ARSENIC EXPOSURE IN UTERO ON OXIDATIVE DNA DAMAGE AND REPAIR IN YOUNG CHILDREN

Chronic arsenic exposure in humans is a major public health concern globally. Maternal arsenic exposure through drinking water during the prenatal stage can cause adverse health effects in childhood.

Previous Thai studies have shown that prenatal arsenic exposure can alter gene expression profiles of newborns, as well as cause epigenetic changes in DNA methylation patterns. Analysis of cord blood from newborns of exposed mothers showed a number of changes in the expression of genes involved in various biological networks such as stress responses, inflammation, metal exposure, and apoptosis, as well as epigenetic changes in DNA methylation at the promoter region of the tumor suppressor gene, p53. These findings suggest that exposure to arsenic early in life may therefore increase risks for disease development.

A major limitation in studying the health risk of arsenic exposure in young children is the impracticality of obtaining blood samples as biological specimens for molecular and cellular studies. Emerging evidence in the past few years has prompted an interest in using saliva as an alternative to serum that can be investigated for diagnostic purposes. Preliminary studies in both animals and humans have shown that total arsenic in saliva has a significantly positive correlation with total arsenic in urine. Monitoring of arsenic exposure and early effects via salivary biomarkers will allow researchers to more easily study the consequence of exposure which reflects the underling mechanism of toxicity in young children.

To gain a better understanding of the mechanism through which arsenic induces toxicity in children, a follow-up study was conducted at Chulabhorn Research Institute, Thailand, on a cohort of children who were exposed to arsenic *in utero* and who have continued to live in the same arsenic-contaminated areas of Ron Phibul District, Nakon Sri Thammarat Province, Thailand. Matched controls were born and lived in this geographical area but with lower levels of arsenic contamination. Researchers used non-invasive urinary and saliva-based assays to assess arsenic exposure and to investigate oxidative DNA damage by measuring salivary 8-OHdG, urinary 8-OHdG and salivary *hOGG1* expression. Therefore, it was hypothesized that exposure to arsenic *in utero* and in early childhood could be associated with an increased level of oxidative DNA damage and reduced level of repair capacity, which may result in a decreased level of oxidative DNA damage that is detected in urine.

In the present study, the measured levels of arsenic in most of the drinking water samples at the exposed sites were lower than the WHO guideline level of 10 µg/l, but higher than those at the control sites. Significantly higher levels of all biomarkers of exposure, including arsenic in saliva and urine, and arsenic accumulation in toenails and fingernails, were found in the arsenic-exposed children. This would seem to indicate that arsenic contamination in drinking water was an important route of arsenic exposure in this population. Furthermore, a significant correlation between arsenic levels in non-drinking water and various biomarkers of exposure was also observed. Despite the fact that children may be exposed to arsenic in both drinking and non-drinking water, many reports suggest that arsenic can rarely penetrate the skin during bathing and showering. Therefore, children may be indirectly exposed to arsenic in non-drinking water by several other routes, e.g. through normal daily activity (brushing teeth) and food consumption (eating foods processed with non-drinking water). Since non-drinking water is used for cooking, children were likely exposed to arsenic in non-drinking water through food. The level of arsenic in non-drinking water at the exposed site was much higher than that of the control site; therefore, there should be a heightened awareness that although corrective measures have been implemented, people living in the area are still exposed to arsenic at levels that may pose health problems later in life.

In this study, significant associations between salivary biomarkers of effects (*hOGG1* expression and 8-OHdG) and levels of arsenic exposure in drinking water were observed. Similar trends in the correlation pattern were found between these salivary biomarkers of early biological effects and levels of salivary and urinary $_i$As, and total arsenic accumulation in nails. Reduction of *hOGG1* expression in saliva was significantly negatively correlated with an increase in levels of arsenic in water, levels of $_i$As in saliva and urine and arsenic accumulation in nails. In contrast, a significantly positive correlation between levels of 8-OHdG in saliva and various parameters, including arsenic concentration in water, levels of $_i$As in saliva and urine, as well as arsenic accumulation in nails, was observed. Moreover, the multiple regression analysis results showed that concentrations of total arsenic in saliva had a significant association with salivary 8-OHdG, urinary 8-OHdG and salivary *hOGG1* expression.

The results suggest that persistent exposure to arsenic in water from the prenatal period may lead to reduction of *hOGG1* repair capacity, which results in an increase of 8-OHdG as observed in saliva. It has been noted that $_i$As species are more toxic than organic species in the body. This is in agreement with the Thai study results: multiple regression analysis showed that among the various arsenic species, $_i$As in both saliva and urine had the greatest significant positive association with salivary 8-OHdG and negative association with salivary *hOGG1* expression. The results suggest that the $_i$As species measured in urine and saliva could be responsible for the biological changes detected in the Thai study. Therefore, saliva could be useful for human biomonitoring, as a biological sample for biomarkers of arsenic exposure and early biological effects, especially in children.

Source: Toxicology and Applied Pharmacology, Vol. 273, Issue 3, Pages 569-579, December 2013

BISPHENOL A AND HUMAN CHRONIC DISEASES

Bisphenol A (BPA) is one of the highest volume chemicals produced worldwide. Current estimation indicates that over 6 billion pounds were produced annually in the manufacturing of polymers (such as polycarbonate, epoxy resins), polyvinyl chloride plastics and flame retardant tetrabromobisphenol-A. Polycarbonate is used in materials of foodstuffs (such as baby bottles, reusable plastic bottles, plates, goblets, cups, microwave ovenware, and storage containers) and the epoxy resins are used for internal coating of food and beverage cans. Currently, the applications of polycarbonate and epoxy resins were extended into other uses of life such as sunglasses, thermal papers, building materials, CD-ROM, medical devices, and dental materials. Thus, over 100 tonnes of BPA are released into the atmosphere every year of production.

Aquatic environment, air and soil can be a source of human BPA exposure, but food is believed to be the major source of exposure. Indeed, under various conditions, BPA can leach out of containers and pass to the food or beverage which is then a source for human exposure. Researchers have detected 0.234 ng/ml (~1 nM) of BPA after 5 days of water incubation at polycarbonate plastic water bottles at room temperature and this amount can increase when hot water was used. The authors concluded that detectable concentrations of BPA leach from everyday plastic containers and that heating can increase this leaching. In the same context, food cans often contain BPA during the sterilization process at high temperature or when heated for use.

In addition to consumption of contaminated drinks and foodstuffs, dermal exposure was suggested through paper contact especially thermal printed paper which is typically used in point of sale receipts (fast food restaurants, retailers, grocery stores, gas stations, and post offices). BPA was detected in thermal paper with detectable concentration recorded in many developed countries (Belgium, Denmark, Sweden, Switzerland and US). This suggests the dermal route as an important additional exposure source for the general population, which warrants further inclusion in the overall risk assessment of BPA.

A recently published review presents data collected from a wide range of epidemiological studies which are sufficiently robust, in the view of the researchers, to raise concerns about the potentially deleterious impact of BPA on human development and chronic human disease induction such as diabetes, obesity, reproductive disorder, cardiovascular diseases, birth defects, chronic respiratory and kidney diseases, and breast cancer.

The review discussed its mechanisms of action, especially genetic, epigenetic and endocrine disruption mechanisms with the possible involvement of oxidative stress, mitochondrial dysfunction and cell signaling.

Clearly additional fundamental non-human primates and clinical research is required to understand more the mechanisms of BPA action that looks too complicated. Indeed, despite that mouse and rat have been shown to be excellent models to understand human chronic diseases mechanisms, a variety of differences among species have been reported. Also, as a preventive and precautionary principle, urgent further investigations are required, particularly to developing fetuses and young children as they may be the most susceptible to adverse effects of this ubiquitous compound in developed as well as in developing countries which need more attention even through public sensitization programs. On the other hand, in reality humans are exposed to a mixture of pollutant, so the possibility of additive and synergistic effects of BPA with other prevalent compounds should not be overlooked. Also, actual tolerable daily intake must be revised because several *in vivo* studies have reported significant effects in animals with doses below the published LOAEL of 0.05 mg/kg BW/day.

Source: Environmental International, Vol. 64, Pages 83-90, March 2014

THE ROLE OF FOOD CONTACT CHEMICALS ON DISEASES OF COMPLEX AETIOLOGY

In the developed world chronic diseases are responsible for around two-thirds of deaths, with about 16% of such deaths occurring before age 60. While most chronic, non-communicable diseases are rightly considered 'diseases of complex aetiology' (and, therefore, have multiple causes), there is strong evidence linking these disorders with chronic exposure to environmental pollutants. The WHO and the United Nations Environment Programme (UNEP) recently concluded in their 'State of the Science on Endocrine Disrupting Chemicals 2012' report that EDCs are a global public health threat. EDCs and other neotoxicants are commonly used, or present, in food contact materials (FCMs); their safety for this use has often not been established. The direct health consequences of this exposure to neotoxicants via FCMs are unknown. Since most foods are packaged and the entire population is likely to be exposed, it is of utmost importance that gaps in knowledge are reliably and rapidly filled.

Unravelling the role of FCMs in the development of chronic disease is of high scientific and public interest. In contrast to other challenges in nutritional and environmental epidemiology, chemical exposures from FCMs offer the benefit of a relatively discrete and measurable route of exposure. The authors of this study propose, specifically, that in addition to using food frequency questionnaires and other dietary assessment methods and technologies, dietary habits should additionally be characterized according to FCMs and supplemented by biomonitoring efforts. Such a task will include analyses of the uses of materials in contact with food throughout the food supply chain (processing, packaging, storage) and food packaging in stores, at home, the workplace and other settings. Furthermore, studies should also measure – through validated instruments and procedures – the frequency of consumer practices, such as storage in freezers, heating foods in plastic dishes and containers, use of plastic films, as well as packaging preferences when buying foods and beverages. In Europe, for example, the FACET database can

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Health concerns over the toxicity of phthalates

Phthalates are known as plasticizers to give plasticity for rigid materials in many products. Nowadays the industry generates billions of pounds of phthalates every year. Phthalates are widespread contaminants in both indoor and outdoor environments. These toxicants can be delivered into the body via inhalation, dietary intake, and skin absorption. Dermal absorption occurs at a significant rate for phthalates. Humans can be exposed to phthalates from building materials, household furnishing, soil, and dust. Furthermore, phthalates are employed in skin care products for their roles as stabilizers, binders, emulsifiers, and lubricants. One study recently demonstrated that hand and body lotions are the main contributors to dermal exposure, especially for diethyl phthalate (DEP). Since phthalates are not chemically bound to most of the products, they are easily released from products to skin.

A recent study has explored the effect of topically-applied phthalates on skin absorption and toxicity. Immunohistology, functional proteomics, and Western blotting were employed as methodologies for validating phthalate toxicity. Among 5 phthalates tested, di(2-ethylhexyl)phthalate (DEHP) showed the highest skin reservoir. Only diethyl phthalate (DEP) and dibutyl phthalate (DBP) could penetrate across skin. Strat-M® membrane could be used as a permeation barrier for predicting phthalate penetration through skin. A recent study showed the accumulation of DEHP in hair follicles was ~15 nmol/cm^2, which was significantly greater than DBP and DEP. DBP induced apoptosis of keratinocytes and fibroblasts via caspase-3 activation. This result was confirmed by downregulation of 14-3-3 and immunohistology of TUNEL. On the other hand, the HSP60 overexpression and immunostaining of COX-2 suggested inflammatory response induced by DEP and DEHP.

A major concern of phthalate exposure on skin is the possibility of carcinogenesis. Some proteins examined in the present report are biomarkers of epidermal malignancy, including HSP27 and cytokeratin. The negligible change or significant downregulation of these proteins by phthalates demonstrated that phthalates may not cause skin carcinogenesis after a consecutive exposure of 7 days. The experimental results of this report indicate a different response on skin toxicity by DBP and DEP/DEHP. The skin reaction of DBP was mediated by modulating apoptosis and proliferation without a significant inflammation. DEP and DEHP produced inflammatory responses and a moderate differentiation. DEP and DEHP showed a comparable change on most of the biomarkers tested. Since skin deposition of DEP was much lower than that of DEHP, this suggested a more prominent toxicity of DEP compared to DEHP. The report is limited to local skin behavior after topical phthalate application. The limitation of the study was the insufficient replicates in the *in vivo* experiment, resulting in the deficiency for valid statistics. Further study on a large group for animals or humans is necessary to validate the findings.

The toxicity caused by phthalates is an issue with increasing importance. Consumers receiving skin care products and cosmetics are always exposed to more than one phthalate. This may result in a synergistic or additive effect of skin damage. Researchers had used validated methodologies for evaluating phthalate toxicity on skin. The results demonstrated that different phthalates revealed different absorption levels, with DEHP showing the greatest accumulation into skin. There was also no common trend of alteration of skin protein expression by exposure to different phthalates. A significant apoptosis was observed for DBP but not DEP and DEHP. On the other hand, both DEP and DEHP exhibited greater inflammatory stimulation than DBP. The experimental results presented in this work possess clinical relevance for finding effective biomarkers for phthalate toxicity. Strat-M® could be helpful to predict phthalate penetration across skin. HSPs, 14-3-3, and cytokeratin may serve as sensitive biomarkers for differentiating skin toxicity of various phthalates. Proteomic mapping supplied information about the effect of phthalates on skin toxicity, especially calcium homeostasis. This report serves to establish knowledge about skin toxicity elicited by phthalates.

Source: Food and Chemical Toxicology, Vol. 65, Pages 105-114, March 2014

The Role of Food Contact Chemicals on Diseases of Complex Aetiology

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support such efforts: this newly established database from the EU-funded research project Flavorings, Additives and Food Contact Materials Exposure Task (FACET) contains levels of food packaging migrants from FCMs and links them with food consumption data. Subsequently, statistical analyses would integrate this type of information with data traditionally used in nutritional, environmental and molecular epidemiology.

Innovative research could also expand knowledge on toxic mechanisms – for example, on oestrogenic, androgenic, thyroid and glucocorticoid effects of chemicals migrating from FCMs; on the homeostasis of glucose and lipid metabolism, energy homeostasis and insulin resistance; on the role of agonists and antagonists of nuclear receptors in modulation of nuclear receptor function and endocrine diseases, including non-nuclear steroid membrane receptors and non-steroid receptors; on metabolic and mitochondrial dysfunction, inflammation, adipogenesis and adipose macrophages.

Also, given the economic and cultural influences on food consumption, social epidemiology should develop a research agenda on FCMs, health and well-being.

Integrating knowledge about FCM chemical composition and migration into food in epidemiological studies is in the view of the researchers an opportunity and a duty for epidemiologists. Eventually, such research will strengthen primary prevention policies by reducing chemical exposures resulting from a manageable source. It will also advance basic and applied knowledge on the molecular and physiological mechanisms that link some environmental chemicals and human diseases.

Source: Journal of Epidemiology and Community Health, Available from – http://dx.doi.org/10.1136/jech-2013-202593 (19 February 2014)

A COHORT COMPARISON OF AIR POLLUTION EFFECTS ON FETAL AND CHILD DEVELOPMENT IN CHINA

Air pollution is a serious health concern for the population of China living in urban areas, due to the presence of coal-fired power plants, which currently produce nearly 75% of the country's electricity. Combustion of coal is a major source of air pollution in China. Emissions from the burning of coal contain carcinogenic substances such as particulate matter (PM) and polycyclic aromatic hydrocarbons (PAHs), as well as neurotoxicants such as mercury.

While these exposures are harmful for adults, they are particularly detrimental to health during early childhood, as the fetus and child have increased susceptibility to environmental pollutants from increased absorption and slower clearance of toxicants compared to adults.

The present study was conducted in the city of Tongliang, which has a population of approximately 810,000 and is situated in a small basin approximately 3 km in diameter. A coal-fired power plant located south of the town center operated during the dry season from 1 December to 31 May each year before 2004 to compensate for insufficient hydroelectric power during that time period. Analysis of 72-h average concentrations showed that the highest concentrations of PAHs were found in winter, reflecting the relationship between coal-fired power plant operation and meteorological conditions.

In May 2004, the Tongliang County Government determined that the shutdown of the power plant would significantly improve local health and have minimal adverse social and economic impacts, and the power plant was closed and replaced by the national grid system of electrical energy.

In partnership with Chongqing Children's Hospital, the Columbia Center for Children's Environmental Health has carried out two prospective cohort studies in Tongliang, China to examine the impact of exposure to emissions from the coal-fired power plant on children's health. The first cohort was recruited prior to the power plant shutdown in 2002, and the second cohort was recruited following the shutdown in 2005. Here researchers test the hypothesis that the shutdown of the power plant has had a significant beneficial health impact on children born in the Tongliang area. It was anticipated that the levels of outdoor emissions, and in particular, PAH, would decrease following the power plant shutdown, and translate to improved health outcomes.

PAH-DNA adducts are widely used as a risk marker because they provide an informative individual biologic dosimeter and risk marker for exposure to PAH. Therefore, researchers expected to find lower cord PAH-DNA adduct levels and more favorable birth/child neuro-development and physical outcomes in the second cohort compared to children in the first cohort enrolled when the power plant was still operational. The lipid soluble characteristic of PAHs allows these molecules to be stored in the mother's fat tissue and further transferred to the developing fetus, so PAH metabolism may vary based on additional factors such as the mother's nutritional status and polymorphic characteristics of PAH metabolism pathways. These factors are being evaluated for influence on fetal/child physical and neuro-development in a subsequent cohort recruited in 2007 in Tongliang to investigate the long term benefits of shutting down the Tongliang power plant.

This cohort comparison study has the advantage of being the first to evaluate the impact of a coal-fired power plant shutdown on children. Furthermore, the use of B[a]P-DNA adducts as the biomarker of exposure allowed researchers to quantitatively measure the biologically effective dose of B[a]P for newborns in cohort I and cohort II. Infants were monitored after birth for up to 30 months, providing data on the effects of pre- and post natal exposure on growth rate. However, the study was limited by the modest number of study subjects (150 in cohort I and 158 in cohort II) and therefore the ability to control for other environmental interactions. Nonetheless, averaged air pollution data showed a significant positive correlation with increased B[a]P-DNA adduct levels. Prior studies have linked PAHs and PAH-DNA adducts with developmental deficits and increased risk of cancer in children in China and other countries.

Further studies are needed to examine the potential health effects of modern "clean power plants". In addition to the unique opportunity to examine subjects before and after the closure of the power plant, continuing work with a 2007 cohort in Tongliang will further emphasize the benefits of reduced exposure. The findings of this study are encouraging in that direct governmental interventions to remove a polluting coal-burning source can have rapid and direct benefits to children's health. This in turn is a strong motivation for China to further invest in renewable and energy efficient fuels, a necessity for its future.

Source: Environmental Pollution, Vol. 185, Pages 90-96, February 2014

EFFECTS OF LONG-TERM EXPOSURE TO THE HEAVY METAL CHROMIUM

Living organisms are exposed to complex mixtures of natural and man-made compounds for the length of their life. Mixtures of toxic and carcinogenic compounds, including heavy metals, polycyclic aromatic hydrocarbons (PAHs), and halogenated compounds, are ubiquitous and widespread in the environment.

One of the major problems in current toxicology and its application to risk assessment is the lack of a sound methodology to deal with the health effects of such chemical mixtures. Data are scarce, and this scarcity points at the need to evaluate critically the assumptions used to determine risk from exposure to complex toxicant mixtures, ultimately leading to an understanding of the health effects of exposure. While many studies have dealt with the mechanisms of action and effect of individual classes of compounds, little work has examined how the individual responses to the single toxic constituents of a mixture bears on the response to a mixture of the components or how the mechanisms operative at one dose of exposure can be extrapolated to the biological responses at different doses. Previous studies are limited to mixtures of similar compounds and are of little use when dealing with complex mixtures of multiple components, because combined exposures generate substance-specific changes in gene expression that cannot be attributed to a single mechanism.

Two compounds often found together in the environment are the heavy metal chromium (Cr) and the PAH benzo[a]pyrene (B[a]P). In a new study, researchers have examined how long-term exposure to a low concentration of Cr(VI) affects the transcriptional response to B[a]P, a second toxicant with an unrelated mechanism of action. Growth of mouse hepatoma cells for 20 passages in medium with 0.1 or 0.5 μM Cr(VI) increases DNA damage and apoptosis while decreasing clonogenic ability. Treated cells also show transcriptome changes indicative of increased expression of DNA damage response and repair genes. In them, B[a]P activates cancer progression pathways, unlike in cells never exposed to Cr(VI), where B[a]P activates mostly xenobiotic metabolism pathways. Cells grown in Cr(VI) for 20 passages and then cultured for an additional 5 passages in the absence of Cr(VI) recover from some but not all the chromium effects. They show B[a]P-dependent transcriptome changes strongly weighted toward xenobiotic metabolism, similar to those in B[a]P-treated cells that had no previous Cr(VI) exposure, but retain a high level of Cr(VI)-induced DNA damage and silence the expression of DNA damage and cancer progression genes.

The gene expression response to Cr(VI) in earlier studies using tissue culture cells does not differ significantly from the response previously seen in animal studies, although the amount of Cr(VI) needed to cause those responses differs widely. Mice exposed to Cr(VI) in drinking water in the range of 0–520 ppm (equivalent to 0–2 mM) for 90 days showed several thousand significant gene expression profile changes in intestinal epithelium at the high doses, but very few changes at doses below 100 ppm (400 μM). At the 100 ppm, however, the transcriptome changes and the primary mechanisms for which gene expression was altered were the immune response, cell cycle, DNA damage repair, and oxidative stress responses, consistent with the responses that were found in cells treated with 0.5 μM Cr(VI) for 20 passages. It is remarkable that the transcriptomic changes are so consistent, even though the *in vitro* concentration is 800-times lower than the *in vivo* dose. Many caveats, however, need to be taken into consideration when comparing *in vivo* and *in vitro* data, including the diversity of cell types that respond *in vivo* compared to a single lineage *in vitro*, and the uncertainties associated with the difference between the exposure dose to a mouse and the concentration of treatment to a tissue culture cell.

Continuous treatment with Cr(VI) also causes significant changes in the cells response to B[a]P. In the absence of Cr(VI), while some pathways related to carcinogenicity and inflammatory responses are also affected, the ones most significantly altered by B[a]P treatment are the xenobiotic response pathways. This is not the response to an additional treatment with B[a]P that cells exposed to Cr(VI) have. Previous treatment with 0.5 μM Cr(VI) for 20 passages causes the response to B[a]P treatment to change the expression of genes mostly related to cancer progression pathways and much less the typical xenobiotic response, normally seen in B[a]P treated cells. The gene expression response activates the corresponding upstream regulators, leading to up-regulation of the DNA damage response due to activation of pathways related to the genotoxic topoisomerase inhibitors camptothecin and topotecan and down-regulation of growth factor signaling cascades, such as VEGF and HGF. This raises the central conclusion that the effects of Cr(VI) and B[a]P co-exposure are both different and more significant than just the sum of the effects of either single exposure.

The present study thus finds that extended treatment with a very low concentration of Cr(VI) induces cellular damage and disrupts the cellular transcriptome. As a consequence, other transcriptomic responses, in this case the response induced by B[a]P, is also disrupted, causing an outcome unpredictable from the effect caused by that either single treatment. Furthermore, consistent with previous *in vivo* work, long treatments with low Cr(VI) concentration silence the activation of DNA damage repair pathways. It remains to be determined the mechanisms operative in the repression process.

Source: Toxicology, Vol. 316, Pages 14-24, February 2014

ASSOCIATION OF POLYFLUOROALKYL CHEMICALS AND EARLY MENOPAUSE

Polyfluoroalkyl chemicals (PFCs) are man-made compounds that have been used in a number of common consumer and industrial products such as food containers; stain- and water-resistant protection for clothing, furniture, and carpets; paints; fire-fighting foam; and photographic emulsifiers. PFCs are ubiquitously present and persistent in the environment, and although there are demographic, geographic, and temporal differences, exposures in the general population are widespread. Four PFC analytes – perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), and perfluorohexane sulfonate (PFHxS) – are commonly detected in humans. Unlike traditional persistent organic pollutants, which are lipophilic and stored primarily in fat tissue, PFOS and PFOA are both lipophobic and hydrophobic. After absorption, they persist in the body by forming chemical bonds to proteins in serum, rather than accumulating in lipids. Serum levels of PFCs reflect long-term exposures to these contaminants with estimated geometric mean half-lives of 7.3 years for PFHxS, 4.8 years for PFOS, and 3.5 years for PFOA. However, in a more recent study, a shorter median half-life for serum PFOA (2.3 years) was estimated.

PFCs are potential endocrine disruptors, and effects of PFOS and PFOA on endocrine function have been reported in animal studies. Less is known about associations between PFCs and human endocrine function.

In a new study, researchers investigated associations between multiple PFCs (PFOS, PFOA, PFNA, and PFHxS) and age at natural and surgically induced (hysterectomy) menopause using National Health and Nutrition Examination Survey (NHANES) data. NHANES collected information regarding the age at which women experienced menopause, which allowed investigation of the relationship between serum levels and time since menopause and the possibility of reverse causality.

The study reported that higher body burdens of PFCs were associated with earlier onset of natural menopause. Associations were strongest between serum levels of PFNA and PFHxS and the rate of natural menopause. PFNA and PFHxS have not been studied previously with respect to menopause, which is of concern because PFNA and PFHxS have not declined over time in the same manner as PFOA and PFOS; geometric mean serum levels of PFNA are increasing (e.g., from 0.55 to 1.49 ng/mL between survey years 1999-2000 and 2007-2008), and serum levels of PFHxS increased in 2007-2008 compared with previous years. Results of the present study show positive associations between PFOS and PFOA and earlier menopause, consistent with previous reports in the literature.

The consistency and robustness of these findings suggest a relationship between PFCs and menopause, although the underlying mechanism of that association remains unknown. In these cross-sectional data, it is not clear whether the association observed between PFCs and menopause is causal, if results are due to noncausal influences such as biases from confounding or misclassification, or if results are due to accumulation of PFCs after menopause. Regardless of the underlying cause, women appear to accumulate PFCs more rapidly after they are no longer menstruating. These results, along with the ubiquitous nature of exposure and persistence of PFCs in the environment, support the need for continued monitoring of serum levels in the general population as well as further studies of the reproductive health effects of PFCs.

Source: Environmental Health Perspectives, Vol. 122, No. 2, Pages 145-150, February 2014

CALENDAR OF EVENTS

International Training Courses in Environmental Toxicology at Chulabhorn Research Institute, scheduled for 2014 - 2015

	Training Course	Date	Duration	Closing Date
1.	Environmental Immunotoxicology and Reproductive Toxicology	October 20-31, 2014	2 weeks	August 27, 2014
2.	Environmental and Health Risk Assessment and Management of Toxic Chemicals	December 2014	2 weeks	September 24, 2014
3.	Environmental Toxicology	May 2015	2 weeks	February 24, 2015

Fellowships: A limited number of fellowships are available that will cover roundtrip airfare, accommodation (on site) and meals, training material, and health insurance.

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More information and application:
please visit - http://www.cri.or.th/en/ac_actcalendar.php

The ICEIT NEWSLETTER is published quarterly by the International Centre for Environmental and Industrial Toxicology of the Chulabhorn Research Institute. It is intended to be a source of information to create awareness of the problems caused by chemicals. However, the contents and views expressed in this newsletter do not necessarily represent the policies of ICEIT.

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