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IDENTIFYING IMPORTANT LIFE STAGES FOR MONITORING AND ASSESSING RISKS FROM EXPOSURES TO ENVIRONMENTAL CONTAMINANTS

A significant challenge associated with monitoring and assessing individual- and population-level exposure to and risk from exposure to environmental chemicals is associated with the need to rigorously consider changes in behavior and physiology that are related to age and life stage. Age- and life stage-related differences will determine windows of highest exposure as well as the appropriate distribution of exposure factors required to address specific exposure scenarios. Age and life stage differences in how people interact with the environment may be a major determinant for identifying the individual or population most vulnerable to risks from particular exposures to environmental contaminants. Identifying the most vulnerable age range or life stage for a particular population and exposure scenario requires a better scientific basis. Currently available approaches are limited in scope and potentially in applicability to the full range of geographic, social, cultural and economic diversity in populations worldwide. In addition, there is a need to better link or coordinate hazard and exposure assessment (the need to identify the most vulnerable based on windows of greatest susceptibility as well as windows of highest exposure, and then to incorporate that knowledge in a population-based risk assessment). Therefore, the World Health Organization (WHO) convened a group of experts to review these issues and provide guidance on how to better identify critical life stages for use in exposure and risk assessment.

The objective of this exercise was to propose a fit-for-purpose set of life stages independent of exposure context and exposure scenario. In this context, the group considered the following steps towards development and application of common life stages for exposure assessment:

- Define age bins by carefully identifying the particular characteristics that distinguish them.
- Decide how finely the overall life stage of childhood should be divided into age bins.
- Describe how additional factors, such as sex, culture and geography, might modify the significance of standard age bins.
- Recognize that there may be cases in which a specific factor (e.g. mouthing behavior) is a more significant indicator of exposure than age.
- Identify the most pressing gaps in the base of scientific knowledge that would justify standard age bins and in the exposure factor data required to use the age bins for risk assessment.

A summary has now been published of important exposure-related issues to consider in determining the most appropriate age ranges and life stages for risk assessment. The authors propose a harmonized set of age bins for monitoring and assessing risks from exposures to chemicals for use globally. The focus is on preconception through adolescence, though the approach should be applicable to addressing additional life stages. Information collated here was developed as follows. A review of previous efforts to establish standardized age bins was conducted, and previously proposed bins were used as a starting point for harmonization. Important developmental changes underpinning extant binning approaches were identified. A literature review was conducted to identify potential modifying factors and impacts on development, exposure and vulnerability to risk. The influence of social structure and geography on

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IDENTIFYING IMPORTANT LIFE STAGES FOR MONITORING AND ASSESSING RISKS FROM EXPOSURES TO ENVIRONMENTAL CONTAMINANTS

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exposure factors was considered, and proposed age bins were evaluated based on important contextual elements.

To harmonize exposure assessment for comparison across time, place and culture, WHO has recommended a standard framework within which to analyze population-specific information. Defining standard age ranges for children will also facilitate collection of data and analyses of aggregate exposure and cumulative risk.

Given the range of scientific and policy-related needs for a harmonized set of age groups, the following tiered set of early life age groups is recommended for international use to facilitate some level of consistency with recently developed age grouping guidance currently in use in some regions:

Tier 1: Adopt guidance similar to the US EPA's recommended childhood age groups.

Tier 2: Consolidate some of the age groups defined above in order to reduce the burden of developing age-specific exposure factor data for different countries or regions.

Tier 1 is preferred in those cases where significant differences in exposure early in life can greatly impact health risks from acute or subchronic exposure to toxins. For example, fluid consumption on a body weight basis is on average almost 3 times greater shortly after birth (birth to <1 month) than for infants 6 to <12 months of age and almost twice the time-weighted average for the entire first year of life.

The above recommendation builds on several recent activities and fills gaps identified in recent publications that focus on assessing risks from exposures of children to environmental contaminants. The US EPA document titled *Guidance on Selecting Age Groups for Monitoring and Assessing Childhood Exposures to Environmental Contaminants* presents recommended age bins for children based on physiology and behavior. The scope of this document narrowly focuses on birth through 18 years of age and is designed specifically to promote a more uniform approach for exposure assessments conducted across US EPA program offices and regions. Prenatal and preconception periods were identified as important periods for consideration in assessing health risks from early life exposures, and these life stages were added to the US EPA-recommended age bins in the document titled *A Framework for Assessing Health Risks of Environmental Exposures to Children.* The WHO document titled *Principles for Evaluating Health Risks in Children Associated with Exposure to Chemicals* cites the US EPA guidance document in the exposure section. However, the lack of harmonization in determining age ranges for life stages became apparent during development of the WHO document. In a few instances, life stages defined at the beginning of the document consistent with WHO terminology were slightly different from the US EPA-recommended exposure bins that were used in the exposure chapter of the WHO document. Even with the focus on children in these three documents, there is no uniform approach for identifying the important life stage (age range), exposure factors specific to the exposure/risk assessment question of interest or the characteristics of a particular population that might modify these. In addition, very few institutions outside the United States have addressed this issue, and there are likely some different factors that might be important for non-US populations that should be considered for a harmonized approach.

While there is no single "correct" means of choosing a common set of age groups to use internationally in assessing early life exposure and risk, use of a set of defined age groups is recommended to facilitate comparisons of potential exposures and risks around the globe.

Application of these age groups for robust assessment of exposure and risk for specific populations will then require country- or region-specific exposure factor information as well as local environmental monitoring data.

Source: Regulatory Toxicology and Pharmacology. Available from - http://dx.doi.org/10.1016/j.yrtph.2013.09.008 (4 October 2013)

Maternal Diet: Prenatal Protection against Impact of PAHs

Environmental exposures *in utero* may have adverse effects on health both immediately and in later life. Measurement of biomarkers in cord blood improves exposure assessment and may improve our understanding of biological mechanisms during this critical window of exposure and vulnerability.

Bulky DNA adducts are a widely accepted and sensitive biomarker of the biologically effective dose of genotoxic agents in complex environmental exposures, including those in ambient air, tobacco smoke, and diet. They reflect individual exposure, absorption, and metabolic activation of heterogeneous adduct-forming compounds, in combination with the ability to repair induced DNA damage, and may be predictive of cancer risk.

Bulky DNA adducts are commonly detected in human DNA by ^{32}P-postlabeling combined with multidimensional thin-layer chromatography. Among common environmental genotoxic agents, polycyclic aromatic hydrocarbons (PAHs) cause DNA damage that is readily detectable as bulky DNA adducts, although the chemical nature of the DNA damage that leads to adduct formation is not known with certainty. A positive correlation between DNA adducts in blood and PAH exposure has been reported in adult populations exposed to high levels of PAHs in ambient air or food, which suggests that bulky DNA adducts reflect DNA damage caused by genotoxic PAHs. Bulky DNA adducts and more specific PAH-related DNA adducts have been detected in human umbilical cord white blood cells, in human placenta and in *ex vivo* human placental perfusions, which suggests that PAHs and other environmental genotoxic agents are capable of forming DNA adducts *in utero*.

Consumption of fruits and vegetables is considered beneficial for health and may protect against cancer through antioxidative effects and other properties related to dietary intake of fiber, folate, and other beneficial nutrients.

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ADVERSE HEALTH EFFECTS OF PARTICULATE MATTER IN AIR POLLUTION

Particulate matter (PM) in air pollution has been associated with adverse health effects such as respiratory and cardiovascular diseases as well as lung cancer mortality and is thus of great public health concern. PM is a major air pollutant in many big cities, especially where traffic is congested. Vehicle exhaust emissions are mainly responsible for the particulate air pollution in urban areas and diesel exhaust is considered a major contributor. Recently, diesel exhaust has been classified by the International Agency for Research on Cancer (IARC) as a group 1 human carcinogen. Exposure to diesel exhaust has been well established for its association with an increased risk of lung cancer.

PM derived from vehicle combustions is mostly in fine and ultra-fine fractions. The small size of particles facilitates their ability to get into the respiratory tract and reach the alveoli where the PM are retained resulting in adverse health effects. Fine particles ($PM_{2.5}$) are composed of various carcinogenic compounds including polycyclic aromatic hydrocarbons (PAHs) and metals which are biologically active components resulting in induction of oxidative DNA damages. The particles or their chemical components induce reactive oxygen species (ROS) which may subsequently lead to cellular oxidative stress. The oxidative stress can induce oxidative DNA lesions such as 8-hydroxy-deoxyguanosine (8-OHdG) and DNA strand breaks. Associations between the levels of 8-OHdG in lymphocyte DNA and in urine and levels of exposure to PM and to particle-bound PAHs and carcinogenic metals have been reported. 8-OHdG is a marker of oxidative stress to DNA. If left unrepaired, it can lead to a heritable mutation and cancer initiation.

Furthermore, PM-induced ROS has been shown to mediate inflammatory responses through activation of redox-sensitive transcription factors resulting in increased synthesis of pro-inflammatory cytokines such as interleukin-6 and -8 (*IL-6* and *IL-8*). The pro-inflammatory cytokines are involved in the amplification of inflammatory reactions. Persistent excessive inflammation is important in development of lung damage, vascular dysfunction, and cancer. Clara cell protein 16 kDa (CC16) has been ascribed an anti-inflammatory function to protect the lung from excessive inflammation. However, evidence on alteration of the lung protein expression as a result of PM exposure and associations between the lung protein and the inflammatory cytokines are still limited. Circulating lymphocytes are easily assessable for human biomarker studies. It could be useful as a surrogate for the lung tissue to predict adverse health outcomes from PM exposure. Nevertheless, no study has been conducted to evaluate the similarity in the response of lymphocytes and lung cells.

Accordingly, the present study aimed to investigate effects of PM on induction of oxidative DNA damage and inflammation by using lymphocytes *in vitro* and in humans exposed to PM in the environment. Human lymphoblasts cell line (RPMI 1788) were treated with diesel exhaust particles (DEP) (SRM 2975) at various concentrations (25-100 µg/ml) to compare the extent of responses with alveolar epithelial cells (A549). ROS generation was determined in each cell cycle phase of DEP-treated cells in order to investigate the influence of the cell cycle stage on induction of oxidative stress. The oxidative DNA damage was determined by measurement of 8-hydroxy-deoxyguanosine (8-OHdG) whereas the inflammatory responses were determined by mRNA expression of *interleukin-6* and *-8* (*IL-6* and *IL-8*), *Clara cell protein (CC16)*, and *lung surfactant protein-A (SP-A)*.

The results showed that RPMI 1788 and A549 cells had a similar pattern of dose-dependent responses to DEP in terms of particle uptake, ROS generation with highest level found in G2/M phase, 8-OHdG formation, and induction of *IL-6* and *IL-8* expression. The human study was conducted in 51 healthy subjects residing in traffic-congested areas. The effects of exposure to $PM_{2.5}$ and particle-bound PAHs and toxic metals on the levels of 8-OHdG in lymphocyte DNA, *IL-8* expression in lymphocytes, and serum CC16 were evaluated. 8-OHdG levels correlated with the exposure levels of $PM_{2.5}$ and PAHs, but this was not the case with *IL-8*. Serum CC16 showed significantly negative correlations with B[a]P equivalent levels, but positive correlation with Pb.

In conclusion, the *in vitro* study demonstrated that DEP can induce ROS generation, oxidative DNA damage, and expression of pro-inflammatory cytokines (*IL-6* and *IL-8*) in lymphocytes with a similar pattern to the lung target cells. Lymphocytes could be used as a surrogate to assess PM-induced oxidative DNA damage and inflammatory responses in the lung. Interestingly, the most prominent increase in ROS generation of the cells in G2/M phase revealed a novel finding that DEP-induced oxidative stress was dependent on cell cycle stage. The susceptibility of G2/M cells to DEP in terms of oxidative stress is most likely reflected in the severity of DNA damages and possibility of the unrepaired DNA lesions in the daughter cells.

Furthermore, the human study showed that increased oxidative DNA damage can be detected in circulating lymphocytes of people exposed to traffic-related PM. However, *IL-8* expression in people exposed to relatively high levels of PM should be further investigated. Moreover, people residing in traffic-congested areas are possibly at risk of health effects from PM exposure. Preventive measures to reduce the particulate air pollution in ambient air are needed to mitigate the health effects.

Source: International Journal of Hygiene and Environmental Health, Vol. 217, Issue 1, Pages 23-33, January 2014.

INVESTIGATION OF POTENTIAL ENDOCRINE ACTIVITY

The high prevalence of disorders related to the endocrine system, e.g. fertility problems and congenital malformations of reproductive organs, has been of growing concern for several years. Genetic, environmental and lifestyle factors are likely to be involved in these adverse effects, and one of these factors is developmental exposure to endocrine active compounds (EACs).

Humans are exposed to a mixture of several EACs, and during the last decade, scientific and regulatory focus has gradually shifted towards taking mixture effects into account.

In studies where experimental animals have been exposed simultaneously to several EACs, e.g. pesticides, substantial mixture effects on reproductive development have been seen, even though each of the individual compounds was present at low doses, where no effects were seen. In addition, there are indications that cumulative exposure to EACs such as pesticides may play a role causing adverse effects on human development. In epidemiological studies possible association between risk of cryptorchidism and maternal pesticide exposure has been reported. Because the adverse effects of some pesticide mixtures occur at exposure levels at which the single pesticides do not cause adverse effects, it raises concerns about their potential combined impact on human health. Thus, there is a need for continued research on the effect of combined exposure in order to gain more knowledge on mixture effects.

The present study forms part of a larger project, in which an initial screening of 13 currently used pesticides was performed, applying a battery of *in vitro* assays, including assays for effects on the estrogen receptor (ER), the androgen receptor (AR), the aryl hydrocarbon receptor (AhR), the thyroid hormone receptor (TR), and steroidogenesis. The aim of the screening was to reveal potential mechanisms of action as well as to determine the potency of the pesticides. The selection of test compounds was based on a list of pesticides that were registered and approved for use in Denmark (except for malathion prohibited in 2008) as well as their endocrine disrupting potential, as described both in the open literature and in draft assessment reports (DARs). The five pesticides chosen for the current study represent different classes of pesticides, and included the fungicides bitertanol and propiconazole, the insecticides cypermethrin and malathion, and the herbicide terbuthylazine. The selection of pesticides was based on their potency and efficacy in the initial *in vitro* tests, with a special focus on data from the H295R steroidogenesis assay. The five pesticides were mixed in two different mixtures. A mixture named "Mix 3" consisting of: bitertanol, propiconazole, and cypermethrin mixed in the ratio 1:1:1 and a mixture called "Mix 5" consisting of all five pesticides mixed in a 1:1:1:1:1 ratio.

In the present study the aim was to investigate the potential endocrine disrupting effects of the selected pesticide mixtures *in vitro* using the H295R steroidogenesis assay, and *in vivo* using an *in utero* exposure rat study. In the *in vitro* experiment all five single pesticides were tested in addition to the two mixtures. For the *in vitro* study the intention was not to perform any mixture modeling or mixture predictions, but rather to compare the effect of the single pesticides to the effects of the mixtures to see if the researchers by intuition were able to predict the qualitative response of the mixtures.

In the *in vivo* study, pregnant rats were dosed with the two pesticide mixtures from gestation day (GD) 7 to 21. At GD 21 fetuses were removed by cesarean section, and various endpoints were measured to examine potential endocrine disrupting effects of the mixtures.

It is an interesting observation that the levels of the three pesticides present in both Mix 3 and Mix 5 were lower when they were combined with terbuthylazine and malathion in Mix 5. This indicates ADME (absorption, distribution, metabolism and excretion) interactions resulting in lower exposure when more chemicals are given at relatively high doses (10 mg/kg). Whether this is due to an affected absorption, distribution, metabolism, and/or excretion of the pesticides remains unknown. However, the lower levels of the same pesticides in Mix 5 compared to Mix 3 indicate that this effect is not primarily due to saturated metabolism. The bitertanol levels in Mix 3 increase in a non-linear fashion and this might be explained by saturated metabolism. However, this nonlinearity is not observed for bitertanol in Mix 5, indicating that the presence of terbuthylazine and/or malathion reduce bitertanol levels or lead to more complex ADME interactions.

It is likely that discrepancies between *in vitro* and *in vivo* results could partly be due to ADME interactions and metabolism of parent compounds that occur *in vivo*, but not *in vitro*. Nevertheless, for Mix 5 alone there seems to be reasonable agreement between *in vivo* and *in vitro* observations, as aromatase induction is evident both *in vitro* and *in vivo*. Terbuthylazine alone has an overall stimulatory effect on testosterone production, but causes a more potent and efficient increase in estradiol levels, which is in agreement with the *in vitro* data on Mix 5 showing a clear indication of increased aromatase activity.

The present study concludes that all five single pesticides, as well as both mixtures, had the ability to affect steroidogenesis *in vitro*, and at the highest dose Mix 5 also exerted endocrine activity in dams as well as female fetuses, in terms of disrupted hormone levels and affected the aromatase mRNA level.

Overall, the human H295R cell assay can give important hints on potential chemically-induced effects on steroidogenesis that can be further investigated *in vivo*. Supplementing the examination on steroidogenesis with a battery of *in vitro* assays covering various mechanisms of action will provide a good basis for generating hypotheses on relevant mechanisms and *in vivo* effects.

Source: Toxicology and Applied Pharmacology, Vol. 272, Issue 3, Pages 757-766, November 2013.

Maternal Diet: Prenatal Protection against Impact of PAHs

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In the present study, researchers investigated the association between bulky DNA adduct levels and birth weight in 612 newborns and further assessed whether maternal consumption of fruits and vegetables during pregnancy modified this association.

The study population was composed of neonates from Denmark (32%), Spain (29%), England (18%), Greece (11%), and Norway (10%). Mothers were predominantly white Europeans, multiparous, and nonsmoking. Most children were born at term and weighed > 2,500 g at birth. Some study population characteristics differed significantly between the Northern and Southern European populations (e.g., maternal smoking; 8% vs. 18%, respectively), whereas characteristics such as maternal prepregnancy BMI and dietary supplement use were similar. The daily median fruit and vegetable intake of the Southern European mothers was 58 g higher than that of Northern European mothers, but the difference was not statically significant. The difference in fruit and vegetable intake between Southern and Northern European mothers was also not statically significant after adjustment for individual total energy intake.

The association of bulky DNA adduct levels with birth weight differed according to maternal intake of fruits and vegetables and intake of fruits high in vitamin C, although interactions were only marginally significant. The estimated difference in birth weight between the highest and lowest tertiles of adduct levels was greater among births to mothers with low intakes of fruits and vegetables than among births to mothers with high intakes. Consumption of dietary supplements during pregnancy was common in both Northern and Southern Europeans (85% and 90%, respectively) and did not appear to modify or confound associations between adduct levels and birth weight.

Low birth weight is an important outcome because it is associated with greater risk of neonatal mortality, hypertension and cardiovascular disease, diabetes, certain cancers, reduced and/or delayed postnatal growth, and cognitive development. The findings suggest that environmental exposures that result in the *in utero* formation of bulky DNA adducts also may affect prenatal growth, and that this potential effect may be reduced by high maternal fruit and vegetable consumption.

Source: Environmental Health Perspectives, Vol. 121, No. 10, Pages 1200-1206, October 2013.

The Role of Glyphosate in Human Breast Cancer Cell Growth

Glyphosate, *N*-(phosphonomethyl) glycine, is widely used as an active ingredient of herbicide products to control weeds in cropped and non-cropped fields around the world. In addition, glyphosate formulations have been used extensively in genetically modified glyphosate-resistant plants. The herbicidal activity of glyphosate is rather specific on the targets with the inhibition of the shikimate pathway which only presents in plants and micro-organisms.

Glyphosate is considered as a non toxic herbicide because of its LD_{50} (the concentration that caused 50% deaths); >4 g/kg. However, the reproductive toxicities of glyphosate have been extensively studied in both animals and humans. Up to now, the endocrine disrupting effects of glyphosate were not observed in the *in vivo* but the *in vitro* studies and the epidemiological studies have still conflicted in those findings due to their differences in the experimental designs, methodology and confounding factors. The synergistic effects of glyphosate and surfactants in its herbicide formulations have been concerned especially the endocrine disrupting activity. Most studies found that the adjuvants or surfactants in most formulations were more toxic and could enhance the toxic effects of glyphosate. Glyphosate at concentrations used in agriculture (21-42 mM) was found to be toxic to human embryonic and placental cells. Roundup®, a popular formulation could disrupt the synthesis of hormones in the mouse MA-10 Leydig tumor cell line. Glyphosate has been shown to disrupt the animal cell cycle in urchin eggs based on its surfactant carrying in commercial formulation. Recently, it was reported that at lower non-toxic concentrations of Roundup® and glyphosate (<1 µg/L), the main endocrine disruption is a testosterone decrease by 35%. Most potential adverse health effects were reported on the commercial glyphosate formulations. The expression of estrogen-regulated genes relating to tumor formation and tumor growth in hormone dependent human breast cancer MCF-7 cells were reported to be disrupted. Furthermore, synergistic effects between glyphosate and estrogen (17β-estradiol or E2) have been demonstrated. Glyphosate was reported to have a disrupting effect on estrogen receptor alpha (ERα) and beta (ERβ) transcriptional activities in HepG2 cells transiently transfected with ERE-TK-luciferase and on androgen receptor (AR) in MDA-MB453-kb2 cells. These toxic effects were reported to be more frequent with glyphosate-based herbicides than that with glyphosate alone.

Now a new study aims to evaluate the estrogenic effects of glyphosate alone at the range of concentrations that has been reported in environmental conditions and exposed humans.

The study shows that glyphosate exerted proliferative effects only in human hormone-dependent breast cancer, T47D cells, but not in hormone-independent breast cancer, MDA-MB231 cells, at 10^{-12} to 10^{-6} M in estrogen withdrawal condition. The proliferative concentrations of glyphosate that induced the activation of estrogen response element (ERE) transcription activity were 5-13 fold of control in T47D-KBluc cells and this activation was inhibited by an estrogen antagonist, ICI 182780, indicating that the estrogenic activity of glyphosate was mediated via ERs. Furthermore, glyphosate also altered both ERα and ERβ expression. These results indicated that low and environmentally relevant concentrations of glyphosate possessed estrogenic activity. Glyphosate-based herbicides are widely used for soybean cultivation, and the results also found that there was an additive estrogenic effect between glyphosate and genistein, a phytoestrogen in soybeans. However, these additive effects of glyphosate contamination in soybeans need further animal study.

Source: Food and Chemical Toxicology, Vol. 59, Pages 129-136, June 2013.

Association between Perfluorooctanoic Acid Exposure and Kidney and Testicular Cancers

Perfluorooctanoic acid (PFOA, or C8) is a synthetic chemical used since the late 1940s in manufacturing industrial and household products. It is persistent in the environment and has a long human half-life. PFOA is found at low levels in the serum of most people living in the United States, with higher levels observed in occupationally exposed workers. Exposure sources in the general population are not well established, but likely include diet, drinking water, food packaging, and household products. PFOA was reported to induce liver, testes, and pancreatic tumors in male rats over a 2-year period. However, no evidence was found of hepatocellular, testicular, or pancreatic tumors in male monkeys exposed to PFOA for 26 weeks and observed for 90 days after exposure. Exposure levels used in the animal studies were higher than human levels typically seen from drinking water or occupational exposure. Because of PFOA's potential for environmental persistence, long human half-life, and possible toxicity, there is rising concern about whether it might be associated with human cancers.

There have been two PFOA-cancer incidence studies among general populations. One of these studies enrolled 57,053 cancer-free Danish adults 50-65 years of age; researchers measured PFOA plasma concentrations during enrollment and followed participants for approximately 10 years for incident prostate, pancreas, liver, and bladder cancers. Positive associations between PFOA and prostate and pancreatic cancers were reported but were not significant, and no significant linear trends were seen for any of the four cancers. A case-control study of 31 breast cancer cases from the Inuit population reported no relationship between PFOA and breast cancer. PFOA levels are typically low and widespread in general populations.

The DuPont chemical plant in Washington, West Virginia, began using PFOA in its manufacturing process in 1951. The plant released PFOA into the Ohio River and air beginning in the 1950s, peaking in the 1990s, and decreasing emissions after 2001. PFOA emitted from the plant entered the groundwater, which was the public drinking water source.

In 2001, residents living near the plant filed a class action lawsuit alleging health damage due to PFOA-contaminated drinking water. A pretrial settlement required DuPont to provide funding for an independent community health study called the C8 Health Project, and also resulted in the creation of the C8 Science Panel, which was tasked with determining whether there was a probable link between PFOA and disease in the community living near the plant.

The C8 Health Project surveyed Mid-Ohio Valley residents in 2005-2006. The survey collected medical history and also measured serum PFOA concentrations. The median serum PFOA concentration in this population was 28 ng/mL in 2005-2006, compared with 4 ng/mL in the United States overall.

Using the C8 Health Project cohort in combination with a DuPont worker cohort, the C8 Science Panel conducted subsequent interviews in 2008-2011 to gather disease incidence data.

Now a new study has examined cancer incidence in Mid-Ohio Valley residents exposed to PFOA in drinking water due to chemical plant emissions.

The cohort consisted of adult community residents who resided in contaminated water districts or worked at a local chemical plant. Most participated in a 2005-2006 baseline survey in which serum PFOA was measured. Researchers interviewed the cohort in 2008-2011 to obtain further medical history. Retrospective yearly PFOA serum concentrations were estimated for each participant from 1952 through 2011. Self-reported cancers were validated through medical records and cancer registry review. Researchers estimated the association between cancer and cumulative PFOA serum concentration using proportional hazards models.

The present study estimated relative risks of incident cancers in association with cumulative PFOA exposure in a large community with a range of exposure levels. More than 2,500 validated cancers covering 21 different cancer types were included in the analysis, making it one of the largest cohorts ever used to examine PFOA and cancer. The findings indicate that PFOA exposure was positively associated with kidney and testicular cancer in this Mid-Ohio Valley population. Because this is largely a survivor cohort, results for highly fatal cancers must be interpreted with caution.

Source: Environmental Health Perspectives, Vol. 121, No. 11-12, Pages 1313-1318, November-December 2013.

Genetic and Oxidative Damage in Workers Exposed to Coal

Coal is an important fossil fuel used for energy generation. During coal extraction, large quantities of coal dust particles are emitted, contributing to environmental pollution. Brazil has approximately 12×10^9 tons of coal reserves, of which approximately 90% are in Rio Grande do Sul (RS), the southern-most Brazilian state. This coal is of low quality, and extraction is performed in open-cast coal mines. The Candiota region, situated in southeast RS, has the largest coal reserves in Brazil. The coal is used locally to generate electricity at the largest thermal power complex in the state. Consequently, a broad range of pollutants from coal and its derivatives is added to the chemical load of the atmosphere in the region.

Coal is a mixture of a variety of chemicals, especially hydrocarbons, which can give rise to polycyclic aromatic hydrocarbons (PAHs). All technological processes associated with open fire or temperatures between 400°C and 600°C, which may lead to the formation of PAHs, should be considered potentially hazardous. Some PAHs, such as benz[a]anthracene, chrysene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene and benzo[g,h,i]perylene, are found in the Candiota region. In addition to hydrocarbons, coal contains high concentrations of various metals that are related to coal and to activities such as mining and burning fossil fuels. Exposure to many types of PAH compounds and metals can lead to DNA damage.

Coal miners are constantly exposed to coal dust and its derivatives. Therefore, characterizing and estimating the risks of exposure are of utmost importance to the safety of individuals working in a coal mining environment. There is an increased risk for developing several diseases following exposure to coal dust and products of combustion. A number of studies have shown that the parameters of oxidative damage are altered following inhalation of industrial particles such as coal. Studies with coal mineworkers have demonstrated higher

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GENETIC AND OXIDATIVE DAMAGE IN WORKERS EXPOSED TO COAL

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levels of DNA and chromosomal damage, as evaluated via assays of chromosomal aberration, micronucleus (MN) and sister chromatid exchange.

In Brazil, some studies have demonstrated genotoxic effects of coal exposure in different species such as wild rodents, bats and land snails. In a previous study, researchers evaluated whether prolonged exposure to coal dust could lead to an increase in the genomic instability, cell death and MN frequency of the basal cells in humans.

The aim of the present study was to complement previous findings by evaluating the potential genotoxic effects of coal exposure and oxidative stress on the lymphocytes of Candiota individuals exposed to coal as part of their occupation.

The results showed that the exposed group had a significantly increased damage index and damage frequency, as assessed using the comet assay, and increased MN and nucleoplasmic bridge frequencies, as assessed using the MN assay, compared with unexposed individuals. Significant and positive correlations between MN frequencies in the lymphocytes and buccal cells of control and exposed individuals were observed. The exposed individuals presented lower average levels of thiobarbituric acid reactive substances (TBARS) and catalase activity (CAT), while the mean superoxide dismutase activity (SOD) levels were higher in this group. The exposed group also had higher hematocrit levels. No correlation between DNA damage and inorganic elements, was found; however, there was a correlation between the damage index and zinc.

One of the challenges facing investigators has been identifying the individual compounds that may be responsible for the possible adverse effects associated with exposure to environmental agents. Although the significance of increased genotoxic effects is difficult to predict for individual subjects, the positive findings of biomonitoring studies suggest a genotoxic hazard at the group level. Genotoxic damage caused by chemical compounds can also be influenced by the individual inheritance of variant polymorphic genes involved in the metabolism of chemical compounds and in DNA repair mechanisms. The evidence of a genetic hazard related to exposure to coal and its derivatives strongly suggests the need for educational programs for coal miners and for the implementation of protective measures.

Source: Mutation Research, Vol. 758, Issue 1-2, Pages 23-28, December 2013.

The Protective Effect of Grape Seed Procyanidin Extract against Cadmium-induced Renal Oxidative Damage in Mice

Cadmium (Cd) is one of the most important toxic metals in the environment and can cause damage to numerous organs of the body. Environmental levels of Cd have increased due to tobacco smoking, and industrial and agricultural practices, including Cd-nickel battery manufacturing, electroplating and paint pigment manufacturing. Moreover, Cd has a long biological half-life in humans (10-30 years) and has been classified as a category I human carcinogen by the International Agency for Research on Cancer (IARC). The toxicity of Cd is associated with the dose and duration of exposure. Acute exposure to Cd induces primarily hepatic and testicular damage, whereas chronic exposure results in renal injury and osteotoxicity. In addition, Cd-poisoning can cause pulmonary edema, hemorrhage, immunotoxicity, and lethality.

Previous studies have suggested that oxidative stress plays an important role in the mechanism underlying Cd toxicity. Oxidative stress may cause glutathione to become depleted, altering the activity of antioxidant enzymes and changing the structure of the cellular membrane through lipid peroxidation. Cd was reported to stimulate the formation of reactive oxygen species (ROS), such as O_2^- and NO, which enhance lipid peroxidation (LPO), leading to disturbances in the antioxidant defense system of organisms. Consequently, protein and DNA damage may occur.

Currently, the search for suitable reagents to counteract the toxic effects of Cd is the focus of numerous studies. The potential of many antioxidants to prevent Cd toxicity, such as coenzyme Q_{10}, β-carotene, and vitamin E, has been investigated. Many treatment protocols for Cd-poisoning have been investigated, though few have succeeded in clinical trials.

Grape seed procyanidin extract (GSPE) is a natural compound that is found in high concentrations in fruits, vegetables, and tea leaves, as well as the seeds of many plants, including grapes and apples. GSPE belongs to a class of phenolic compounds that take the form of oligomers or polymers of polyhydroxy flavan-3-ol units, such as (+)-catechin and (−)-epicatechin. GSPE has been demonstrated to possess a wide array of pharmacological and biochemical actions, including anti-inflammatory, anti-carcinogenic, and antioxidant properties. In many countries, including the United States, Japan, and Korea, GSPE has been used as a nutritional supplement. GSPE has been reported to be a potent free radical scavenger that is more effective than vitamin E and has been reported to reduce oxidative stress. Previous studies have suggested that GSPE may potentially be a therapeutic substance for the treatment of inflammation and oxidative damage.

Now, in a new study, researchers have assessed the protective effect of GSPE on Cd-induced renal damage using animal experiment. After 30 days, the oxidative damage of kidney was evaluated through measurement of superoxide dismutase (SOD), glutathione peroxidation (GSH-Px) and malondialdehyde (MDA). Since, oxidative stress could lead to apoptosis, the renal apoptosis was measured using flow cytometer. Moreover, the expression of apoptosis-related protein Bax and Bcl-2 was analyzed by immunohistochemistry and Western blot.

The results showed that Cd led to the decrease of SOD and GSH-Px activities, and the increase of MDA level, induced renal apoptosis. However, the co-administration of GSPE attenuated Cd-induced lipid peroxidation, and antagonized renal apoptosis, probably associated with the expression of Bax and Bcl-2. These data suggested that GSPE has protective effect against renal oxidative damage induced by Cd, which provides a potential natural chemopreventive agent against Cd-poisoning.

Source: Environmental Toxicology and Pharmacology, Vol. 36, Issue 3, Pages 759-768, November 2013.

ANNOUNCEMENT

International Symposium on "RECENT ADVANCES IN CANCER THERAPEUTICS"
October 13-15, 2014, Chulabhorn Convention Center, Bangkok, Thailand

Organized by Chulabhorn Research Institute in collaboration with Fritz Bender Foundation

Sessions:

I. Genetic Profiles of Cancer
II. Therapeutic Targets and Drugs
III. Experimental Therapeutics: Models
IV. Tumor-Host Relationships
V. Translational Investigations

Invited Speakers (partial list):

- Anne-Lise Borresen-Dale, Norway
- Carlos Caldas, UK
- Bruce Chabner, USA
- Lynn G. Fuen, USA
- Michelle D. Garrett, UK
- Alex Matter, Singapore
- Enrico Mihich, USA
- Mathuros Ruchirawat, Thailand
- Niramol Savaraj, USA
- Tatsuhiro Shibata, Japan
- Zoltan Szallasi, Denmark
- Axel Ullrich, Germany
- Kurt Zanker, Germany

For more information, please visit: **http://ract2014.cri.or.th**

CALENDAR OF EVENTS

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

	Training Course	Approximately Date	Duration	Closing Date
1.	Environmental Toxicology	May 1-9, 2014	2 weeks	March 31, 2014
2.	Environmental Immunotoxicology and Reproductive Toxicology	October 20-31, 2014	2 weeks	August 27, 2014
3.	Environmental and Health Risk Assessment and Management of Toxic Chemicals	December 2014	2 weeks	September 24, 2014
4.	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.

International Training Courses on "Environmental Immunotoxicology and Reproductive Toxicology" October 20-31, 2014

Faculty: Judith T. Zelikoff, Ph.D., New York University, USA
Nancy Denslow, Ph.D., University of Florida, USA
Norbert E. Kaminski, Ph.D., Michigan State University, USA

This course consists of 2 parts. The first part provides an overview of blood cells and the mammalian immune system, including a detailed description of all three arms of the immune response, how toxic chemicals can impact normal immune system homeostasis to result in adverse health outcomes, cellular/functional methodologies for determining the potential immunotoxicity of a given chemical, and how immunotoxicology relates to other scientific fields such as drug development and risk assessment. The second part provides an introduction to hormonally active agents and their mechanisms of action; routes of exposure, bioaccumulation, distribution and metabolism; effects on reproduction and development in humans and animals; and methods for studying changes in gene expression.

Requirement: Participants should have an understanding of the fundamental principles oftoxicology, and some basic knowledge on the biology of the immune and reproductive systems.

Contact: Chulabhorn Research Institute (CRI)
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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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