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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".

INTERNATIONAL TRAINING COURSE ON ENVIRONMENTAL TOXICOLOGY

Organized by Chulabhorn Research Institute
18 – 28 July 2005

This international training course was part of Chulabhorn Research Institute's ongoing training program for human resource development in environmental toxicology. It was attended by 19 participants from 10 countries in the Asia Pacific region.

Ten participants, from Cambodia, China P.R., Fiji, Hong Kong, Nepal, Philippines, Sri Lanka and Vietnam, were supported by Thailand International Development Cooperation Agency; and a further nine participants from Lao P.D.R. were supported by the same agency under the Thai-Lao Project.

The course was designed to provide participants with a comprehensive background of major groups of toxic substances encountered by humans and animals in food and in the environment as well as through exposure at the workplace. These toxicants include mycotoxins, naturally occurring toxins, N-nitroso compounds, solvents, plastics, pesticides, pollutants and radiation.

The main focus of the course was on the chemistry of toxicants, their fate and distribution in the environment, the mechanisms of their actions and toxic manifestation in living organisms including toxic syndrome in human beings.

The training course was coordinated by Dr. *Khunying* Mathuros Ruchirawat, Vice President for Research at the Chulabhorn Research Institute, and the faculty consisted of a team of internationally renowned experts: Professor Ronald C. Shank, Director, Environmental Toxicology Program, University of California at Irvine, U.S.A.; Professor John H. Duffus, Director, Center of Toxicology, Edinburgh, U.K.; Professor Leonard Ritter, Executive Director, Department of Environmental Biology, University of Guelph, Ontario, Canada; and Dr. Roger Walk, Director, Worldwide Scientific Affairs, Philip Morris, U.S.A.

Arsenic and Prostate Cancer

Although arsenic carcinogenesis has many other targets, a significant association has also been observed between prostate cancer and chronic arsenic exposure. Arsenic can cause malignant transformation of human prostate epithelial cells *in vitro*, and these CAsE-PE (chronic-arsenic-exposed prostate epithelial) cells produce aggressive, carcinoma-like tumors when inoculated into nude mice. There is also evidence that arsenic can enhance tumor progression. For instance, oral exposure to arsenic in mice not only increases the incidence but also greatly increases the progression of skin cancers associated with ultraviolet irradiation. Furthermore, transplacental exposure to arsenic is an effective carcinogen in mice, resulting in malignant tumors of the liver and lung. In this model system, arsenic also appears to act as a tumor progressor because it greatly increases malignant liver tumor multiplicity. Although arsenic exposure is associated with prostate cancer in humans, the role of arsenic in prostate cancer progression is undefined.

The normal prostate gland requires androgen for growth and maintenance of differentiated function and will undergo regression if androgen is withdrawn. Prostate cancer therapy often involves orchiectomy and pharmacologic intervention to diminishing availability of androgen at the androgen receptor (AR) within prostate cancer cells. However, prostate cancer cells often lose the need for androgen as a survival, growth, or differentiation factor and become androgen independent. Although poorly understood, this progression to androgen independence is clearly a critical step in the development of advanced prostate cancer. Androgen-independent prostate cancers are typically more advanced and difficult to treat, and acquisition of such independence has been called a “death sentence” for prostate cancer patients.

Altered AR levels or activity can be key elements in acquired androgen independence in prostate cancer. AR is a nuclear transcription factor that normally binds androgen to activate its signaling pathway. Prostate cancer cells can achieve functional AR signaling in the presence of greatly diminished androgens in a variety of ways. AR gene amplification and overexpression can make cells hypersensitive to low levels of androgen, and many prostate cancers show overexpression of AR. In addition, AR mutations have been recognized that change the ligand specificity of AR such that it can be activated by nonandrogens and even antiandrogens. Furthermore, ligand-independent activation of the AR pathway appears to occur in some instances, creating, in essence, a bypass of AR. For instance, certain growth factors, such as insulin-like growth factor-1, keratinocyte growth factor, and epidermal growth factor (EGF), as well as HER2/neu, a member of the EGF-receptor family of receptor tyrosine kinase, can activate AR-dependent genes in absence of AR ligand. Thus, evidence suggests that altered AR levels, activity, or function can play a major role in the development of androgen-refractory prostate cancer cells, although an AR bypass can also be important. In men the primary circulating androgen is testosterone. In the prostate, testosterone is converted to the more potent androgen 5-α-dihydrotestosterone (DHT) by the enzyme 5α-reductase (5α-R). DHT is 3-10 times more potent than testosterone in activating AR-regulated downstream events. There is evidence that a significant portion of human prostate cancers overexpress 5α-R type 1. Androgens may be converted to estrogens by the enzyme 5α-aromatase (5α-A). This aromatase is expressed in the human prostate, suggesting a local role for estrogen. Indeed, estrogen can elicit direct actions affecting the growth of prostate cells and can affect estrogen receptor (ER)-mediated gene transcription. Estrogens have been implicated in the promotion of aberrant prostate growth and do not necessarily always work through indirect inhibition of androgen pathways. In animal models, it has been well established that estrogen may play an important role in prostate carcinogenesis. As in other tissues, the effects of estrogen on the prostate are likely transduced primarily by ERs. Prostate cells can be a direct target of estrogen regulation, because they contain both ER-α and ER-β. Recent evidence indicates that antiestrogens can perturb prostate cancer formation and progression and that this effect is at the level of the ER within prostate cells.

In the current study, researchers examined the growth of normal and arsenic-transformed human prostate epithelial cells. The arsenic-transformed cells had been exposed continuously to sodium arsenite and, after 29 weeks of exposure, produced malignant tumors when inoculated into nude mice. Both cell lines were observed in two different media. One medium included the complete range of steroids, including ample amounts of androgen and growth factors. The other lacked normal amounts of sex hormones and growth factors.

The experiment showed that, consistent with malignant cell growth, prostate cells chronically exposed to arsenic grew more rapidly than control cells in both media. In the steroid-rich medium, the arsenic-transformed cells proliferated approximately twice as fast as the unexposed control cells. In the steroid-depleted medium, the arsenic-transformed cells proliferated about 2.5 times faster than control cells.

Arsenic exposure therefore appeared to be associated with the acquisition of androgen independence in prostate cells. However, the observed arsenic-induced androgen independence did not occur by any previously studied mechanism; it was not linked to overexpression of androgen receptors or receptor mutations that facilitate cell growth via nonandrogens.

A clue may lie in earlier experiments, in which the same researchers observed a marked increase in production of K-*ras* (an oncogene associated with prostate cancers) in arsenic-transformed cells. K-*ras* is a key part of a growth-stimulating pathway in the prostate that is stimulated by androgens. K-*ras* was clearly correlated with arsenic-induced carcinoma-like growth and androgen independence. The researchers speculate that arsenic may by-pass the androgen receptor and directly cause aberrant K-*ras* activation.

Source: Environmental Health Perspectives, Vol. 113, No. 9, September 2005.

The Interaction Between Arsenic Exposure and Urinary Arsenic Speciation on the Risk of Peripheral Vascular Disease

About 40 million people in different parts of the world are exposed to arsenic via drinking water. Exposure to inorganic arsenic through drinking artesian water is the most possible cause of the so-called blackfoot disease (BFD), a unique peripheral vascular disease (PVD) identified in the endemic area along the southwestern coast of Taiwan. The disease frequently ends up with dry gangrene and spontaneous amputation of affected extremities, with an underlying pathological change of severe systemic atherosclerosis.

In drinking water, arsenic is usually found in the form of inorganic arsenate (As^{V}) or arsenite (As^{III}), depending on the pH and the presence of oxidizing and reducing substances. The metabolism of inorganic arsenic involves 2 steps of chemical reactions: reduction and oxidative methylation. Arsenate is reduced to arsenite before it can be further metabolized. Arsenite is then oxidatively methylated to monomethylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}). The methylation process of arsenic is catalyzed by a 42-kDa protein encoded by the *cyt19* genes of mouse and human genomes and the methyl donor has been identified as *S*-adenosylmethionine. Previously, methylation of inorganic arsenic has always been considered as a detoxification mechanism because MMA^{V} and DMA^{V} have relatively low toxicity, and are rapidly excreted in the urine. However, recent studies have confirmed the existence of trivalent intermediates and products of monomethylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}), which are more toxic than inorganic arsenite. The capacity to metabolize inorganic arsenic differs among individuals; and its biologic effects on various organ systems depend not only on the ingested dosage, but also on the capacity of the individuals to metabolize and detoxify the related compounds. To achieve a more accurate assessment of arsenic methylation capacity, it is necessary to determine the specific arsenic species derived from inorganic arsenic, which are excreted in the urine. Studies evaluating the association between various urinary arsenic metabolites and clinical outcomes are still rare. Some recent studies have documented that subjects with higher cumulative arsenic exposure (CAE) and higher urinary MMA^{V} percentage or lower urinary DMA^{V} percentage suffered from a higher risk of skin cancer and bladder cancer among residents of the BFD areas. Previous studies evaluating the association between inorganic arsenic and PVD have focused on the estimated ingested dosage of total arsenic from drinking water. Whether the metabolism of arsenic could have an effect on the risk of developing PVD is an interesting issue that has not been studied before. Now a new study aims at evaluating the impact of the

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ARSENIC EXPOSURE AND CARDIOVASCULAR DISEASE

Chronic exposure to arsenic is well known to be the cause of cardiovascular diseases such as hypertension.

Previous epidemiologic studies have reported that peripheral vascular resistance and systemic blood pressure were elevated in populations that had ingested arsenic-contaminated drinking water. Researchers have observed that chronic administration of arsenite to rats and rabbits caused a significant increase in vascular peripheral resistance. These studies suggest the possibility that arsenic may disrupt normal vasomotor function, leading to hypercontraction of blood vessels. Indeed, a previous study demonstrated that arsenic could inhibit acerylcholine-induced vascular relaxation via inhibition of nitric oxide synthase in vascular endothelial cells.

In order to further investigate the effect of arsenic on blood vessels, researchers have now examined whether arsenic affected the contraction of aortic rings in an isolated organ bath system.

The study found that treatment with arsenite, a trivalent inorganic species, increased vasoconstriction induced by phenylephrine or serotonin in a concentration-dependent manner.

Among the arsenic species tested – arsenite, pentavalent inorganic species (arsenate), monomethylarsonic acid (MMA^{V}), and dimethylarsinic acid (DMA^{V}) – arsenite was the most potent. Similar effects were also observed in aortic rings without endothelium, suggesting that vascular smooth muscle plays a key role in enhancing vasoconstriction induced by arsenite. This hypercontraction by arsenite was well correlated with the extent of myosin light chain (MLC) phosphorylation stimulated by phenylephrine. Direct Ca^{2+} measurement using fura-2 dye in aortic strips revealed that arsenite enhanced vasoconstriction induced by high K^{+} without concomitant increase in intracellular Ca^{2+} elevation, suggesting that, rather than direct Ca^{2+} elevation, Ca^{2+} sensitization may be a major contributor to the enhanced vasoconstriction by arsenite. Consistent with these *in vitro* results, 2-hr pretreatment of 1.0 mg/kg intravenous arsenite augmented phenylephrine-induced blood pressure increase in conscious rats. These results suggest that arsenite increases agonist-induced vasoconstriction mediated by MLC phosphorylation in smooth muscles and that calcium sensitization is one of the key mechanisms for the hypercontraction induced by arsenite in blood vessels.

Source: Environmental Health Perspectives, Vol. 113, No.10, October 2005.

The Effects of Benzene Exposure on Peripheral Blood Mononuclear Cell Gene Expression in a Population of Workers in a Shoe Factory

Benzene is an established cause of leukemia. However, the mechanisms of benzene-induced hematotoxicity and leukemogenesis, as well as the risk benzene poses at low levels, remain unclear.

To shed further light on these mechanisms and to better understand the risks posed by benzene in the workplace, a new study has been conducted in Tianjin, China to examine the effects of benzene exposure on peripheral blood mononuclear cell (PBMC) gene expression in a population of shoe-factory workers with well-characterized occupational exposures to benzene using cDNA microarrays and real-time polymerase chain reaction (PCR).

PBMC RNA was stabilized in the field and analyzed using a comprehensive human array, the U133A/B Affymetrix GeneChip set. A matched analysis of six exposed-control subject pairs was performed. A combination of robust multiarray analysis (RMA) and ordering of genes using paired *t*-statistics, along with bootstrapping to control for a 5% familywise error rate, was used to identify differentially expressed genes in a global analysis. This resulted in a set of 29 known genes being identified that were highly likely to be differentially expressed. These analyses were repeated on a smaller subset of 508 cytokine probe sets and it was found that the expression of 19 known cytokine genes was significantly different between the exposed and control subjects. Six genes were selected for confirmation by real-time PCR, and of these, *CXCL16, ZNF331, JUN,* and *PF4* were the most significantly affected by benzene exposure, a finding that was confirmed in a larger data set from 28 subjects. The altered expression was not caused by changes in the makeup of the PBMC fraction. Thus, microarray analysis along with real-time PCR confirmation reveals that altered expressions of *CXCL16, ZNF331, JUN,* and *PF4* are potential biomarkers of benzene exposure.

This study demonstrates that microarray analysis can be a useful tool for discovering genes of potential mechanistic interest or biomarkers of exposure and early effect in molecular epidemiological studies of populations exposed to potential carcinogens. It also demonstrates that only small numbers of paired study subjects are required to identify differentially expressed genes.

The small-sample protocol used here also limits the amount of high-quality RNA required, meaning that archived samples, stored by partial isolation and stabilization of the RNA in the field, are amenable to analysis. These studies therefore provide a model for biomarker discovery in chemically exposed human populations, although with lower exposed populations it may be necessary to study more subject pairs, with 15 pairs probably being ideal. Because the price of global gene expression arrays is decreasing, such studies are becoming more feasible.

Source: Environmental Health Perspectives, Vol. 113, No. 6, June 2005.

The Interaction Between Arsenic Exposure and Urinary Arsenic Speciation on the Risk of Peripheral Vascular Disease

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interaction between arsenic exposure dosage and urinary arsenic species on the development of PVD among residents in the BFD-hyperendemic area.

The study subjects were recruited from residents in three BFD-hyperendemic villages in Putai Township of Chiayi County located along the southwest coast of Taiwan. BFD prevalence ranged from 9.6 to 13.6 per 1000 in these villages, with a median arsenic concentration of artesian well water ranging from 0.70 to 0.93 mg/L. A tap water supply system was implemented in the study villages in the early 1960s, but its coverage remained low until the early 1970s. Artesian well water was no longer used for drinking and cooking after the mid-1970s. The population with an age of 30 years or older in the studied villages as registered in the household registration office was 2258. Among them, 1571 (70%) were eligible and lived in the study villages 5 or more days a week. From September to December 1988, a total of 1081 (69%) of the eligible subjects were interviewed. All of the 1081 subjects were invited to participate in the first health examination during January and February 1989 and 941 (87%) subjects actually participated. Bi-annual health examinations were then carried out. The urinary samples used for the assay of arsenic metabolites in the present study were collected during the first health examination. The Doppler ultrasound examination for diagnosis of PVD was performed and blood samples were collected during the third health examination in February 1993. A total of 479 subjects having both urinary samples and receiving Doppler ultrasound examination were recruited for the present study.

The study concludes that PVD risk in BFD-hyperendemic area in Taiwan is associated with a higher exposure dosage of arsenic and a lower capacity to methylate inorganic arsenic to DMA^{V}. These observations could explain partly why some subjects with a high exposure dosage would not develop clinical PVD. However, the association between PVD and the undetected trivalent forms of methylated metabolites in this study awaits further clarification.

Source: Toxicology and Applied Pharmacology, Vol. 206, August 2005.

RESEARCH AT CRI

Environmental and Occupational Exposure to Benzene in Thailand

Benzene is classified as a human carcinogen, and an association between exposure to benzene and the development of leukemia is well established. Exposure to benzene in the environment and in certain occupational settings has been a subject of concern in Thailand, particularly since it was observed that the incidence of leukemia has increased in the past few decades.

Benzene is an important component of gasoline. In Thailand, the limit for benzene content in gasoline is set at 3.5%, while in some industrialized countries, such as the USA, the content is only 1%. Benzene is also used in the petrochemical as well as many other chemical industries. Sources of benzene in the environment, to which the general public may be exposed, are cigarette smoke, burning of coal, and traffic emissions. In many occupational settings, exposure may be from benzene used as a solvent, as a raw material, or in gasoline itself.

Environmental and occupational monitoring of benzene exposure and the use of various biomarkers to study benzene exposure and potential toxicity can help to identify the high-risk groups and to determine whether this risk is due to the relative high exposure levels, as in occupational exposures, or the inherently greater susceptibility of the individual, such as in children.

A study was undertaken by researchers at the Laboratory of Environmental Toxicology at Chulabhorn Research Institute to investigate exposure to benzene in the environment, i.e. from traffic-related air pollution in Bangkok, and in various occupations, such as in gasoline service attendants and petrochemical factory workers. Environmental and personal air sampling were used to assess the exposure. Measurement of blood benzene levels and/or urinary *trans, trans*-muconic acid (MA) provides information on the internal doses as well as individual variation in metabolism. DNA damage as measured by the Comet assay, and DNA repair capacity as measured by the cytogenetic challenge assay, provide information on possible early biological effects of benzene exposure and may be indicative of health risks.

In the study involving traffic-related air pollution, seven heavily congested areas of Bangkok were chosen as study locations for roadside and school levels of benzene, namely the Pratunam, Banglampu, Chakrawad, Pratumwan, Hualampong, Surawongse and Victory Monument areas. Street vendors who set up their stalls directly at the roadside and whose work period was approximately 8 h were recruited as subjects. Temple areas located within 500 m of the selected main roads were chosen as control sites, and monks and nuns who spent most of their time in the temples were selected as the controls. Through information provided in the questionnaires, factors such as age and lifestyle (e.g. non-smoking status, medication, type of diet, etc.) were taken into consideration to ascertain that they were well matched between controls and study groups. Schools in Bangkok located within 500 m of the main roads were selected as study sites, while others situated in Chonburi (a provincial area located approximately 110 km from Bangkok) were used as control sites. Students were age-, gender-, and education level-matched between the control and study groups. An explanation of the study was given to all subjects and an informed consent was obtained from all the participants (or parents in the case of the school children).

Attendants from gasoline service stations in Bangkok and workers in the quality control unit of petrochemical factories involved with the production of aromatic compounds, such as benzene and toluene, were recruited in this study. Gasoline service station attendants worked 8-h shifts and only refueled gasoline. They were not involved in any other responsibilities during their workday. The control subjects were age-, gender-, and lifestyle (non-smoking status, medication, type of diet, etc.)-matched workers in an occupational setting unrelated to the use of benzene.

Results from this study showed that various different activities which required people to work at varying distances from main roads in Bangkok caused them to have different levels of exposure to benzene. Cloth and grilled-meat vendors had significantly higher individual exposures to benzene than controls (monks and nuns), while Bangkok school children were also exposed to significantly higher levels of benzene than rural school children. In terms of potential health effects, these greater exposures led to an increase in DNA damage and a decrease in DNA repair capacity, although we cannot rule out the potential impact of concurrent exposures to other genotoxic compounds. These observed effects indicate that exposed individuals are potentially at greater risk for diseases such as cancer. Finally, the fact that similar effects were observed in Bangkok school children as in occupationally-exposed adults, who were exposed to far greater levels of benzene, provides an indication that children may be more susceptible to the effects of genotoxic environmental contaminants.

Source: Chemico-Biological Interactions, Vol. 153-154, May 2005.

IMMUNOMODULATORY EFFECTS OF FUNGICIDE EXPOSURE ON VINEYARD WORKERS

Ethylenebisdithiocarbamates (EBDCs) are widely used in agriculture as fungicides. Evidence has emerged in recent years to suggest that these conpounds and other dithiocarbamates have an immunomodulatory effect.

One compound of this group, the sodium diethyldithiocarbamate (Imuthiol), has been shown to be a potent *in vivo* immunomodulator, influencing maturation and activation of T cells, natural killer (NK) cell activity, IgG secretion, and prolonging immunological memory.

Based on these properties, Imuthiol has been experimented as immunostimulator for the therapy of different clinical immunodeficiency conditions, including HIV infections; however, the results of double-blind placebo-controlled trials failed to show any beneficial effects. In workers occupationally exposed to an EBDC compound, the ethylenebisdithiocarbamate of manganese and zinc (Mancozeb), an increase in serum IgG, IgE, and α_2-macroglobulin was observed. Studies carried in a group of workers engaged in Mancozeb production demonstrated an increase in T-cell proliferative responses, suggesting an immunostimulator effect in conditions of low-level, prolonged occupational exposure. On the other hand, ethylenebisdithiocarbamate of zinc did not show any immunoenhancing effect in response to T-cell mitogens, since only a cytocidal effect on spleen lymphocytes was shown, whereas sodium methyldithiocarbamate has also been reported to cause a significant immunosuppression in mice following *in vivo* exposure. At present, given the number of conflicting reports, there is insufficient evidence either in humans or animals to confirm, or to rule out, the hypothesis that EBDC compounds can act as immunomodulators in occupational or environmental exposures.

Thus the present study has been carried out with the aim of evaluating the immune system of a group of agricultural workers occupationally exposed to Mancozeb.

The study was carried out in Northern Italy, in a rural area named "Oltrepo Pavese", on 13 vineyard workers (12 males and 1 female; mean age: 41, range: 25-54) and 13 matched healthy controls (12 males and 1 female; mean age: 40, range: 25-50). Agricultural workers were engaged in pesticide application on vineyard. In the period of the investigation (May-June), workers applied by tractor, three to four times, a Mancozeb-based plant-protection product, with a daily use of 5-10 kg of active ingredient. Applications lasted 1-2 days each and were made at 10- to 14-day intervals. Matched controls, never exposed to Mancozeb or to other plant protection products, were selected in the same geographical area.

Exposure was assessed through the determination of ethylenethiourea (ETU) in urine. Complete and differential blood count, serum immunoglobulins, complement fractions, autoantibodies, lymphocyte subpopulations, proliferative response to mitogens, natural killer (NK) activity, and cytokine production were measured.

Post-exposure samples showed ETU urine concentration significantly higher than pre-exposure and control groups. A significant increase in CD19+ cells, both percentage and absolute number, and a significant decrease in the percentage of CD25+ cells were found in post-exposure samples compared to controls. A statistically significant increase in the proliferative response to phorbol myristate acetate plus ionomycin (PMA + ionomycin) was observed in the post-exposure group compared to controls and baseline, while a significant reduction in LPS-induced TNF-α release in post-exposure samples was observed.

Overall, the results of the studies suggest that low-level exposure to Mancozeb has slight immunomodulatory effects, and point out a method adequate to reveal immune-modifications in workers occupationally exposed to potential immunotoxic compounds, based on a whole blood assay.

In particular, the use of whole blood makes possible the assessment of the functionality of the immune system without preparation artifacts; is time effective and requires only an small amount of sample; and is equal in all functional assays. It is essential still, however, to quantify either the relative or the absolute white blood cells and lymphocyte subsets. Indeed, the use of whole blood may mask an excess or reduction of a cell type number, which may lead to analytical bias.

Source: Toxicology and Applied Pharmacology, Vol. 208, November 2005.

CHLORPYRIFOS EXPOSURE AND SEX-SELECTIVE HYPERLIPIDEMIA AND HYPERINSULINEMIA IN RATS

Chlorpyrifos, one of the most widely used organophosphorus pesticides, is increasingly restricted in the United States because of its adverse effects on fetal and neonatal brain development. Nevertheless, organophosphates, including chlorpyrifos, still account for up to 50% of all insecticide application worldwide. Although the systemic toxicity of these pesticides resides in their ability to inhibit cholinesterase, other mechanisms contribute to their developmental neurotoxicity, notably the targeting of cell signaling cascades governing neuronal and hormonal signals that are essential to cell differentiation and homeostatic regulation. Of these, the pathway synthesizing cyclic AMP (cAMP), controlled by adenylyl cyclase, appears to be among the most prominent sites for disruption by chlorpyrifos.

Adenylyl cyclase and cAMP also participate in important metabolic cardiovascular and hormonal functions in the periphery, and researchers recently found that neonatal chlorpyrifos exposure leads to disruption of cardiac and hepatic cell signaling in adulthood. Perhaps most critically, chlorpyrifos-exposed males showed hyperreactivity of hepatic adenylyl cyclase to stimulation of β-adrenoceptor or glucagon receptors, inputs that are responsible for promoting gluconeogenesis and the consequent rise in circulating glucose levels. If these cellular alterations do indeed result in changes in hepatic function and responsiveness, then neonatal chlorpyrifos exposure might be expected to elicit long-term hyperglycemia and associated metabolic abnormalities, or alternatively, insulin hypersecretion might be required to offset the promotion of gluconeogenic signals.

Now, a new study has demonstrated that male rats exposed neonatally to chlorpyrifos display hyperinsulinemia and hyperlipidemia in adulthood.

In the study, 1 mg/kg/day of chlorpyrifos was administered to rats on postnatal days 1 to 4. This regimen was below the threshold level for systemic toxicity.

When tested in adulthood, chlorpyrifos-exposed animals displayed elevations in plasma cholesterol and triglycerides, without underlying alterations in nonesterified free fatty acids and glycerol. This effect was restricted to males. Similarly, in the postprandial state, male rats showed hyperinsulinemia in the face of normal circulating glucose levels but demonstrated appropriate reduction of circulating insulin concentrations after fasting. These outcomes and sex selectivity resemble earlier findings at the cellular level, which identified hepatic hyperresponsiveness to gluconeogenic inputs from β-adrenoceptors or glucagon receptors. The results thus indicate that apparently subtoxic neonatal chlorpyrifos exposure, devoid of effects on viability or growth but within the parameters of human fetal or neonatal exposures, produce a metabolic pattern for plasma lipids and insulin that resembles the major adult risk factors for atherosclerosis and type 2 diabetes mellitus.

Source: Environmental Health Perspectives, Vol. 113, No.10, October 2005.

EFFECTS OF LOW-LEVEL CHRONIC EXPOSURE TO CADMIUM ON BONE METABOLISM OF MALE RATS

Bone disorders are one of the main adverse health effects of chronic cadmium (Cd) intoxication in humans and experimental animals. Nowadays, an increasing interest has been focused on the bone effects of Cd, since there is evidence from epidemiological studies that relatively low exposure to this metal may pose a risk for skeletal damage. Decreased bone mass due to environmental exposure to Cd has been reported especially in elderly women. However, in both sexes, an inverse correlation between Cd concentration in blood and/or urine and the bone mineral status, indicating a dose-effect relationship for the bone effect of Cd, has been noted. Cd has been suggested as an environmental risk factor for osteoporosis, but the critical level of the exposure, the risk of bone damage, and the gender-dependent differences in the vulnerability to the bone injury at low and moderate environmental exposure are still unknown. Generally, there is a lack of comprehensive data concerning whether health effects, including skeletal damage, due to environmental exposure to various contaminants are prevalent or manifested differently in men and women.

Recently, based on a female rat model of human exposure, researchers have reported that low-level chronic exposure to Cd has an injurious effect on the skeleton. The purpose of the current study was to investigate whether the exposure may also affect bone metabolism in a male rat model and to estimate the gender-related differences in the bone effect of Cd. Young male Wistar rats received drinking water containing 0, 1, 5, or 50 mg Cd/l for 12 months. The bone effect of Cd was evaluated using bone densitometry and biochemical markers of bone turnover. Renal handling of calcium (Ca) and phosphate, and serum concentrations of vitamin D metabolites, calcitonin, and parathormone were estimated as well. At treatment with 1 mg Cd/l, corresponding to the low environmental exposure in non-Cd-polluted areas, the bone mineral content (BMC) and density (BMD) at the femur and lumbar spine (L1-L5) and the total skeleton BMD did not differ compared to control. However, from the 6th month of the exposure, the Z score BMD indicated osteopenia in some animals and after 12 months the bone

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New Insights into the Etiology of Brain Tumors

A research team has reported an association between a genetic variant for d-aminolevulinic acid dehydratase (ALAD, an enzyme involved in the synthesis of heme) and an increased risk of developing meningioma, a tumor that occurs in the membranes covering the brain and the spinal cord.

The team discovered a connection between *ALAD2*, an *ALAD* polymorphism carried by people with higher than normal concentrations of lead in their blood, and meningioma. The study was with 573 patients with brain tumors from hospitals in Arizona, Massachusetts, and Pennsylvania. Of this total, 151 had meningioma, 355 had glioma (a cancer that grows from glial cells in the brain), and 67 had acoustic neuroma (a tumor of the auditory nerve). These patients were compared to 505 control subjects who were admitted to the same hospitals for conditions that did not involve tumors.

The *ALAD* genotype – based on the *ALAD1* and *ALAD2* alleles – was determined for each patient and each control subject. Possible links between the *ALAD2* allele and the brain tumors were investigated using unconditional logistic regression.

The statistical analyses revealed that people who carried the *ALAD2* allele (heterozygotes and homozygotes) were 1.6 times more likely than the *ALAD1* homozygotes to develop meningioma.

This modest but significant association was stronger in males, who were 3.5 times more likely to develop meningioma if they had the variant allele.

Confirmation of these findings will require replication in other studies with a larger number of meningioma cases. If risk of meningioma is truly increased in individuals with the *ALAD2* allele, the question arises as to whether the effect depends upon exogenous chemical exposures that act on the heme synthesis pathway or is independent of such exposures. A direct effect of the *ALAD2* polymorphism might be indicated if the *ALAD2* allele has lower enzyme activity than the *ALAD1* allele, given that the precursor ALA is thought to be neurotoxic and genotoxic. However, ALAD enzyme activity does not appear significantly different for the two alleles. Alternatively, it is possible that the increased risk of meningioma in *ALAD2* individuals arises in the presence of chemicals that influence the heme synthesis pathway. Several chemicals have been shown to inhibit ALAD enzyme activity, including lead, trichloroethylene, bromobenzene, and styrene. Polymorphic differences in enzyme binding or chemical uptake have been examined most extensively for lead, and individuals with the *ALAD2* allele are generally reported to have higher blood lead levels than are individuals with the *ALAD1* allele.

The observation that the relationship between *ALAD2* and risk of meningioma was stronger in men than in women could be due to biologic differences or differential exposure to a chemical agent modified by *ALAD* genotype. Given the small number of male meningioma cases with the variant allele, there is also the possibility that the observed effect modification is due to chance.

Although this study had limited power for evaluating risk with respect to subtypes of tumor, it remains one of the largest case-control studies of brain tumors to date. Aside from a small percentage of brain tumors that can be explained by familial syndromes or exposure to ionizing radiation, very little is known about the etiology of brain tumors. In order to clarify the role of lead (or other chemicals) in the observed relationship between *ALAD* genotype and risk of meningioma, it will be important to conduct a detailed exposure assessment and evaluate the joint effect of exposure and *ALAD* genotype in this, or another, study population.

Source: Environmental Health Perspectives, Vol. 113, No. 9, September 2005.

EFFECTS OF LOW-LEVEL CHRONIC EXPOSURE TO CADMIUM ON BONE METABOLISM OF MALE RATS

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resorption very clearly tended to an increase. The rats' exposure corresponding to human moderate (5 mg Cd/l) and especially relatively high (50 mg Cd/l) exposure dose- and duration-dependently disturbed the processes of bone turnover and bone mass accumulation leading to formation of less dense than normal bone tissue. The effects were accompanied by changes in the serum concentration of calciotropic hormones and disorders in Ca and phosphate metabolism. It can be concluded that low environmental exposure to Cd may be only a subtle risk factor for skeletal demineralization in men. The results together with previous findings based on an analogous model using female rats give clear evidence that males are less vulnerable to the bone effects of Cd compared to females.

Source: Toxicology and Applied Pharmacology, Vol. 207, September 2005.

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