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ESTROGENIC AND ANTI-ANDROGENIC ACTIVITIES OF 4-NITROPHENOL IN DIESEL EXHAUST PARTICLES

Air pollution is a grave problem throughout the world, and diesel exhaust particles (DEP) are a leading contributor. DEP contain thousands of compounds that have hazardous effects on human health, including lung cancer, allergic rhinitis, and bronchial asthma-like disease. Another important feature of DEP is their endocrine-disrupting effects and potential adverse impact on both male and female reproductive function. Previous studies have reported that diesel exhaust suppresses spermatogenesis in adult mice and rats. In addition, pregnant C57BL mice injected with DEP extract showed significant increases in abortion rate and uterine weight. These *in vivo* findings show that DEP contain compounds that can modulate estrogenic and anti-androgenic activities. *In vitro* studies also have shown that DEP possess estrogenic, anti-estrogenic, and anti-androgenic activities. However, the specific compound(s) responsible for these phenomena remains unclear. To address this question, researchers recently isolated four nitrophenol derivatives: 4-nitrophenol (PNP), 2-methyl-4-nitrophenol, 3-methyl-4-nitrophenol, and 4-nitro-3-phenylphenol from DEP. These derivatives have vasodilatory activity, as well as estrogenic and anti-androgenic activities *in vitro*.

Uterotrophic and Hershberger assays were used to study the estrogenic and anti-androgenic activities of PNP *in vivo*. In ovariectomized immature female rats injected subcutaneously with 1, 10, or 100 mg/kg PNP daily for 7 days, significant *($p<0.05$)* increases in uterine weight were seen in only those receiving 10 or 100 mg/kg PNP. Furthermore, in castrated immature male rats implanted with a silastic tube (length, 5 mm) containing crystalline testosterone and injected subcutaneously with 0.01, 0.1, or 1 mg/kg PNP daily for 5 days, those receiving the doses of 0.1 mg/kg showed significant *($p<0.05$)* weight decreases in seminal vesicles, ventral prostate, levator ani plus bulbocavernosus muscles, and glans penis. Plasma follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels did not change in female rats but were significantly *($p<0.05$)* increased in male rats treated with 0.1 mg/kg PNP.

The present results suggest that PNP has an anti-androgenic effect in the Hershberger assay that is mediated through the androgen receptor, and the inhibitory mechanism of androgenic activity may be related to the hypothalamus-pituitary axis and other hormones that are involved in testosterone synthesis and metabolism.

The results also are important from an environmental perspective. Many countries exhaust vast amounts of DEP into the atmosphere, and previous reports indicated that 1 kg DEP contains 15 mg PNP. The exact concentration of PNP in the environment is unknown because the research on the isolation of the compounds found in DEP has just begun. PNP, one of the chemicals isolated from DEP, is also a degradation product of the insecticide parathion, which is used as a fumigant, acaricide, and pre-harvest soil and foliage treatment for a wide variety of

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POLYBROMINATED DIPHENYL ETHERS IN HOUSE DUST IN SINGAPORE

Environmental health problems resulting from the exposure to polybrominated diphenyl ethers (PBDEs) have become a matter of increasing concern in recent years. PBDEs are added to polymer resins and plastics to suppress fire and they can be found in household furnishings, TV sets, stereos, computer circuit boards and casings, foams and upholstery.

Due to concerns over potential toxicological effects in humans, penta- and octa-BDE mixtures have been banned in the European Union and will be likewise banned in the United States in 2008. However, in Asia the demand for PBDEs has been increasing, and all BDE commercial mixtures are used without regulation.

Sources of human exposure to PBDEs are still poorly understood. As PBDEs are not covalently bonded into the polymer matrix of many plastic products they are particularly susceptible to leaching and dissemination into the environment. Contaminated food consumption is undoubtedly a key exposure route, especially for compounds which are known to undergo biomagnification, such as BDE 47. As PBDEs have been widely added to products which are predominantly used indoors, higher levels of PBDEs in the indoor than the outdoor environment are apparent. Since PBDE degradation processes are typically slow, and people spend most of their time indoors, the internal environment is a particularly important source of human exposure. Transportation as particle bound contaminants on airborne dust represents an important pathway for low-volatility PBDEs in the human body. House dust is an especially important exposure pathway for young children via inhalation, ingestion or direct skin contact.

Because of the current lack of data on levels of these contaminants in the home environment, a study was recently undertaken to ascertain prevailing levels of PBDEs in residential house dust in 31 homes on the main island of Singapore. The objective was to determine both the concentration and the profile of PBDEs in the house dust, and further assess human exposure to these contaminants via dust ingestion and inhalation.

PBDEs were detected in all 31 dust samples and the number of BDE congeners detected per home range between 3 and 8. The most abundant BDE congeners found were BDE 47, 99 and 209; with a median value of 20, 24 and 1000 ng/g dust, respectively. BDE 209 contributed 88% to the median of all the congeners, and BDE 47 and 99 contributed 1.8% and 3.5%, respectively. Different congener profiles were observed between this and studies conducted elsewhere, which is consistent with the use of different commercial PBDEs around the world. No significant correlations between PBDE dust levels and residential characteristics (number of TVs and computers, floor area or flooring material) were observed. The daily intake of PBDEs via the inhalation pathway was estimated. House dust may be regarded as the most important exposure route of PBDEs for children.

Future work in assessing human exposure to PBDEs will focus on perinatal exposure, since infants and young children are deemed to be more susceptible where exposure can even begin in uterus via placental transfer and to the newborn infant via breast milk feeding.

Source: Chemosphere, Vol. 66, January 2007.

UN Warning on E-waste "Mountain"

A recent study by the Basel Action Network has concluded that whereas traditionally much electronic waste from the world's richest countries found its way to Asia, tighter regulations now mean that more and more is ending up in Africa with 100,000 computers a month entering the Nigerian port of Lagos alone each month for recycling. However, between 25-75% of these and other items including old TVs and mobile phones are defunct and simply constitute e-waste. When they are burnt, the most common disposal method, this releases toxic fumes, and chemicals such as barium and mercury are leached into the soil.

UNEP estimates that up to 50 million tonnes of waste from discarded electronic goods is now generated annually. The decreasing cost of replacing computers, mobile phones and other electronic gadgets, and the speed with which technology goes out of date, mean that there is more and more to be disposed of.

HAZARDOUS ELECTRONIC WASTE

- **Lead** in cathode ray tubes and solder
- **Arsenic** in older cathode ray tubes
- **Antimony trioxide** as flame retardant
- **Polybrominated flame retardants** in plastic casings, cables and circuit boards
- **Selenium** in circuit boards as power supply rectifier
- **Cadmium** in circuit boards and semiconductors
- **Chromium** in steel as corrosion protection
- **Cobalt** in steel for structure and magnetivity
- **Mercury** in switches and housing

Source: Basel Convention for the Environmentally Sound Management of Electronic Wastes – World Forum on E-waste, 30 November 2006.

Occupational Exposure to Polycyclic Aromatic Hydrocarbons in Airport Workers

Airport ground staff perform different tasks such as aircraft fuel tank, aircraft routine maintenance procedures, airplane parking/towing, baggage charge/discharge, that can induce exposure to complex chemical mixtures including several polycyclic aromatic hydrocarbons (PAHs) produced by vapors or combustion of commercial Jet-A-A1 fuels and by combustion of diesel/gasoline engines of runway shuttles and baggage trolleys operating in the vicinity of the planes. This occupational exposure is very complex and still poorly characterized. Moreover the exposure conditions are highly variable since they are influenced by environmental factors (wind, heat, etc.) associated to different climatic and meteorologic conditions.

Although the airport workers are exposed to low levels of PAHs there is a possibility of long-term health effects following chronic exposure by inhalation or skin contamination. The exposure can in fact occur through dermal contact with raw fuel and/or aerosol; dermal contact with clothing and gloves saturated with fuel; inhalation of Jet-A vapors or exhaust and diesel combustion.

Over 2 million military and civilian personnel per year are occupationally exposed to jet propulsion fuels (JP-8, JP-8-100, JP-4 or JP-5), or to the civil aviation equivalents Jet-A or Jet-A1.

A recently conducted study aims to characterize the exposure in a large civil airport by environmental monitoring of 23 PAHs, including the 16 priority PAHs published by the U.S. Environmental Protection Agency (EPA) and other PAHs known as products of jet fuel partial combustion. The exposure was also assessed by biological monitoring of the most used biomarker of PAH absorbed dose, urinary 1-hydroxypyrene (1-OHP). The aim of the study was also to evaluate the possible early genotoxic and oxidative effects of airport pollution, using micronucleus test and Fpg-modified comet assay, that allows the evaluation of early direct and oxidative DNA damage, on lymphocytes and on exfoliated buccal cells (direct target tissue for inhalable substances). Genotoxic effects were also evaluated by standard chromosomal aberrations and sister chromatid exchange analyses. Very few data are in fact available on health effects of airport pollution on civil aviation workers and studies in the field are very necessary for the further development of the global aviation system.

Environmental monitoring showed different PAH levels, prevalently 2-3 ring PAH, in the three working areas with the highest level in airport apron (27,703 $\mu g/m^3$ total PAHs), characterized by the prevalence of methylnaphthalenes and acenaphthene presumably associated to jet-fuel combustion of the aircrafts. However, total PAHs value was in this area much lower than threshold limit value (TLV) proposed for occupational total PAH exposure by ACGHI (0.2 mg/m^3). While in terminal/office area, as expected, the lowest PAH levels were found. This is the first study that in a civil airport analyses such a large number of PAHs, chosen in relation to the different sources of PAH emission present in this specific environment. No data are in fact available on PAHs monitoring in civil aviation airports probably due to inability of commonly used methods to measure trace amounts of each PAH. The sensitive method of analysis allowed detection of very low doses of PAHs and to correctly define PAH airport exposure. The urinary 1-OHP did not show significant differences between exposed and controls probably associated to low daily exposure to PAH.

The environmental PAH monitoring in three different airport working areas and urinary 1-OHP levels of exposed workers furnish a useful contribution to the characterization of airport exposure actually still not well defined and could contribute to define the exposure of a given category of workers in relation to their tasks, furnishing a useful model adaptable to other, similar working situations.

Source: Toxicology, Vol. 223, June 2006.

ESTROGENIC AND ANTI-ANDROGENIC ACTIVITIES OF 4-NITROPHENOL IN DIESEL EXHAUST PARTICLES

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crops, both outdoors and in greenhouses worldwide. Accumulation of PNP in air, soil, and water could have serious deleterious effects on wildlife and human health through disturbance of endocrine and reproductive systems.

In conclusion, PNP shows estrogenic activity in immature female rats, as well as anti-androgenic activity (in light of the decreased weights of testosterone-stimulated accessory sex glands and increased plasma FSH and LH levels) in male rats. The results clearly demonstrate that PNP is an endocrine-disrupting chemical.

Source: Toxicology and Applied Pharmacology, Vol. 217, November 2006.

Exposure Assessment of Benzene in Thai Workers, DNA-Repair Capacity and Influence of Genetic Polymorphisms

Since benzene is known to be a human leukemogen and able to cause adverse health effects such as hematopoietic toxicity and bone marrow depression, it has been replaced to a large degree by other, less toxic solvents. However, in many parts of the world some industrial processes still make controlled use of benzene or use it in a mixture, e.g., in unleaded gasoline and as adhesive solvent in glue and shoe factories. The general population is exposed to benzene from automotive emissions.

Benzene exposure can be assessed through the use of biomarkers, e.g., as unmetabolized benzene in exhaled breath, urine and blood, or as urinary metabolites, e.g., *trans,trans*-muconic acid, *s*-phenylmercapturic acid and phenol. These biomarkers are useful in characterizing internal exposure to benzene, but markers of potential carcinogenic effects, e.g., cytogenetic effects, are also very important. The relationship between benzene exposure and cytogenetic effects has been explored in a number of studies. At exposure levels of 1-10 ppm, a slightly increased number of chromosomal aberrations, mainly chromatid deletions and gaps, were found in lymphocytes of oil transportation workers and workers in aromatic production plant. DNA damage, determined by single-cell gel electrophoresis in lymphocytes of gasoline service attendants exposed to benzene at a mean level of 93.75 ppb (0.32 $\mu g/m^3$; 8h TWA), has been reported with an average tail moment of 1.90 µm compared to 0.94 µm in the unexposed group.

Recently, the challenge assay was claimed to be a sensitive method for detecting an abnormal DNA-repair capacity in workers exposed to mutagens. By challenging cells with UV or X-rays, abnormal repair capacity in the challenged cells of toxicant-exposed populations compared to non-exposed or low level exposed populations could be detected. However, repair capacity in workers exposed to low levels of benzene using the challenge assay has not yet been reported.

Benzene is bio-transformed by *CYP*2E1 in the liver to benzene oxide and then by non-enzymatic re-arrangement to phenol, which can be further hydroxylated to hydroquinone, catechol and 1,2,4-trihydroxybenzene. The di- and tri-hydroxy metabolites can be further oxidized in the bone marrow by myeloperoxidase to benzoquinones, which are detoxified by *NAD(P)H*: quinone oxidoreductase 1 (*NQO*1) to less toxic hydroxybenzenes. Workers with a 'rapid' *CYP*2E1 enzymatic activity (estimated by the fractional excretion of chloroxazone) and two copies of the *NQO*1 609C allele had a 7.6-fold increased risk of benzene poisoning --- as measured by white blood cell and platelet count --- compared with workers with a 'slow' *CYP*2E1 enzymatic activity and one or two wildtype alleles of *NQO*1. Benzene oxide can be further hydrolyzed by epoxide hydrolase generating benzene glycol, which can be dehydrogenated to catechol or ring-opened to form *trans,trans*-muconaldehyde, which is the precursor of *trans,trans*-muconic acid that is excreted in urine. *GSTT*1*2/*2 or the null genotype has been reported to increase susceptibility to benzene and to cause myelodysplastic syndrome. *XRCC*1 is involved in base-excision repair. Workers who carry the *XRCC*1 399Gln allele may be at greater risk due to a decrease in DNA-repair capacity resulting from a decreased rate of base-excision repair. Higher sister chromatid exchange frequencies were found in current smokers who carried the *XRCC*1 399Gln allele.

To assess the potential risk of workers in Thailand occupationally exposed to benzene and to obtain a better understanding of inter-individual variation in susceptibility, the link between internal dose, repair capacity and genetic susceptibility was investigated. The polymorphisms of several genes involved in benzene activation (*CYP*2E1), detoxification (*NQO*1, *GSTT*1), and DNA repair (*XRCC*1, codon 399) were studied.

The study subjects were 31 laboratory workers at a petrochemical factory and 31 gasoline service attendants. Control subjects were 34 workers from a mail sorting service center. Occupational exposures to benzene were assessed using biomarkers of exposure in blood and urine. Induction of DNA-repair capacity was assessed as a biomarker of early effect. The effects of polymorphisms in a metabolizing gene (*CYP*2E1), in detoxification genes (*NQO*1 and *GSTT*1), and in a DNA-repair gene (*XRCC*1, codon 399) on biomarker levels were evaluated. The mean individual benzene exposure of laboratory workers (24.40 ppb) and that of gasoline service attendants (112.41 ppb) were significantly higher than in controls (1.39 ppb). Blood benzene levels of laboratory workers (169.12 ppt) and gasoline service attendants (483.46 ppt) were significantly higher than those of the controls (43.30 ppt). *Trans,trans*-muconic acid levels in post-shift urine samples collected from laboratory workers (0.14 mg/g creatinine) and gasoline service attendants (0.20 mg/g creatinine) were significantly higher than in urine samples of controls (0.04 mg/g creatinine). The level of benzene exposure was correlated with blood benzene levels and post-shift urinary *trans,trans*-muconic acid concentrations. As a biomarker of early effect, DNA-repair capacity was assessed by use of the cytogenetic challenge assay, i.e., chromosomal aberrations in peripheral lymphocytes were assessed after challenging blood cultures with 1 Gy gamma radiation. A significantly lower DNA-repair capacity --- determined as dicentrics in laboratory workers (0.17 per metaphase cell) and in gasoline service attendants (0.19 per metaphase cell) compared with controls (0.12 per metaphase cell) --- was observed. The frequency of deletions in laboratory workers (0.22 per metaphase cell) and gasoline service attendants (0.39 per metaphase cell) was significantly higher than in control workers (0.16 per metaphase cell). An increase in radiation-induced dicentrics and deletions indicated a

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DIFFERENTIAL SENSITIVITY OF BLOOD, PERIPHERAL, AND CENTRAL CHOLINESTERASES IN BEAGLE DOGS FOLLOWING DIETARY EXPOSURE TO CHLORPYRIFOS

Guidelines that limit pesticide residues on foods are developed and enforced by public health agencies worldwide. These guidelines incorporate data from many sources into the risk assessment process, including high-dose animal toxicity studies. In the case of organophosphorus insecticides such as chlorpyrifos (CPF), these animal studies typically use inhibition of the enzyme acetylcholinesterase (AChE) as a toxicologic endpoint, since neural AChE is generally accepted as the putative toxicologic target of organophosphorus insecticides. AChE is a serine esterase responsible for terminating the biological activity of acetylcholine, a neurotransmitter found at some neural synapses and neuromuscular junctions. Dietary exposure guidelines for CPF are based on No Observable Effect Levels (NOELs), *i.e.,* doses at which no identifiable inhibition of either red blood cell (RBC) or brain AChE inhibition occurs. Inhibition of RBC AChE is used only as an index of exposure and is not considered a toxic effect. The current NOEL for inhibition of dog RBC AChE by dietary CPF is 0.1 mg/kg/day. A comprehensive analysis of human acute dietary exposure assessments for CPF has been recently reported, and predicted exposure levels fall well-below 0.001 mg/kg/day. Pre-school children's exposure to CPF is low. Complex exposure models have used real-world, measured levels of CPF and its degradation product 3,5,6-trichloro-2-pyridinol (TCP) in food, air, and surfaces. These models indicated the median child's CPF exposure was roughly 3-30 ng/kg/day.

For reasons likely related to cost and convenience, few studies in the literature have examined the effects of dietary CPF on AChE in mammals. Most published reports have used gavage or subcutaneous dosing routes at high doses of CPF. Data from these studies have limited usefulness in human risk assessment, as they represent physiologic conditions that rarely occur in nature, except under extreme conditions (*e.g.,* suicidal ingestion of pesticide formulations). Inhibition of mixed cholinesterase (mChE, also called total cholineseterase; ChE) activity following dietary exposure to CPF has been reported in rats and dogs. These studies have consistently reported a large difference in sensitivity to inhibition between RBC and brain mChE activity, which has been used as a surrogate estimate of AChE.

Determinations of mChE activity in biological tissue samples may not accurately reflect actual levels of AChE inhibition, as mChE activity is comprised of many enzyme activities, including butyrylcholinesterase (BuChE). Human serum BuChE, when tested *in vitro*, is more than 400-fold more sensitive than human RBC AChE to inhibition by chlorpyrifos oxon, the active metabolite of CPF, making mChE a potentially-biased estimate of true AChE activity. To generate more appropriate data for use in human risk assessment, it is important to isolate true AChE activity from measurements of mChE by reducing the confounding influence of BuChE, which can be accomplished using BuChE-specific enzyme inhibitors.

AChE is found in a wide variety of tissues such as brain, RBCs, skeletal muscle, autonomic ganglia, cardiac tissue, etc. Recent concerns regarding whether current dietary exposure guidelines based upon protection of brain AChE adequately protect peripheral tissue AChE from inhibition have motivated some regulatory agencies to reconsider the relative sensitivity of peripheral tissue AChE. To answer the question: "Is peripheral tissue AChE more sensitive to inhibition by dietary exposure to CPF than brain AChE?," a series of studies were designed to examine three cholinesterase activities (mChE, AChE, and BuChE) in the brains, blood components (RBC and plasma), and peripheral tissues (heart, diaphragm, skeletal muscle, and autonomic ganglia) of Beagle dogs exposed to CPF in their diets.

Two studies were performed to find out whether exposure limits that protect brain AChE will protect peripheral tissue AChE after exposure to CPF, an organophosphate insecticide. In a methods-development study, male dogs (3/dose) were exposed to 0.0, 0.3, 0.6, or 1.2 mg/kg/day CPF in their diets for 4 weeks. mChE, AChE, and BuChE activities were measured in plasma, RBC, brain, left atrium and ventricle, diaphragm, quadriceps, and nodose ganglia. Plasma, brain and peripheral tissue BuChE was inhibited at all dose levels. While RBC AChE was inhibited at all doses, brain and peripheral AChE activities were unaffected. In the main study, dogs (4/sex/dose) were exposed to 0.0, 0.5, 1.0, or 2.0 mg/kg/day CPF in their diets for six weeks and RBC AChE was significantly inhibited at all doses in both sexes. Diaphragm, quadriceps, and nodose ganglia AChE was unaffected by treatment. Brain AChE was decreased by ~6% compared to controls in high-dose groups, and this was considered a threshold effect. Left atrium AChE in high-dose dogs was 25.5% less (males) and 32.1% greater (females) than controls; these differences were attributed to chance. While peripheral tissue and brain AChE were not affected following exposure to 1.0 mg/kg/day, RBC AChE was inhibited at all doses. These results show that RBC AChE is more sensitive than brain or peripheral tissue AChE to inhibition by CPF, and that protection of brain AChE would protect peripheral tissue AChE.

Source: Regulatory Toxicology and Pharmacology, Vol. 47, April 2007.

EFFECTS OF LOW-EXPOSURE TO CADMIUM ON OSTEOPOROTIC RAT FEMORAL BONE

Cadmium (Cd) is a toxic substance that is widely distributed in the environment and has a long biological half-life in organs. The kidneys, liver, bones, and respiratory and cardiovascular systems are the most important target organs for Cd toxicity and Cd exposure can cause itai-itai disease, kidney tubular dysfunction, cancer, and bone damage. Reduced bone mineralization and increased risk of vertebral, hip, and forearm fractures have been reported after low to moderate exposure to Cd.

The mechanism of Cd-induced bone effects is not clear but several different possibilities have been suggested. Cd-induced bone effects may be mediated via renal tubular dysfunction. The normal activation of vitamin D in the kidney also may be reduced and leads to decreased Ca absorption from the gut and impaired bone mineralization. But recent studies including *in vitro* experiments carried out on bone cultures show that Cd acts directly on the activity and metabolism of bone cells and hydroxyapathyte formation.

Biomechanical integrity of bone is one of the most important factors related to bone strength and tendency to fracture. Bone ultimate strain, ultimate stress, stiffness, and toughness are predictor parameters for the evaluation of bone fragility and are measured by biomechanical test in this study. Although many studies have been conducted to clarify the mechanisms of Cd-induced bone damage, only a few studies investigated the effects of Cd on biomechanical parameters of bone. Also, the critical level of Cd exposure that leads to fracture is still unknown.

Cd is more accumulated and more toxic in growing skeleton than in mature skeleton. Disorders of bone properties that occur during skeletal growth at a young age will affect its properties in later life. In addition, itai-itai disease is considered to be the most severe form of chronic Cd intoxication and 90% patients are postmenopausal women.

In the light of these studies, a group of researchers at Mersin University Medical School in Turkey have suggested that Cd might have a different effect on normal and osteoporotic bone and designed the present study to investigate this issue.

For this purpose, 12-week-old Sprague-Dawley female rats were assigned randomly to a control group, a Cd group, and an ovariectomy (OVX) + Cd group. OVX + Cd rats underwent bilateral ovariectomy via ventral incision. Twelve weeks after OVX, cadmium chloride was given to rats (Cd and OVX + Cd groups) as intraperitoneal (ip) injection of 0.5 mg/kg three times a week for 18 weeks and distilled water was given to control group via ip route for 18 weeks. Bone mineral density (BMD) was measured at mid-diaphysis femoral region by dual-energy X-ray absorbsiometry. Cross-sectional area of the femoral shaft was evaluated by computerized tomography. Biomechanical measurements were performed at the mid-diaphysis of the left femur. Collagen fibers were evaluated at light microscopic level. BMD, cortical thickness, cortical area, and femur length were not changed in Cd and OVX + Cd groups in comparision to controls. In the OVX + Cd group, strength, displacement, energy, stress, strain, and toughness were significantly lower than those of the control group. The Cd concentration of bone was significantly increased in the OVX + Cd group compared to that in the control group. Collagen fiber intensity was decreased in all groups except control group. The results of the present study indicate that the administration of low-dose Cd does not affect normal bone biomechanical parameters, but it has a significant effect on osteoporotic bone.

Source: Ecotoxicology and Environmental Safety, Vol. 66, January 2007.

IRON SUPPLEMENT PREVENTS LEAD-INDUCED DISRUPTION OF THE BLOOD-BRAIN BARRIER DURING RAT DEVELOPMENT

Environmental lead (Pb) intoxication has been known to cause irreversible neurological disturbances by mechanisms remaining to be identified. Children are particularly vulnerable to Pb toxicities for several reasons. They absorb the ingested Pb from the gastrointestinal tract better than do the adults; a greater proportion of systemically circulating Pb gains access to the brain more easily in children, especially those of 5 years old or younger, than in adults; and the developing nervous system in children is far more sensitive to Pb toxicity than the mature adult brains. Evidence in the literature also suggests that at low concentrations, Pb disrupts normal blood-brain barrier (BBB) function, resulting in regionally specific increases in permeability to plasma proteins.

The homeostasis of brain microenvironment, which is essential for its normal function, is maintained by the BBB. The BBB is formed by highly specialized endothelial cells whose tight junctions between adjacent cells restrict the paracellular diffusion of hydrophilic molecules. The tight junctions are the intricate combination of transmembrane and cytoplasmic proteins linked to an actin-based cytoskeleton system. Typical tight junction proteins include claudin-1, occludin, and zonula occluden-1 (ZO-1). Claudins constitute the backbone of tight junction strands by forming dimers and binding homotypically to claudins on adjacent cells to produce the primary seal of the tight junctions. Occludin, a 65 kDa protein existing in various phosphorylated forms, functions as a dynamic regulatory protein, whose presence in the membrane is correlated with decreased paracellular permeability. The cytoplasmic proteins interacting with transmembrane strands include zonula occludens proteins (ZO-1 and ZO-2), cingulin, AF6, and 7H6 antigen. It is noteworthy that the structure of occludin consists of two extracellular loops, which project into the paracellular space and interact with claudins, and one cytoplasmic domain, which interacts with ZO-1. The multiple functions of occludin render it indispensable for the tightness of the BBB and more vulnerable to insults of toxicants.

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IRON SUPPLEMENT PREVENTS LEAD-INDUCED DISRUPTION OF THE BLOOD-BRAIN BARRIER DURING RAT DEVELOPMENT

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Iron (Fe) is an essential trace element in mammals. Research in the past has demonstrated that there is an intimate relationship between Pb exposure and Fe metabolism in biological systems. For example, Fe deficiency can lead to an augment in Pb absorption; vice versa, Fe supplement can reduce the Pb absorption from the intestine. However, the question as to whether or not Fe supplement may protect against Pb toxicity on the BBB has never been investigated.

Thus the hypothesis tested in this report was that Fe supplementation may prevent against Pb-induced disruption of the BBB permeability during rat development.

Male weanling Sprague-Dawley rats were divided into four groups. Three groups of rat were exposed to Pb in drinking water containing 342 µg Pb/mL as Pb acetate, among which two groups were concurrently administered by oral gavage once every other day with 7 mg Fe/kg and 14 mg Fe/kg as $FeSO_4$ solution as the low and high Fe treatment group, respectively, for 6 weeks. The control group received sodium acetate in drinking water. Pb exposure significantly increased Pb concentrations in blood by 6.6-fold ($p<0.05$) and brain tissues by 1.5-2.0-fold ($p<0.05$) as compared to controls. Under the electron microscope, Pb exposure in young animals caused an extensive extravascular staining of lanthanum nitrate in brain parenchyma, suggesting a leakage of cerebral vasculature. Western blot showed that Pb treatment led to 29-68% reduction ($p<0.05$) in the expression of occludin as compared to the controls. Fe supplement among Pb-exposed rats maintained the normal ultra-structure of the BBB and restored the expression of occludin to normal levels. Moreover, the low dose Fe supplement significantly reduced Pb levels in blood and brain tissues. These data suggest that Pb exposure disrupts the structure of the BBB in young animals. The increased BBB permeability may facilitate the accumulation of Pb. Fe supplement appears to protect the integrity of the BBB against Pb insults, a beneficial effect that may have significant clinical implications.

Source: Toxicology and Applied Pharmacology, Vol. 219, February 2007.

House Dust as a Possible Route of Environmental Exposure to Cadmium and Lead in the Adult Population

Cadmium (Cd) and lead (Pb) are heavy metals with high toxicity, which during life accumulate in the human body. The 24-h urinary excretion of Cd is a biomarker of lifetime exposure, while the concentrations of Cd and Pb in blood reflect more recent exposure. Commonly held points of view are that populations living in areas historically polluted by Cd or Pb are mainly exposed by consumption of crops or animal produce originating from contaminated soils. Young children with pica behavior can ingest considerable amounts of contaminated soil particles. Tobacco smoke and polluted air are additional sources of exposure. Although house dust prominently contributes to the Pb body burden of children, fewer studies have investigated to what extent indoor particulate matter might contribute to the environmental exposure of the general adult population.

Now researchers in Belgium have investigated the relations between biomarkers of exposure to Cd and Pb, and the metal loading rates in house dust in the adult residents of an area with a soil Cd concentration of > 3 mg/kg (n = 268) and a reference area (n = 205). They determined the metal concentrations in house dust allowed to settle for 3 months in Petri dishes placed in the participants' bedrooms. The continuously distributed vegetable index was the first principal component derived from the metal concentrations in six different vegetables. The biomarkers of exposure (blood Cd 9.2 vs. 6.2 nmol/L; 24-h urinary Cd 10.5 vs. 7.0 nmol; blood Pb 0.31 vs. 0.24 µmol/L), the loading rates of Cd and Pb in house dust (0.29 vs. 0.12 and 7.52 vs. 3.62 ng/cm^2/92 days), and the vegetable indexes (0.31 vs. -0.44 and 0.13 vs. -0.29 standardized units) were significantly higher in the contaminated area. A two-fold increase in the metal loading rate in house dust was associated with increases ($p<0.001$) in blood Cd (+2.3%), 24-h urinary Cd (+3.0%), and blood Pb (+2.0%), independent of the vegetable index and other covariates. The estimated effect sizes on the biomarkers of internal exposure were three times greater for house dust than vegetables.

If confirmed, these findings may have important implications for the assessment and management of the associated health risks. Experts increasingly recognize that house dust might substantially contribute to the body burden, particularly in children. However, many algorithms to estimate health risks, such as the Dutch CSOIL model, do not specifically account for exposure via house dust. In areas with contaminated soils, the Cd content of vegetables can be reduced by stabilizing Cd in the soil by organic matter or other additives, or by increasing the pH. Removal of contaminated soil is a more invasive and expensive approach. Such measures are currently being implemented or planned for in the Noorderkempen area. However, as suggested by the present and other studies, preventive measures that do not decrease exposure to house dust might not substantially reduce the biomarkers of internal exposure. In an intervention study, which aimed to reduce exposure of children living in a smelter area, simple techniques, such as hand washing and dust control were implemented. An average drop of the blood levels by 50% occurred immediately and persisted for 1 year. Because the reduction was achieved in the presence of elevated soil concentrations and Pb paint in many of the homes, the researchers concluded that controlling house dust is critical in reducing the body burden of heavy metals.

In conclusion, in the adult population, house dust is potentially an important and long-lived source of exposure to heavy metals in areas with contaminated soils, and should be incorporated in the assessment of health risks.

Source: Environmental Research, Vol. 103, January 2007.

ASSOCIATION OF EXPOSURE TO COOKING OIL FUMES WITH LUNG CANCER

For over twenty years, lung cancer has been the leading cause of death in Taiwanese women. However, the phenomenon cannot be ascribed to cigarette smoking since less than 10% of Taiwanese women are smokers. The cause has been shown to be cooking oil fumes (COF).

A recent report indicates that inhibitor of apoptosis protein 2 (IAP2) induced by COF may contribute to the survival and proliferation of A549 lung cancer cells. In a new study to further verify whether other antiapoptosis problems including IAP1, X-linked IAP (XIAP), and survivin were linked with lung cancer cell survival and proliferation, these IAPs expressions in A549 cells after treatment with COF and its two major components, benzo[*a*]pyrene (BaP) and 2,4-decadienal (2,4-DDE) were evaluated by Western blotting.

The data showed that IAP2 was significantly induced by COF, BaP, and 2,4-DDE, but XIAP was decreased by COF and 2,4-DDE, but not by BaP. Even though different effects of COF and 2,4-DDE on IAP2 and XIAP protein expressions were observed, the caspase-3 expression was diminished by COF and 2,4-DDE. In addition, induction of IAP2 and phosphorylated Akt proteins by COF and 2,4-DDE were simultaneously abolished by 2-(4-morpholinyl)-8-phenyl-1-4H-benzopyran-4-one-hydrochloride (LY294002). Flow cytometry and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) analysis showed that the proportion of A549 cells at the S-phase was increased significantly after treatment with COF or 2,4-DDE. The cell proliferation induced by COF is associated with the attenuation of p21Cip/Waf1 expression. Therefore, increases of IAP1, IAP2, survivin, and cyclin D1 expressions and decreases of XIAP, caspase-3, and $p21^{Cip/Waf1}$ expressions might partly contribute to the survival and proliferation of lung cancer cells after exposure to 2,4-DDE and COF. The lung cancer cell growth promoted by COF might support previous epidemiological reports indicating that exposure of COF was associated with lung cancer development among Chinese women, and the results of the present study may be helpful in understanding the possible mechanism in COF-associated lung cancer development.

Source: Mutation Research, Vol. 628, April 2007.

Exposure Assessment of Benzene in Thai Workers, DNA-Repair Capacity and Influence of Genetic Polymorphisms

(Continued from page 4)

lower DNA-repair capacity in benzene-exposed workers. The influence of genetic polymorphisms on the biomarkers was assessed. Benzene-exposed workers who carried *CYP*2E1*1/*5 or *5/*5 genotypes excreted slightly higher levels of *trans,trans*-muconic acid than workers who carried the *CYP*2E1*1/*1 genotype. In this study, *NQO*1 and *GSTT*1 genotypes did not have any effect on the levels of *trans,trans*-muconic acid. In the case of *XRCC*1, laboratory workers with 399Arg/Gln or Gln/Gln had a lower DNA-repair capacity --- measured as radiation-induced frequency of dicentrics and deletions --- than those with the 399Arg/Arg genotype. The results of the study show that biomarkers of internal dose and early biological effect in people occupationally exposed to benzene are influenced by genetic polymorphisms in susceptibility genes.

The study concludes that even at relatively low levels of benzene exposure, workers may have an increased risk for genotoxicity that is due in particular to a decrease in repair capacity. Monitoring levels of benzene exposure through biomarkers in susceptible populations is useful in identifying workers who are at risk. Biomarkers of early effects such as DNA-repair capacity are necessary for evaluating the health risk.

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