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AIR POLLUTION-RELATED PROTHROMBOTIC CHANGES IN PERSONS WITH DIABETES

Urban pollution, especially by particulate matter (PM), contributes to respiratory and cardiovascular morbidity and mortality. To a large extent, the increase in mortality linked to PM ≤ 10 µm in aerodynamic diameter (PM_{10}) is attributable to cardiovascular diseases. Persons with diabetes who also have cardiovascular disease appear to be more sensitive to the effects of air pollution on daily mortality. Previous studies have found stronger associations between increased levels of PM and hospitalizations for heart disease among those who had diabetes compared with those who did not. The risk of coronary heart disease, stroke, and peripheral arterial disease is increased in persons with diabetes. Both atherosclerosis and thrombosis appear to contribute to this increased cardiovascular risk. Therefore, research on environmental factors that may aggravate the disease, and on the mechanisms underlying this, has substantial public health relevance.

One of the problems in epidemiologic studies is estimating individual exposure to PM. In the present study, the chronic exposure to PM was estimated at an individual level by determining the carbon load of airway macrophages. This approach is based on the fact that airway macrophages are the primary phagocytotic cells of inhaled PM. The amount of carbonaceous PM extracted from the lung at autopsy reflects the chronic exposure to PM. The present study hypothesized that exposure to PM causes prothrombotic changes in persons with diabetes, possibly via systemic inflammation.

Persons with both type 1 and type 2 diabetes were consecutively recruited from the diabetes outpatient clinic at the University Hospital, Leuven, Belgium. This clinic is a dedicated clinic for the routine 3- to 6-month follow-up of patients with diabetes. All patients were invited to participate on days when the investigator was present. They were included if they were ≥ 18 years of age and were nonsmokers. The study was carried out on different days from February 2007 through September 2008. On the study day, patients completed a questionnaire to obtain information on age, occupation, socioeconomic status, exposure to environmental tobacco smoke, alcohol use, use of medication, use of oral contraception, menopausal status, place of residence, and means of transportation to the hospital. Socioeconomic status was coded and condensed into a scale with scores ranging from 1 to 3. Use of antiplatelet medication was coded as use of either none or one or more of the following substances: acetylsalicylic acid, clopidogrel, ticlopidine, or dipyridamole. Distances from the home address to major roads were calculated through geocoding. Living close to a major road was defined as living within 100 m of an N-road or an E-road. Of the 186 recruited subjects, 137 (74%) took part in the examination. The 49 patients that did not participate had the same age and sex distribution as the 137 participants. Sufficient numbers of airway macrophages to assess the area occupied by carbon were obtained from 80 of the 119 patients (18 of the 137 patients failed to produce sputum). A blood sample could not be obtained from 11 subjects,

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AIR POLLUTION-RELATED PROTHROMBOTIC CHANGES IN PERSONS WITH DIABETES

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and platelet function analysis was not successful in 28 subjects. Ultimately, 63 subjects had data for both the carbon load of airway macrophages and the platelet function analysis.

In the study, the carbon load in airway macrophages was associated with modeled 6-month average PM_{10} exposure at the patient's home. However, the blood leukocyte count was not significantly associated with the modeled 6-month average PM_{10} concentration, although it was with the carbon load of airway macrophages. This suggests that the latter biomarker of chronic exposure might be a better reflection of personal exposure to PM. For the modeled previous day, week, month, 3-month, and annual average PM_{10} at the patient's residence, no correlations were found with the carbon load of airway macrophages.

The findings have important implications for understanding the biological mechanisms of air pollution on cardiovascular health and its clinical relevance, because both a prothrombotic tendency and systemic inflammation play an important role in atherosclerosis and cardiovascular disease. The clinical relevance of the findings in persons with diabetes is evident from the observation that a realistic increase in recent PM air pollution exposure was associated with a change in platelet function toward a greater prothrombotic tendency. The magnitude of this change was about two-thirds (in opposite direction) of the average effect of antiplatelet medication.

Source: Environmental Health Perspectives, Vol. 118, No. 2, Pages 191-196, February 2010.

Effects of Atrazine in Amphibians: Feminization in Male African Clawed Frogs

Atrazine is one of the most widely used pesticides in the world. Approximately 80 million pounds are applied annually in the United States alone, and atrazine is the most common pesticide contaminant of ground and surface water. Atrazine can be transported more than 1,000 km from the point of application via rainfall and, as a result, contaminates otherwise pristine habitats, even in remote areas where it is not used. In fact, more than a half million pounds of atrazine are precipitated in rainfall each year in the United States.

In addition to its persistence, mobility, and widespread contamination of water, atrazine is also a concern because several studies have shown that atrazine is a potent endocrine disruptor active in the ppb (parts per billion) range in fish, amphibians, reptiles, and human cell lines, and at higher doses ppm (parts per million) in reptiles, birds, and laboratory rodents. Atrazine seems to be most potent in amphibians, where it is active at levels as low as 0.1 ppb. Although a few studies suggest that atrazine has no effect on amphibians under certain laboratory conditions, in other studies, atrazine reduces testicular volume; reduces germ cell and Sertoli cell numbers; induces hermaphroditism; reduces testosterone; and induces testicular oogenesis. Furthermore, atrazine contamination is associated with demasculinization and feminization of amphibians in agricultural areas where atrazine is used and directly correlated with atrazine contamination in the wild.

Despite the wealth of data from larvae and newly metamorphosed amphibians, the ultimate impacts of atrazine's developmental effects on reproductive function and fitness at sexual maturity, which relate more closely to population level effects and amphibian declines, have been unexplored.

In the present study, researchers examined the long-term effects of atrazine exposure on reproductive development and function in an all male population of African clawed frogs *(Xenopus laevis)*, generated by crossing ZZ females (sex-reversed genetic males) to ZZ males. The advantage of using this population is that 100% of the animals tested were genetic males. As a result, all hermaphrodites and females observed are ensured to be genetic males that have been altered by endocrine disruption. The study examined sex ratios, testosterone levels, sexual dimorphism, reproductive behaviors, and fertility in males exposed to 2.5 ppb atrazine throughout the larval period and for up to 3 years after metamorphosis.

All of the control animals reared to sexual maturity were males, on the basis of external morphology, whereas only 90% of the atrazine-treated animals appeared male at sexual maturity (on the basis of the presence of keratinized nuptial pads on the forearms and the absence of cloacal labia). The other 10% of atrazine-exposed animals lacked visible nuptial pads on the forearms and had protruding cloacal labia, typical of females. Upon dissection of two of the apparent females and laparotomy in another two, researchers confirmed that animals with cloacal labia were indeed females from the present study, on the basis of the presence of ovaries. To date, two atrazine-induced females have been maintained, mated with control males, and produced viable eggs. The resulting larvae were all male when raised to metamorphosis and sampled, confirming that atrazine-induced females were, in fact, chromosomal males. Furthermore, atrazine-induced females lacked the *DM-W* genes, further confirming that these atrazine-induced females were indeed chromosomal males. These ZZ females expressed gonadal aromatase, as did true ZW females (from the study's stock colony), but ZZ males (control or treated) did not.

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Effects of Atrazine in Amphibians: Feminization in Male African Clawed Frogs

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Previous studies showed that atrazine adversely affects amphibian larval development. The present study demonstrates the reproductive consequences of atrazine exposure in adult amphibians. Atrazine-exposed males were both demasculinized (chemically castrated) and completely feminized as adults. Ten percent of the exposed genetic males developed into functional females that copulated with unexposed males and produced viable eggs. Atrazine-exposed males suffered from depressed testosterone, decreased breeding gland size, demasculinized/feminized laryngeal development, suppressed mating behavior, reduced spermatogenesis, and decreased fertility. These data are consistent with effects of atrazine observed in other vertebrate classes. The present findings exemplify the role that atrazine and other endocrine-disrupting pesticides likely play in global amphibian declines.

Source: Proceedings of the National Academy of Sciences, Vol. 107, No. 10, Pages 4612-4617, March 2010.

EXCRETION OF PFOA AND PFOS IN MALE RATS

Due to their unique physicochemical properties, perfluorinated compounds (PFCs), such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), have been widely used in a diverse variety of industrial and commercial products. Previous investigations have reported the widespread presence of perfluorinated acid and related fluorochemicals in wild animals and blood of the general population. Their persistence, high bioaccumulation, and potential toxicity in organisms have attracted much attention to this class of compounds, resulting in the withdrawal of PFOS from the marketplace.

Increasing numbers of studies have indicated the toxic effects caused by this kind of peroxisome proliferators in exposed laboratory animals. PFOA and PFOS caused reductions in body weight, decreases in weight gain rate, and interference in lipid metabolism in rats. The liver is considered the primary target organ for both acute and chronic exposure of these two fluorochemicals, and their hepatotoxicity in rodents has been extensively studied, which mainly includes increased liver weight, induction of hepatocellular hypertrophy and lipid vacuolation, increased incidence of hepatocellular adenoma, and hypocholesterolemia. Additionally, the potential neurotoxicity, genotoxicity, molecular toxicity, and reproductive and developmental toxicities of PFOA and PFOS were demonstrated in recent toxicological studies. The pharmacokinetics of PFCs in animals has received increasing attention, which is of fundamental significance with regard to their potential biological effects. Previous pharmacokinetic studies on PFOA and PFOS were mostly conducted on mammals (rats, dogs, and monkeys), birds, and humans. The research found that both of them were well absorbed after ingestion. More than 95% of the total carbon-14 was absorbed within 24 hours after a single oral dose by gavage of ^{14}C-PFOA or ^{14}C-PFOS. Moreover, a relatively pronounced gender difference in PFOA elimination has been observed in several animals, such as rats and beagle dogs, but not in mouse, primate, or human. For example, the serum elimination half-lives of PFOA were approximately 5.68 and 0.08 days in male and female rats, respectively, following intravenous (iv) administration. By exposing beagle dogs, researchers calculated plasma half-lives of 473 and 541 hours in two males and 202 and 305 hours in two females following a single iv injection. A universally accepted viewpoint is that estradiol promotes urinary excretion of PFOA, whereas testosterone decreases its excretion. Compared to PFOS, striking differences among species existed in the pharmacokinetics of PFOA. The combined properties of rapid absorption and poor elimination could contribute to the bioaccumulation of PFOA or PFOS. It has been determined that the accumulation of PFOA in tissues of rodents, as well as PFOS, is principally in liver, kidney, and blood.

To date, limited toxicological studies are available on the examination of excretion of PFCs under consecutive exposure. In light of the ubiquity and persistence of PFCs in both the environment and organisms, the current study was undertaken to compare the excretion of PFOA versus PFOS in male Sprague-Dawley rats during a 28-day subchronic exposure.

The faster elimination rate in urine compared to feces indicated that urinary excretion is the primary clearance route in rats for either PFOA or PFOS. During the first 24 hours after administration of PFOA (5 and 20 mg/kg body weight/day), about 24.7-29.6% of the oral dose was excreted through urine and feces, while for PFOS, the excretion amounts were only 2.6-2.8% of the total gavaged doses (5 and 20 mg/kg body weight/day). The excretion rates of both PFCs increased with increasing exposure doses. The higher elimination rate of PFOA through excretion indicated its lower accumulation in rats, thus inducing possible lower toxicities compared to PFOS.

Source: Archives of Environmental Contamination and Toxicology, Vol. 58, No. 1, Pages 205-213, January 2010.

EFFECTS OF EXPOSURE TO DIESEL EXHAUST PARTICLES ON HUMAN HEALTH

Numerous epidemiological studies have shown that diesel exhaust particles (DEPs), ubiquitously present in urban areas where there are diesel powered cars and trucks, can have hazardous effects on human health. Studies have reported associations between exposure to DEPs and cardiovascular disease, allergic rhinitis and pulmonary cancer.

It has also been shown that DEP exposure can disturb reproductive function. Studies conducted in animals found that DEP exposure impaired male reproductive function. In male rats, the regulation of testicular function was altered with elevation of serum testosterone following DEP exposure, and in mice, DEP exposure disturbed spermatogenesis, resulting in reduction of daily sperm production.

Although the DEP components responsible for such reproductive effects have not been clarified, it is well known that DEP contains amounts of small particles ranging from nanoparticles to coarse particles. This suggests that much more attention should be paid to the adverse effects of nanoparticle-rich diesel exhaust (NR-DE) included in DEPs. These NR-DE have a larger surface area than larger particles and are thought to be able to penetrate deeply into the respiratory tract, and also translocate from alveoli to the circulation. Recently, the potential reproductive toxicity of NR-DE has become an increasing concern because a relatively low dose of NR-DE exposure clearly increased serum and testis testosterone levels in rats. Because the mechanism of the increase was also not investigated, it is important to evaluate the mechanism by which NR-DE increased testosterone synthesis in the testes or decreased the metabolism in the liver. Testosterone is synthesized in the Leydig cells via several important enzymes, carrier proteins or receptors from *de novo* synthesized cholesterol. Steroidogenic acute regulatory protein (StAR) and cytochrome P450 side-chain cleavage enzyme (P450scc) are involved in these enzymes and carrier protein, respectively. StAR plays a key regulatory role in cholesterol transport from the outer to the inner mitochondrial membrane, and P450scc is responsible for the conversion of cholesterol to pregnenolone.

On the other hand, cytochrome P450 2C11 (CYP2C11) is an enzyme responsible for the testosterone metabolism in the liver. Expression of CYP2C11 is regulated by growth hormone (GH) through insulin-like growth factor I (IGF-I). GH, a 19-21-kDa cytokine polypeptide, stimulates postnatal longitudinal bone growth, induces diverse effects on cell growth, and regulates an important cholesterol carrier protein, StAR, while IGF-I, a protein hormone that mediates the growth promoting effects of GH, regulates P450scc. The activation of these GH signalling was initiated by binding of GH to its receptor, growth hormone receptor (GHR).

An earlier study reported that exposure to low (22.5 ± 0.2 nm in diameter, 15.4 ± 1.0 $\mu g/m^3$ in mass weight, $2.27x10^5/cm^3$ in mean number concentration), and medium (26.1 ± 0.5 nm, 36.4 ± 1.2 $\mu g/m^3$, $5.11x10^5/cm^3$) concentrations of NR-DE for 1 and 2 months (5 h/day, 5 days/week) significantly increased plasma testosterone in male Fischer 344 rats, whereas exposure to a high concentration (27.1 ± 0.5 nm, 168.8 ± 2.7 $\mu g/m^3$, $1.36x10^6/cm^3$) did not. Now a new study attempts to clarify the mechanism of this elevation. Low and medium exposures to NR-DE for 1 and 2 months significantly increased StAR- and P450scc-mRNA and their protein expressions in the testis of rats, in which the elevation pattern was very similar to that of plasma testosterone levels. Interestingly, both exposure levels for 1 month signifcantly increased GHR expression in the testis, and low exposure also increased testicular IGF-I-mRNA levels and hepatic CYP2C11-mRNA and their protein levels in rats. These two factors are thought to be related to GH secretion. Disruption of testosterone biosynthesis by NR-DE exposure may be a mode of action for reproductive toxicity, which may, in part, be regulated by increasing StAR and P450scc expressions via GH signalling.

The frst point of interest in this study is why the mRNA and protein of StAR expressions increased in rats after NR-DE exposures at low and medium concentrations. Interestingly, both concentrations also increased testicular GHR, which is a nuclear receptor mediating between GH and IGF-I signalling, and IGF-I-mRNA levels, though the increase in the latter at medium concentration did not evidence a statistically signifcant difference. GH and IGF-I up-regulate StAR expression and steroidogenesis in Leydig cell progenitors, and plasma testosterone levels and StAR protein were reported to be lower in IGF-I null mice compared to those in the wild-type mice. These results suggest that plasma GH may be elevated similar to the testicular GHR, and might be a causative factor in the increased StAR and testosterone levels after NR-DE exposure. The induction of P450scc in low and medium NR-DE exposed groups was also very similar to that of StAR or plasma testosterone except for the 3-month exposure. The induction of P450scc has also been considered to be up-regulated by IGF-I in Leydig cells. In addition, the expression of P450scc was significantly lower in IGF-I null mice than wild-type mice. Taken together, NR-DE exposure might activate GH signalling which resulted, in part, in an increase in not only StAR but also P450scc expression levels, followed by an increase in the plasma testosterone levels.

A second point for discussion is whether or not the plasma testosterone levels might be regulated not only by testosterone biosynthesis in the testis but also by metabolic deactivation in the liver. Male-specifc CYP isozyme CYP2C11 participates in testosterone hydroxylation at 2 and 16 positions. CYP2C11 itself was regulated by GH, which is secreted in pulsatile fashion from the pituitary gland. This is also supported by reports that CYP2C11 apoprotein and mRNA levels were reduced in male rats after chronic ethanol treatment, where GH secretion and plasma testosterone levels were reduced as well. In the current study, CYP2C11 elevation following exposure to low NR-DE concentration might be mediated by the increased plasma GH. Nevertheless, plasma testosterone levels were increased after exposure to low and medium concentrations of NR-DE. Therefore, increased CYP2C11 by NR-DE might not be important in the elevation of testosterone levels after NR-DE exposure: induction of StAR and P450scc expressions in Leydig cells are more important in the regulation of plasma testosterone levels.

A third issue is that pregnenolone synthesized in the mitochondria is, in turn, converted to progesterone by 3β-hydroxysteroid dehydrogenase (3β-HSD), followed by cytochrome P450-17α-hydroxylase/C17-20 lyase ($P450_{17\alpha}$) to produce androstendione. This hormone is also produced from pregnenolone via dehydroepiandrosterone by $P450_{17\alpha}$ and 3β-HSD. Then, testosterone is produced from androstendione by catalytic action of 17β-HSD. However, there were significant changes only in the mRNA expression levels of 3β-HSD in the medium concentration exposure group and in $P450_{17\alpha}$ in the low concentration

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The Expression of Nerve Growth Factor in Mice Lung Following Low-Level Toluene Exposure

The impact of indoor air, especially volatile organic compound (VOC) levels, on the susceptibility to and severity of respiratory allergies and diseases is a pertinent topic of great interest and importance. Sick building syndrome (SBS) or sick house syndrome is characterized by non-specific complaints, including upper respiratory irritative symptoms, headaches and fatigue. Several reports have found that most SBS patients have allergic diseases. Although few studies have examined the effects of inhaling VOCs on the induction of allergic diseases in relation to indoor air quality, a positive relationship between immunological responses and the inhalation of VOCs has been speculated. However, domestic VOCs are not a major determinant of risk or severity of childhood wheezing illness. Therefore, the association between VOCs and allergic diseases remains controversial. Clarifying which factors regulate sensitivity to the adverse effects of low-level VOC exposure is of great importance in susceptible subjects.

Among the VOCs, toluene is a toxic indoor air pollutant that can occur at relatively high concentrations in homes and buildings. Epidemiologically, occupational exposure to mixed organic solvents (toluene, ethyl acetate, isopropyl alcohol, and ethyl ketone) has been shown to decrease the number of T lymphocytes and to increase the number of B lymphocytes in the peripheral blood. Exposure to alkanes and aromatic compounds, such as toluene, o-xylene, m + p-xylene, 2-, 3- and 4-ethyl-toluene and chlorobenzene, contributed to a skewed type 2 memory response to allergen challenge. A recent study was conducted of the effects of low-level exposure to toluene on airway inflammation in mice and showed that the expression of IL-5 mRNA in the lung and antigen-specific IgE antibody production in the plasma significantly increased in ovalbumin (OVA)-immunized mice. These findings suggested that low level exposure to toluene enhances Th2-dominant immunity.

Neurotrophins contribute to neurogenic inflammation by modulating the activity of sensory neurons and enhance the synthesis and release of neuropeptides. Neurotrophins, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), play important roles in neuroimmune crosstalk in the lung. NGF can influence allergic inflammation and asthma through the release of chemokines and cytokines into the immune system. Increased CCL2, RANTES, and CCL3 in bronchoalveolar lavage fluid were observed in allergic asthmatic patients. Neurotrophins are produced continuously during allergic inflammation and act as long-term modulators. Low-level toluene exposure increased the plasma NGF content in mice but suppressed the expression of NGF receptor tropomyosin-related tyrosine kinase (Trk) A mRNA in lung tissue. Although *in vitro* study showed that the exposure to toluene inhibited the secretion of IFN-γ, IL-4 and IL-13 from human peripheral blood mononuclear cells without causing cytotoxicity, the mechanism by which toluene might facilitate the development of allergic inflammation and the production of neurotrophin remains unclear. Thus, the adverse effects of VOCs, which are major indoor pollutants, on the induction or augmentation of allergic inflammation via neuroimmune interactions have not yet been fully characterized.

In a new study, the researchers sought to determine the effect of low-level tuluene exposure on the expression of NGF using mice with or without OVA sensitization intranasally exposed to toluene for a longer term than in their previous study. They found that compared to the previous studies the long-term exposure of immunized mice to 9 ppm toluene significantly increased the expressions of NGF and TrkA mRNAs in the lung.

However, 90 ppm of toluene inhalation alone significantly increased the expression of NGF mRNA. In addition, immunohistochemical analysis showed that the exposure of immunized mice to 9 ppm toluene resulted in increased NGF-immunoreactivity in bronchial epithelial and smooth muscle cells and in increased TrkA-immunoreactivity in inflammatory cells. This result is consistent with previous studies that respiratory epithelial cells and bronchial smooth muscle cells have been shown to be a major source of NGF, BDNF and NT-3. These results indicate that co-stimulation with OVA sensitization and toluene inhalation augments the production of NGF in the lung. It is possible to speculate that sensitized populations, such as asthmatic populations, may be susceptible to the adverse effects of low-level toluene inhalation.

The findings indicate that low-level toluene exposure upregulates the production of neurotrophin in allergic airway inflammation. However, a dose-dependency in adjuvant activity was not found. Some discrepancies were found between high and low dose effect of toluene on NGF and TrkA expression in the mice lung. No mechanism has been proposed to explain the above stimulatory effect of low-level toluene exposure on NGF production in the lungs. One possibility is that toluene inhalation contributes to neural dysfunction related to sensory stimulation, and that neurotrophin production and homeostatic mechanisms compensate for toxicant-induced changes. Neurotrophins take part in neurogenic inflammation by modulating the activity of sensory neurons and enhancing the synthesis and release of neuropeptides. Another possibility is that in OVA-immunized mice, the immune system may be activated and may be sensitive to low-level toluene. Furthermore, the researchers previously reported that OVA immunization acts as a stressor to activate hypothalamic-pituitary-adrenal axis and then this signal further activates some of the immune cells and neural cells in the lung and this neuroimmune interaction promotes lung inflammation and release of neurotrophins and their receptor expressions.

NGF production after toluene exposure may depend upon type of exposure system. Continuous or frequent exposures to a given stimulus favor the development of tolerance, whereas intermittent exposures favor sensitization. To evaluate the environmental risk of various VOCs in the induction or exacerbation of allergic inflammatory responses, new tools must be developed. The implications of the present findings are significant, not only for dose-responses in terms of toxicology, but also in terms of the reliability of animal model and risk assessment applications. Therefore, the study's repeated and intermittent exposure model is suitable for the detection of sensitivity, the effective dose level and neuroimmune functions following exposure to environmental chemicals at low levels. Further studies are needed to determine whether low-level exposure to other VOCs in indoor air pollutants might also have the ability to exacerbate allergic inflammation.

Source: Toxicology Letters, Vol. 191, Issues 2-3, Pages 240-245, December 2009.

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group for 3-month exposure to NR-DE. These changes appeared not to be important in the plasma testosterone levels after NR-DE exposure.

Fourth, previous studies reported that DEP (3.0 and 5.6 mg/m^3) exposure disrupted male reproductive function with reduction of sperm production in mice and in rat. On the other hand, inhalation of DEP at a concentration of 0.3 or 1 mg/m^3 with 0.4 µm diameter of particles for 8 months increased plasma and testicular testosterone levels. Interestingly, the present study showed results similar to the DEP exposure experiment, although the diameter of NR-DE was 23-27 nm, which was 20 times smaller and the concentration of NR-DE was 20-fold lower than that of the previous study.

The present study underscores the possibility that NR-DE exposure had adverse effects on the reproductive function of adult male Fisher 344 rats. Increased plasma testosterone levels after NR-DE exposure may be derived from the induction of testosterone biosynthesis through elevation of StAR and P450scc in the testis via GH signalling.

Source: Toxicology Letters, Vol. 191, Issues 2-3, Pages 103-108, December 2009.

Removal of Cadmium from Drinking Water

The rising global demand for clean water is challenged by various factors like ever increasing freshwater pollution, more stringent health-based drinking water regulations, and competing demands for clean water from a variety of users. At the same time, the increasing levels of heavy metals in the environment represent a serious threat to human health and ecological systems. In nature, metal ions complex with proteins, humic substances, and biopolymers as well as inorganic colloids such as clay particles. Mobile and soluble toxic metal species are not biodegradable, and thus tend to accumulate in living organisms, causing various diseases and disorders. Among others, cadmium is one of the most toxic nonessential heavy metals present in the environment, even at low concentrations. Cadmium readily bioaccumulates giving rise to adverse health effects in humans such as renal disturbances, decreased lung capacity, bone lesions, cancer, and hypertension. Toxic metal contaminants, like cadmium, exist naturally in many ground waters, aqueous industrial waste streams (electroplating, smelting, alloy manufacturing, pigments, plastic, battery, etc.), as well as surface waters impaired by resource extraction activities (mining, refining).

Numerous processes exist for removing dissolved heavy metals, including ion exchange, precipitation, adsorption, filtration, and electrodialysis. Among various treatment methods, ion exchange and adsorption look the most promising ones when effective, reusable, and relatively low-cost ion exchangers and adsorbents are available. In the past, organic and inorganic particles have been investigated to assess their toxic metal binding abilities. Standard polymeric complexing agents like low molecular weight polyethylenimine, poly(acrylic acid), and alginic acid are known for their significant binding capacity, while relatively new dendritic polymers provide new opportunities to develop high-capacity nanoscale chelating agents for environmental applications. Many researchers also have found that living biomaterials, such as algae, fungus, and bacteria, have the ability to adsorb heavy metal ions from the environment. Among inorganic materials, zeolites are known for their ion exchange properties, but recently zeolite synthesis has advanced to include formation of nanoscale crystals with precisely tailored physical chemical properties; thus, significantly increasing their potential effectiveness in an array of chemical, biomedical, and environmental applications.

To make the toxic metal removal process most efficient and effective, nanoparticles for complexation with metal ions have to possess a few specific characteristics: (1) high affinity toward the target metal, (2) low affinity toward nontarget metal ions, (3) possibility of regeneration, (4) chemical and mechanical stability, (5) low toxicity, and (6) low cost. A critical step is the nanoparticle separation, which could be achieved by low pressure membrane processes in a manner resembling polymer enhanced ultrafiltration. For nano particle-enhanced ultrafiltration, membrane characteristics like molecular weight cut-off, physical chemical properties (pore size, charge, material type, hydrophobicity, etc.), and fouling resistance, as well as energy consumption play an important role.

The current study evaluated removal of cadmium ions from water by nanoparticle-enhanced ultrafiltration using polymer and zeolite nanoparticles. This evaluation considered nanoparticle physical chemical properties, metal-binding kinetics, capacity and reversibility, and ultrafiltration separation for a Linde type A zeolite nanocrystals, poly(acrylic acid), alginic acid, and carboxyl-functionalized poly (amido amine) dendrimers in simple, laboratory-prepared ionic solutions. The three synthetic materials exhibited fast binding kinetics and strong affinity for cadmium, with good regeneration capabilities. Only the zeolite nanoparticles were completely rejected by the ultrafiltration membranes tested. Overall, colloidal zeolites performed similar to conventional metal binding polymers, but were more easily recovered using relatively loose filtration membranes (i.e., lower energy consumption). Further, the superhydrophilic colloidal zeolites caused relatively little flux decline even in the presence of divalent cations which caused dense, highly impermeable polymer gels to form over the membranes. These results suggest zeolite nanoparticles may compete with polymeric materials in low-pressure hybrid filtration processes designed to remove toxic metals from water.

Source: Environmental Science & Technology, Vol. 44, No. 7, Pages 2570-2576, March 2010.

Persistent Organic Pollutants and Heavy Metals in Adipose Tissues of Ethnic Chinese Patients with Uterine Leiomyomas

In recent years, Hong Kong in southern China has experienced serious environmental deterioration due to the rapid urban and industrial development of the Pearl River Delta.

Man-made chemicals, especially those classified as persistent organic pollutants (POPs), such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and also polycyclic aromatic hydrocarbons (PAHs) are major environmental issues in the Pearl River Delta region. Being a large agricultural country, China had been a major producer and consumer of dichlorodiphenyltrichloroethane (DDTs) in the past. Though DDT was banned for agricultural application in China since 1983, it is still allowed for vector control (e.g., controlling mosquitoes which transmit malaria).

PCBs, used as coolants and lubricants in transformers, capacitors, and other electrical equipment due to their nonflammable and insulating properties, were banned worldwide since the early 1970s including in China because of their harmful effects on environmental and human health. PBDEs are used as flame-retardant additives in plastics, electrical appliances, television sets, computer circuit boards, and casing. Since they are mixed into polymers, PBDEs are not chemically bound to plastic or textiles, thus, they may separate from the products easily and enter into the environment. PAHs are often formed during incomplete combustion of coal, oil, gas, tobacco, and grilled meat. Exposure to PAHs usually occurs via cigarette smoke, vehicle exhausts, and municipal trash incineration. Most of these pollutants are able to travel long distances, and they are toxic, persistent, bioaccumulative, and lipophilic. If they enter our food chain, they can be biomagnified, with most of it being stored in adipose tissues resulting in extremely high concentrations in higher trophic organisms including humans.

Uterine leiomyoma (UL; a noncancerous tumor of the uterus) is a common disease among women. The incidence of UL has been shown to be negatively correlated with parity and positively related to time duration from the last pregnancy, and obesity. The body burden of POPs in the mother is transferred to the unborn baby through the umbilical cord and to the infant through breast milk. Cord blood and breast milk have long been used as test materials for studies of pollution-fostered human diseases and regional and global pollution surveillance, respectively. Human exposure to heavy metals/metalloid such as lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As) is mainly through food consumption and inhalation. When heavy metals deposit in the body, they can interfere with the function of the hypothalamic-pituitary-ovarian axis, through direct or indirect modification of the secretion of hormones such as prolactin, adrenocortical steroids, or thyroid hormones.

The current study was designed to (1) quantify the concentrations of POPs and heavy metals in adipose tissues of women with UL compared with a control group, (2) elucidate associations of POPs and heavy metals in subcutaneous fat and visceral fat, and (3) determine any correlations between their body burdens of POPs and heavy metals with their seafood diet, body mass index (BMI), and age.

Samples were collected from ethnic Chinese residents from six hospitals and six cosmetic surgery clinics in Hong Kong. Patients with UL provided both subcutaneous and visceral fat, while women without UL (control group) provided subcutaneous fat through liposuction. Analyses of POPs and heavy metals were conducted using gas chromatography-mass spectrometry and inductively coupled plasma-optical emission spectrometry, respectively. Total Hg content was measured using an atomic fluorescence spectrometer.

The results showed that significantly higher concentrations of DDTs, hexachlorocyclohexane (HCHs), PCBs, PAHs, PBDEs, As, Cd, Pb, and Hg were detected in the subcutaneous fat of patients when compared with those of the control group. Significant correlations were found between pollutant concentrations of subcutaneous and visceral fat in the patient group, with visceral fat containing significantly higher concentrations of As (subcutaneous fat: 0.59 µg/kg fat; visceral fat: 0.73), Cd (0.38; 0.47), Pb (5.24; 5.98), and Hg (9.12; 13.3).

Since UL has a close relationship with estrogen levels in women, and OCPs, PCBs, PAHs, and PBDEs have an estrogen-like effect, these chemicals may correlate with UL. This study showed higher levels of DDT and its metabolites, HCHs, fluoranthene, pyrene, benzo(a)pyrene, PCBs, and BDE-99 in patients with UL than those in the control group. Furthermore, higher concentrations of Cd, Pb, As, and Hg were found in the patient group than those in the control group suggesting that these chemicals may correlate with UL.

These studies demonstrated that these POPs and some heavy metals may have correlations with UL, and their accumulation in the body is positively correlated with seafood diet habit, BMI, and age. In the patient group, higher levels of POPs and some heavy metals were found in visceral fat than in subcutaneous fat confirming the long-held belief that visceral fat is more pernicious and pathogenic than subcutaneous fat.

It is therefore recommended that women minimize their exposure to environmental pollutants as much as possible which includes consuming certain seafoods in moderation, such as fatty fish, carnivorous fish (tuna and swordfish), and shellfish which are known to concentrate POPs and heavy metals, respectively.

Source: Environmental Science and Pollution Research, Vol. 17, No. 1, Pages 229-240, January 2010.

Possible Health Effects of Toxicity from ZnO Nanoparticles in Accidental Exposures

Manufactured nanoparticles are being marketed as having unique properties due to their size, shape, surface area, and composition as compared to bulk material, but there remain concerns regarding toxicities associated with these novel materials. This is especially relevant for oxides of Fe, Si, Ti, and Zn as these compounds are used in foods, cosmetics, and other consumer products. Accidental exposure to nanosized zinc oxide (ZnO) from children accidentally eating sunscreen products is a typical public concern, motivating the study of the effects of nanomaterials in the colon. In a previous study, immortalized human cells were used to study biochemical responses to several pairs of micrometer- and nanosized metal and ceramic oxide particles to test for size-dependent effects. ZnO appeared to induce cell responses at lower exposure concentrations than the other tested oxides. Toxicity from ZnO nanoparticles has been reported in a broad range of biological systems, but the mechanisms are not well understood. In humans, high doses of ZnO can induce metal fume fever, which is characterized by pulmonary toxicity. The toxicity to other organ systems is less clear, but Zn can irritate the gastrointestinal tract, induce emesis, and be associated with an impaired inflammatory response. Zn toxicity in certain organisms is due to the dissolution of Zn ions. ZnO is considered nearly insoluble in water, but solubility increases in acid environments and in the presence of chelators. In a murine model, the development of ZnO tolerance appears to enlist genes like metallothionein that sequester Zn^{2+}.

This study tested the *in vitro* response of human colon-derived RKO cells to two types of ZnO powders. Two commercial ZnO types, one sold as a 8-10 nm powder and the other described as -325 mesh (< 44 µm) powder, were evaluated in human colon derived RKO cells. The powders had a volume-to-surface area ratio equivalent to 40 and 330 nm spheres, respectively. Both materials formed micrometer-sized agglomerates in cell culture media. The nanosized ZnO was more cytotoxic than the micrometer-sized ZnO with LC_{50} values of 15 ± 1 and 29 ± 4 µg/cm^2, respectively. Transfer of Zn from the solid phase to the cell culture media in the presence of RKO cells was time- and concentration-dependent. However, direct particle-cell contact was required for RKO cell cytotoxicity, and the toxicity of particles was independent of the amount of soluble Zn in the cell culture media. The mechanism of cell death includes the disruption of mitochondrial function. Robust markers of apoptosis, Annexin V staining, loss of mitochondrial potential, and increased generation of superoxide were observed when cells were treated with ZnO particulate matter but not when treated with comparable concentration of a soluble Zn salt. Both ZnO samples induced similar mechanisms of toxicity, but there was a statistically significant increase in potency per unit mass with the smaller particles.

It is unclear whether nanoparticulate dietary metal oxides, including ZnO, have chronic effects on the colon. Ingestion of large amounts of ZnO has been reported to cause gastroduodenal corrosive injury in humans without systemic toxicity. Inhaled ZnO can cause pulmonary toxicity but is generally considered to have minimal toxicity in other organs. Increased consumption of fine and ultrafine particulate matter is hypothesized to exacerbate inflammatory bowel disease possibly due to transport of substances adsorbed on the solid surface. A study of patients with Crohn's disease showed that a low microparticle diet was beneficial, but a follow-up study did not confirm the original findings. In rodent studies, particles smaller than 100 nm were taken up by the rat intestinal mucosa and enter systemic circulation, plus inflamed colon cells internalize nanoparticles at a greater rate than normal colon cells. In humans, observations of tissue biopsies from patients with Crohn's disease or cancer contain nanoparticles of exogenous origin.

The concentrations used in this study are high based on treated cell culture well surface area but not implausible on a suspended volume basis. The maximum *in vitro* cell treatment was approximately 100 µg/mL. Assuming 1.5-2 L as the volume entering the colon per day, this concentration could be reached by ingesting 2 g of a lotion containing 10% ZnO. However, unlike TiO_2 and SiO_2, which are also used in cosmetics and sunscreens, ZnO nanoparticles are likely to dissolve in the low pH environment of the stomach.

ZnO in consumer products, such as sunscreens, can lead to ingestion exposure, especially in children. Evidence of cell death and mitochondrial changes in response to ZnO particle contact supports further study in animal models. These colon cell studies of particles sold as novel nanomaterials and as conventional screen-sized powders showed a limited, but observable, size-dependent difference in ZnO particle potency but similar mechanisms of toxicity. Further work is needed to determine if cell uptake, or simply cell contact, is required for cytotoxicity.

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