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AUTISM AND AGRICULTURAL PESTICIDES

"Autism" refers to a set of neurodevelopmental disorders that are characterized by impaired social interaction, restricted communication and repetitive, stereotypic behaviors.

The number of children reported as having autism spectrum disorders (ASD) has risen dramatically since the early 1990s. In the United States, some of this increase is attributable to changes in diagnosis and reporting, although this pattern is not uniform across all states. Symptoms of classic autism do not typically become evident until early childhood, but current evidence is consistent with a pathogenic process originating during fetal development.

Many of the hypotheses regarding ASD pathogenesis involve a functional deficit caused by alterations to specific brain structures occurring *in utero* during defined temporal windows of vulnerability. The lesions in question might result from genetic factors, environmental insults, or a combination of the two. A variety of lesions could give rise to a "final common pathway" to autism; ASD as currently defined may well include multiple disorders that have not yet been successfully differentiated.

A large number of widely used agricultural pesticides have known neurologic effects, raising the possibility that gestational exposure to these compounds could play an etiologic role in ASD and related neurodevelopmental disorders. Most compounds are prone to "drift," and detectable levels in air samples are often measurable at locations beyond the site of application for extended periods afterwards. Elevated levels of agricultural pesticides in household dust and their metabolites in urine have been associated with residential proximity to treated fields.

Studies of pediatric diseases and their associations with residential proximity or parental occupational exposure to pesticides have been accumulating, most notably for cancer and, more recently, neurodevelopmental delay. Many environmental toxicants are conveyed transplacentally, and the blood-brain barrier remains relatively permeable to many of these compounds until well into the first year of life. In general, experimental and epidemiologic evidence concerning pesticides and pediatric neurodevelopment is strikingly lacking, despite considerable knowledge about pesticide toxicity (particularly neurotoxicity).

Now, new studies have been carried out in the California Central Valley to evaluate the hypothesis that maternal residence near agricultural pesticide applications during key periods of gestation could be associated with the development of ASD in children.

Researchers identified 465 children with ASD born during 1996-1998 using the

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A Recent Study Provides Evidence Against a Direct Genotoxic Mode of Action for Arsenic-Induced Cancer

Chronic exposure to arsenic (As), particularly via drinking water, is a major health concern in certain areas of the world. Epidemiological evidence indicates that As is a human skin, lung and bladder carcinogen. The increased cancer risk is thought to be due to the presence of inorganic trivalent arsenite, although both arsenite and pentavalent arsenate are present in drinking water. Exposure to As via drinking water is associated with increased risk of squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and Bowen's disease (squamous cell carcinoma *in situ*), but not melanoma.

Arsenic (arsenite, arsenate) is a well-established human carcinogen but is not a complete animal carcinogen in standard assays. However, arsenite can act as a transplacental carcinogen and as a cocarcinogen. When pregnant C3H mice are exposed to sodium arsenite (42.5 and 85 ppm) in the drinking water, the male offspring had increased hepatocellular carcinoma and benign adrenal tumors while the female offspring had increased ovarian and lung tumors (but no skin tumors).

A recent study showed that low concentrations of arsenite enhanced the tumorigenicity of solar UV irradiation in hairless mice, suggesting As cocarcinogenesis with sunlight in skin cancer and perhaps with different carcinogenic partners for lung and bladder tumors. Cocarcinogenic mechanisms could include blocking DNA repair, stimulating angiogenesis, altering DNA methylation patterns, dysregulating cell cycle control, induction of aneuploidy and blocking apoptosis. Arsenicals are documented clastogens but not strong mutagens, with weak mutagenic activity reported at highly toxic concentrations of inorganic As. Previously, studies showed that arsenite, but not monomethylarsonous acid (MMA[III]), induced delayed mutagenesis in osteosarcoma (HOS) cells. Now, new data has been reported on the mutagenicity of the trivalent methylated arsenic metabolites MMA(III) and dimethylarsinous acid [DMA(III)] at the gpt locus in Chinese hamster G12 cells. Both methylated arsenicals seemed mutagenic with apparent sublinear dose responses. However, significant mutagenesis occurred only at highly toxic concentrations of MMA(III). Most mutants induced by MMA(III) and DMA(III) exhibited transgene deletions. Some non-deletion mutants exhibited altered DNA methylation.

The common view that carcinogenesis occurs primarily via direct acting genotoxic insults to DNA is too simplistic and does not fit the accumulating data for many human carcinogens including As. In the absence of mutagenesis, the challenge of risk assessment is to understand underlying indirect genotoxic mechanisms that may alter the presumptions made regarding thresholds. In the European Union, considerations of indirect genotoxic mechanisms have led to new appreciation of thresholds in risk assessment. Furthermore, hormesis, or protection afforded by very low doses of a carcinogen, also implies thresholds below which non-harmful effects occur. The familiar non-linear U or J-shaped hormesis response curves suggest cellular homeostasis processes that could involve apoptosis, DNA repair, cell proliferation, altered DNA methylation and other non-DNA genotoxic responses. In the As arena, the concept of low dose adaptive responses and hormesis has recently been applied to discussions of As mode of action (MOA). Therefore, risk assessment models for indirect acting carcinogens such as As must account for the likelihood of thresholds resulting from targets other than DNA, as well as adaptive mechanisms.

Source: Toxicology and Applied Pharmacology, Vol. 222, August 2007.

AUTISM AND AGRICULTURAL PESTICIDES

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California Department of Developmental Services electronic files, and matched them by maternal date of last menstrual period to 6,975 live-born, normal-birth-weight, term infants as controls. Proximity to pesticide applications was determined using California Department of Pesticide Regulation records.

A staged analytical design using *a priori* criteria to the results of conditional logistic regressions was employed to exclude associations due to multiple testing error.

Of 249 unique hypotheses, four that described organochlorine pesticide applications – specifically those of dicofol and endosulfan – occurring during the period immediately before and concurrent with central nervous system embryogenesis (clinical weeks 1 through 8) met *a priori* criteria and were unlikely to be a result of multiple testing. Multivariate *a posteriori* models comparing children of mothers living within 500 m of field sites with the highest non-zero quartile of organochlorine poundage to those with mothers not living near field sites suggested an odds ratio for ASD of 6.1. ASD risk increased with the poundage of organochlorine applied and decreased with distance from field sites.

These findings suggest that the possibility of a connection between gestational exposure to organochlorine pesticides and ASD requires further study.

Source: Environmental Health Perspectives, Vol. 115, October 2007.

HEALTH EFFECTS OF SHORT-TERM EXPOSURE TO MOBILE PHONE BASE STATION SIGNALS

Radio frequency electromagnetic fields (rf-emf) do not fall within the ionizing spectrum. Nevertheless, high intensity rf-emf can cause thermal effects with serious implications for human health. In everyday life, however, most humans are not exposed to such high intensity rf-emf and do not possess sensory organs that can detect electric or magnetic fields. The question remains as to whether exposure to low intensity rf-emf, even if undetected, can negatively affect human health. A subgroup of the population has claimed that they are sensitive to rf-emf and this condition, formerly known as Electromagnetic Hypersensitivity, has recently been relabelled in a World Health Organization workshop as Idiopathic Environmental Intolerance with attribution to Electromagnetic Fields (IEI-EMF). In a recent UK survey, it has been reported that around 4% of people claim that they are sensitive to rf-emf to some degree. A variety of negative health effects (e.g., cold and flu-like symptoms) are attributed to exposure to rf-emf from objects such as computers and mobile phones. Previous research has indicated that IEI-EMF individuals report lower levels of well-being compared to healthy individuals and that the symptoms they experience may greatly impact upon their quality of life. However, evidence that IEI-EMF symptoms are indeed caused by rf-emf exposure is yet to be established. A systematic review of 31 blind and double-blind provocation studies yielded no evidence that IEI-EMF individuals could detect the presence of rf-emf, and only seven studies indicated that exposure to rf-emf did affect health indices. In two of these, however, the authors failed to replicate their own findings. Another four studies involved inappropriate use of statistics, while one reported improved mood in the active exposure condition. One unpublished double-blind study specifically examining base station signals did find that exposure to a Universal Mobile Telecommunications System (UMTS) signal resulted in reduced subjective well-being for both sensitive and non-sensitive individuals, while a Global System for Mobile Communication (GSM) base station signal had no effect. However, a recent study conducted in Switzerland was unable to replicate this effect. Another double-blind study has recently reported no negative health effects from exposure to a standard 900MHz GSM handset signal for either sensitive or control participants.

The existing evidence therefore, indicates that exposure to rf-emf signals from mobile phone base stations and handsets has little effect on health, even in those with a perceived sensitivity to rf-emf. Nevertheless, only two double-blind studies have been conducted with base station signals, with contrary results. Given the increase in mobile phone base stations around the world and the level of public concern regarding possible negative health implications, further research is necessary to investigate the short and long-term impact of exposure to rf-emf in both healthy and IEI-EMF groups.

Now, a new study has been carried out to test whether short-term exposure to typical GSM and UMTS base station signals affected a variety of measures of well-being in sensitive and control individuals, using both open provocation and double-blind tests. It was hypothesized that sensitive participants would report more symptoms and lower levels of well-being during GSM and UMTS exposure compared to sham. In addition, sensitive participants should be able to identify above chance level whether the base station was turned "on" or "off". For control participants no difference was expected in the number or severity of symptoms reported during exposure. Previous research has reported higher levels of heart rate, heart rate spectrum ratio, and electrodermal activity in sensitive compared to control individuals. Thus, physiological measurements were also conducted to determine whether exposure to GSM and UMTS base station signals affected objective measures of well-being in both sensitive and control individuals.

In the study, 56 self-reported sensitive and 120 control participants were tested in an open provocation test. Of these, 12 sensitive and 6 controls withdrew after the first session. The remainder completed a series of double-blind tests. Subjective measures of well-being and symptoms, as well as physiological measures of blood volume pulse, heart rate and skin conductance were obtained.

The study found that during the open provocation, sensitive individuals reported lower levels of well-being in both GSM and UMTS compared to sham exposure, while controls reported more symptoms during the UMTS exposure. During double-blind tests the GSM signal did not have any effect on either group. Sensitive participants did report elevated levels of arousal during the UMTS condition, while number or severity of symptoms experienced did not increase. Physiological measures did not differ across the three exposure conditions for either group.

Thus, the study concluded that short-term exposure to a typical GSM base station-like signal did not affect well-being or physiological functions in sensitive or control individuals. Sensitive individuals reported elevated levels of arousal when exposed to a UMTS signal. Further analysis, however, indicated that this difference was likely to be due to the effect of order of exposure rather than the exposure itself.

Source: Environmental Health Perspectives, Vol. 115, November 2007.

Effect of Fly Ash Inhalation on Biochemical and Histomorphological Changes in Rat Liver

Fly ash, defined as an unburnable fraction of coal comprising chiefly aluminosilicate, is generated during the combustion of coal for energy production. Fly ash is now recognized as an environmental pollutant. It is emitted in the atmosphere in various particle sizes and the smallest particles have the longest residence times. The vast majority of fly ash is collected at thermal power plants and disposed predominantly in landfills. However, 1-5% of the fly ash produced during coal combustion is released to the atmosphere in the form of respirable aerosols (aerodynamic diameter <10 µm). The smaller size fraction of ash is thought to be more environmentally relevant for two reasons: (a) industrial scrubbing devices are least effective in the range 0.1-1 µm and (b) smaller particles are enriched with toxic metals. In India, the majority of thermal power plants use bituminous coal as a fuel, which is recognized to release a large quantity (40%) of fly ash. It is estimated that 2 ton of fly ash is produced every minute in a 200-MW capacity generating unit. The annual fly ash production in India during 2000-01 was around 90 million ton and it is likely to cross the 140 million ton barrier by 2020.

A number of genotoxic metals and polycyclic aromatic hydrocarbons have been detected in fly ash particles. The concentration of metals in fly ash is highest in the smallest particles, which also have the longest atmospheric residence time. It is generally accepted that particle size is a major factor in determining the toxic effects of airborne particulate matter. It has been reported in a study undertaken in 1998 that the amount of iron mobilized from coal fly ash was a function of particle size, with the parent coal with the size fraction less than 2.5 µm having more iron mobilized than that with the size fraction 2.5-10 µm. It has also been reported that the tissues extract trace metals much more efficiently from small particles that become deposited in the lung than from larger particles that are deposited higher up in the respiratory system. The fate of particles deposited in the pulmonary region is probably strongly dependent on their mechanical stability, i.e., their rate of dissolution in lung fluids. Extrapulmonary translocation of fly ash has been shown in hamsters and studies have shown that some metals from fly ash were selectively leached in body fluids. The cytotoxic effects of fly ash and metal coated fly ash particles were profoundly influenced by increasing concentration and decreasing particle size.

Now, a new study has been conducted to determine the effect of fly ash inhalation for a 28 day period on the deposition of metal ions and histopathological changes in liver of rats. The results showed an increase in the concentration of metals such as cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), and lead (Pb) in the tissues of exposed rats. The level of metals varied from metal to metal and from organ to organ. Level of serum enzymes such as serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, and alkaline phosphatase were increased in fly ash exposed rats using whole body inhalation exposure as compared to sham controls. Histopathological studies of rat liver exposed to fly ash revealed infiltration of mononuclear cells in and around the portal triads, which seems to be laden with fly ash particles. Hepatocytes showed necrotic changes such as pyknotic nuclei, karyorrhexis, and karyolytic. These changes were more towards the centrolobular areas than the midzonal and periportal areas.

On the basis of the present study, it can be concluded that fly ash, which is an environmental contaminant, is a major source of heavy metals, which could be toxic to the human population. These flndings demonstrate that the toxic metals of inhaled fly ash in rats get translocated into extrapulmonary organs, become deposited, and hence may manifest their toxic effects in different tissues.

Source: Ecotoxicology and Environmental Safety, Vol. 68, September 2007.

EFFECTS OF AMBIENT PARTICLE AIR POLLUTION ON HUMAN HEALTH: A STUDY CARRIED OUT IN WUHAN, CHINA

Ambient air pollution has been associated with a wide range of effects on human health, including measurable decrements in lung function, and increases in respiratory symptoms and diseases, hospital and emergency department admissions, and mortality. A large number of mortality time-series analyses have provided sufficiently convincing evidence that non-accidental mortality, including cardiopulmonary mortality, is significantly associated with ambient particulate matter (PM) exposure in the US, Canada, Eastern Germany, China, Korea, Greece, and Brazil. The estimated PM_{10} effect from recent studies is generally in the range of 1.0-8.0% excess deaths per 50 µg/m^3 PM_{10} increments in 24-h average concentrations.

Despite this large body of available evidence, the establishment of PM exposure as a cause of increased mortality is still controversial because of several uncertainties: the mortality rates attributed to PM exposure are not specific and may also be attributable to other factors such as exposure to other co-pollutants, change of climate or temperature, or personal and socio-demographic factors affecting PM exposure, such as use of air conditioners, level of education, and influence of other co-morbidities. Also, there is insufficient evidence about the general shape of exposure-response relationships between PM and/or other pollutants and observed daily cause-specific mortality; heterogeneity of PM-mortality effect estimates; model misspecification; and lack of biologically demonstrable mechanisms for the observed associations.

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EFFECTS OF DIETARY EXPOSURE TO ACRYLAMIDE

Recent reports of findings of acrylamide in many common foods have sparked renewed interest and concern in assessing human health hazards and the long term risk associated with exposure to vinyl compounds.

Acrylamide is a high-production vinyl compound whose polymeric form is used in the construction and oil industry, in the manufacture of paper, plastics and textiles, as a flocculant in the treatment of waste water, and in cosmetics. Worldwide public concern has been caused by the finding that the same compound is generated in many common foods during cooking at high temperatures. For example, acrylamide reaches parts per million concentrations in French fries, potato and tortilla chips, bread crust, various baked goods, breakfast cereals and coffee. Further studies showed that acrylamide is formed during the Maillard browning reaction from a heat-induced reaction between the amino acid asparagine and the carbonyl group of glucose. A large fraction of ingested acrylamide is converted by cytochrome P450 2E1 (CYP2E1) monooxygenase to glycidamide, an epoxide derivative that is substantially more reactive toward macromolecules than acrylamide itself. Both acrylamide and glycidamide are detoxified by the action of glutathione-S-transferase, which catalyzes the conjugation with glutathione, and glycidamide is additionally inactivated by epoxide hydrolases. This ultimate oxidative metabolite forms DNA adducts, induces mutations in *Salmonella,* and displays mutagenic as well as clastogenic properties in human cells.

Acrylamide is tumorigenic to experimental mice and rats. The long-term carcinogenicity studies demonstrated tumor induction at acrylamide daily doses of 1 mg/kg body weight or higher. In comparison, the average intake of acrylamide in Western populations is in the range of 0.5 µg/kg body weight/day, although children may be more highly exposed. Occupational exposures have been estimated in the daily range of 1 µg/kg body weight/day, whereas cigarette smoking leads to an acrylamide intake of up to ~3 µg/kg body weight/day. Based on rodent studies, conventional low-dose extrapolations have been made for the incidence of human cancer associated with the ingestion of acrylamide contained in foods or beverages. Quantitative estimates of lifetime cancer risks range from 1 to 44 per 10,000 exposed individuals, but acrylamide could not be linked to an increased cancer mortality in any epidemiologic study and therefore it remains difficult to establish the full extent of health risks resulting from the wide appearance of this food carcinogen.

Because serum concentration values from human exposures are missing, hemoglobin adducts in blood, mercapturic acid metabolites in the urine or DNA adducts in target tissues have been proposed as indirect biomarkers to assess the internal burden of acrylamide and its genotoxic metabolite glycidamide. An alternative approach involves the study of active cellular reactions in response to different levels of exposure. Therefore, the present study performed a transcriptomic analysis to identify differentially expressed genes that may be used as a biomarker of acrylamide or glycidamide action in exposed tissues. A standard human breast cell line (MCF7) was selected as the primary target of these transcriptomic experiments because the lifetime rodent experiments yielded mammary gland adenomas and adenocarcinomas in females and the appearance of acrylamide in mammary glands has been demonstrated after ingestion of contaminated food. Despite their unlimited life span, immortalized cell lines maintain a wide range of normal properties. In particular, the selection of MCF7 cells was motivated by their ability to undergo appropriate reactions to genotoxic insults, including activation of the p53 tumor suppressor, cell cycle arrest and apoptosis. Additionally, the human colonic cell line CaCo-2 has been tested as a system that mimics the absorptive barrier of an intestinal epithelium. The transcriptomic approach revealed that glycidamide, the oxidative metabolite of acrylamide, causes broad alterations of genome function, which may potentially modulate the biological endpoint of these compounds.

In this study, the toxicogenomic analysis revealed that glycidamide exerts a plethora of transcriptional effects, but the implication of this study with respect to risk assessment will be understood only when similar experiments are carried out in laboratory animals. Eventually, it may be possible to implement a threshold level that prevents the induction of potentially hazardous transcriptional responses mediated by glycidamide. The present *in vitro* results suggest that low-dose responses, including the induction of epoxide hydrolase 1, may exert beneficial effects by promoting inactivation of the toxicant, and that potentially adverse changes, for example dysregulation of steroid hormone synthesis, are detected at a concentration that exceeds the dietary exposure of the general population.

Source: Toxicology, Vol. 240, October 2007.

Arsenic Exposure: Diabetes and Hypertension in Human Populations

Long-term exposure to ingested arsenic from drinking water has been well documented to be associated with an increased risk of diabetes mellitus and hypertension in a dose-response relationship among residents of arseniasis-endemic areas in southwestern Taiwan and Bangladesh. An increased risk of self-reported hypertension but not diabetes was reported in a community-based study of residents who consumed drinking water with a low level of arsenic. Increased glycosylated hemoglobin level and systolic blood pressure were observed in workers occupationally exposed to arsenic. Inconsistent findings of arsenic and diabetes in occupational studies may result from the healthy worker effect and the variation in exposure measurement, age composition, number of patients, accuracy in diagnosis and classification of underlying causes of death, competing causes of death, and method to detect diabetes. The dose-response relationship and toxicological mechanisms of arsenic-induced diabetes and hypertension need further elucidation.

Inorganic arsenic has been recognized as a human poison since ancient times. The arsenic-related human toxicity is systemic involving a number of organ systems. Acute, subacute and chronic toxic effects of inorganic arsenic exposure through inhalation and ingestion have been reviewed periodically. Ingested arsenic has been well documented to be associated with the development of peripheral vascular disease, ischemic heart disease and cerebrovascular accidents in a dose-response relationship in the endemic areas of arseniasis in southwestern Taiwan. A biological gradient in prevalence of carotid atherosclerosis with increasing exposure to ingested arsenic has also been observed in the same arseniasis-endemic area. Both diabetes mellitus and hypertension are important risk predictors of atherosclerotic diseases. Several epidemiological studies have been carried out to examine the association between long-term arsenic exposure and the development of diabetes mellitus and hypertension. These include community-based and hospital-based studies in both high and low arsenic exposure areas, as well as occupational studies of arsenic exposures from various sources.

A recently published review article has concluded that an increased prevalence of diabetes mellitus and hypertension has been consistently observed among residents in the high arsenic exposure areas in Taiwan and Bangladesh, showing a dose-response relationship with arsenic level in drinking water. Inconsistent findings have been reported from occupational studies and community-based studies in low arsenic exposure areas, which might be biased by the inaccurate measurement of arsenic exposure and health outcome, inadequate number of study subjects, and limited control of confounding factors.

The low dose effect of arsenic on diabetes and hypertension needs further validation from cohort follow-up studies in populations with environmental or occupational exposure to arsenic. Future studies should have the following characteristics: 1) an accurate diagnosis of diabetes mellitus using the fasting plasma glucose test; 2) a precise estimation of total arsenic burden from dietary, environmental and occupational sources during the entire exposure period; 3) a large sample size to ensure an adequate statistical power of the study; 4) an extended duration of follow-up long enough to allow the development of the chronic diseases; 5) an extensive use of biomarkers to assess integral arsenic exposure over time, arsenic methylation capability, and genetic susceptibility to arseniasis; 6) a comprehensive control of possible confounding variables; and 7) an intensive analysis of interaction between age, quantity and duration of exposure, and other risk factors for the chronic diseases under investigation.

In summary, high concentration of arsenic in drinking water is an important risk factor to induce diabetes mellitus and hypertension. The low dose effect and toxicological mechanism need further investigations.

Source: Toxicology and Applied Pharmacology, Vol. 222, August 2007.

EFFECTS OF AMBIENT PARTICLE AIR POLLUTION ON HUMAN HEALTH: A STUDY CARRIED OUT IN WUHAN, CHINA

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In Asia, limited literature has been published on the association between daily mortality and ambient air pollution. Now, a new study has examined the associations of daily cause-specific mortality with daily mean concentrations of PM_{10} in Wuhan, China using 4 years of data (2001-2004). There are approximately 4.5 million residents in Wuhan who live in the city core area of 201 km^2 where air pollution levels are higher and pollution ranges are wider than the majority of cities in the published literature. Researchers used quasi-likelihood estimation within the context of the generalized additive models (GAMs) (natural spline models in *R*) to model the natural logarithm of the expected daily death counts as a function of the predictor variables. They found consistent PM_{10} effects on mortality with the strongest effects on lag 0 day. Every 10 $\mu g/m^3$ increase in PM_{10} daily concentration at lag 0 day was significantly associated with an increase in non-accidental (0.36%), cardiovascular (0.51%), stroke (0.44%), cardiac (0.49%), respiratory (0.71%), and cardiopulmonary (0.46%) mortality. In general, these effects were stronger among the elderly (≥65 years or ≥45 years) than among the young. The exploration of exposure-response relationships between PM_{10} and cause-specific mortality suggests the appropriateness of assuming linear relationships, where the PM_{10} concentration in Wuhan ranged from 24.8 to 477.8 $\mu g/m^3$. The study concludes that there is consistent evidence of acute effects of PM_{10} on cardiopulmonary mortality. A linear no threshold exposure-response relationship is suggested between PM_{10} and the studied cause-specific mortality.

Although researchers were unable to exclude the role of bias and confounding completely in this study, chance, bias, and confounding are unlikely alternative explanations of the observed associations between the daily ambient air pollution levels and the cause-specific mortality in this study.

Source: Environmental Research, Vol. 105, November 2007.

EFFECTS OF EXPOSURE TO DIESEL OIL ON THE NEOTROPICAL FISH PROCHILODUS LINEATUS

Among the different types of pollutants, petroleum products are one of the most relevant to aquatic ecotoxicology. In freshwater ecosystems, one of the largest oil spills occurred in 2001 in Barigui River, in Paraná, southern Brazil, when 50,000 L of crude oil were accidentally discharged. Although these kinds of large oil spills are widely covered in the media, it is believed that the principal source of inland waters contamination from petroleum and its derivatives is due to small and continuous leakages from underground bulk storage tanks, thereby reaching ground water and later rivers. However, little research has been done on the effects of petroleum products on tropical freshwater organisms.

Exposure to crude oil and derivatives can induce a variety of toxic symptoms in experimental animals. Petroleum hydrocarbons can act as a mediator in free radical generation in fish. Studies with the goldfish *Carassius auratus* have shown an increase in antioxidant defenses in animals after exposure to different concentrations of the water-soluble fraction of diesel oil (WSD) for various experimental times. Other studies have also indicated that the exposure of fish to a water-soluble fraction of petroleum derivatives causes different effects in cortisol plasma concentrations, suggesting that these contaminants might interfere in the fish stress response.

Some authors have shown a relationship between exposure to petroleum hydrocarbons and hemolysis and/or hemorrhage while others have observed an increase in hematocrit of fish exposed to a WSD. Some studies have also shown structural damage to organs and tissues related to the exposure of fish to petroleum derivatives.

Despite these previous investigations carried out on petroleum derivatives effects on fish, some toxicological response levels in fish remain poorly understood, revealing the lack of data regarding the stress mechanism, as well as biotransformation and genotoxic responses. In particular, there are only few reports concerning the effects of diesel oil exposure on morphological and physiological parameters in freshwater fish and there is a real need of information about the effects of this fuel oil on neotropical freshwater fish species.

The fish species *Prochilodus lineatus* is native to the south and southeast regions of Brazil and represents a well suited species to environmental monitoring as it is a bottom feeder fish which is in contact with xenobiotics in water and in sediment and also has been shown to be sensitive to variations in water quality.

Thus, considering the growing cases of environmental accidents involving spills of petroleum distillate products into continental waters in the last years in Brazil, a new study has been conducted to investigate biochemical, physiological, and histopathological parameters of *Prochilodus lineatus* exposed to diesel oil as potential biomarkers to assess pollution by these petroleum products and accordingly to get information about the threat imposed by these spills to this neotropical fish species.

In the study, toxicity tests were conducted simulating a diesel oil spill in a tropical environment and juveniles of *Prochilodus lineatus* were exposed to WSD for 6, 24, 96 h, and 15 days. The results showed the activation of biotransformation pathways for xenobiotics, through a time-dependent increase of liver glutathione-S-transferase (GST) activity. The liver can be considered a target organ and of great importance to fish, since it participates in processes such as the biotransformation and excretion of xenobiotics.

In the present study, fish exposed to WSD showed diverse hepatic alterations, some of which can be considered responses to a stressor agent, since they indicate functional activation of this organ.

In summary, the results obtained in this study showed clearly that the WSD can cause important alterations in *Prochilodus lineatus,* at the biochemical level up to the tissue level. At the biochemical level, there was activation of phase II biotransformation pathways for xenobiotics, through a time-dependent increase of GST. It could be shown that WSD causes a decrease in hematocrit and hemoglobin content, very likely due to hemolysis. Furthermore, an increase was observed in glucose levels after acute exposure to WSD, probably mediated by catecholamines. A possible lack of cortisol response could also be associated with WSD, since a reduction in plasma cortisol was seen in fish exposed to the petroleum derivative for 15 days; this effect can lead to the compromise of adaptive stress responses. Moreover, the occurrence of lesions in the gills and even more severe lesions in the liver, should lead to functional damage to both organs, thus interfering directly with fundamental processes for the maintenance of homeostasis in these fish.

Source: Ecotoxicology and Environmental Safety, Vol. 69, January 2008.

Attenuation of eNOS Expression in Cadmium-induced Hypertensive Rats: A Thai Study

Cadmium (Cd) is widely distributed throughout the environment. Industrial and agricultural uses have released Cd into the environment. Its negative impact on human health is an important public health concern. Most of the Cd released into the environment occurs via the smelting of other metals, the burning of fossil fuels and waste materials, and the use of phosphate fertilizers. The high Cd concentrations in the soil and water supply is one of the indicative factors of its content in meat, organ meat, fish, vegetable, fruits as well as tobacco. Chronic exposure to Cd has been related to various toxic effects such as renal dysfunction, hepatic toxicity, osteoporosis, lung, renal, and pancreas cancers. Moreover, it is known that Cd affects the heart and blood vessels causing cardiovascular diseases such as hypertension, atherosclerosis and cardiomyopathy.

Cd has been reported to be a possible risk factor of hypertension in experimental studies. It has been shown that Cd-produced hypertensive effect in rats following repeated exposure to Cd 1 mg/kg (i.p.; 5 days) caused hypertensive responses in anesthetized rats. Recently, Thai researchers also reported that subchronic exposure to Cd via drinking water for 3 months increased systolic blood pressure about 20-30% of control values in rats, and Cd reduced acetylcholine (ACh)-induced cardiac and vascular responses. However, results from human population studies are still controversial. In one study, the association of human exposure to Cd in arctic Finland in relation to area of residence, blood pressure and arterial hypertensive diseases was investigated. The result showed that high blood pressure was related to high blood Cd concentration in people who live in high Cd contaminated areas. In contrast, another study found that there was no relationship between blood pressure and blood or urinary Cd and incidence of hypertension in the general population living in the northeast of Belgium.

M_3 receptor is an important cholinergic receptor located in vascular endothelium of rat aorta. The binding of ACh to muscarinic receptor present on the endothelial cell membrane enhanced nitric oxide (NO) synthesis by endothelial nitric oxide synthase (eNOS). NO diffuses to vascular smooth muscle cell where it activates soluble guanylate cyclase, increasing cGMP levels and causing smooth muscle relaxation. Therefore, the alteration of cholinergic muscarinic function and the lower NO level in blood vessel may contribute to hypertension.

The specific mechanisms responsible for Cd-produced hypertension have not yet been elucidated but several hypotheses have been proposed including an increase of Na^+ retention, interaction with Ca^{2+} channels, activation of sympathetic nervous system, and a decrease of the concentration of vasodilating substances. However, little is known regarding the role of cholinergic muscarinic function involved in Cd-induced hypertension. Thus, a new study conducted by Thai researchers investigated whether vascular muscarinic receptor and its downstream pathway contributes to Cd-induced hypertension in rats. Since exposure to Cd via food and drinking water is one of the important routes in the general population, the study investigated the effect of long-term Cd exposure via drinking water on vasomotor tone and vascular responses to ACh of aortic rings. The expression of muscarinic receptors as well as eNOS of aorta was also determined in this study.

Male Sprague-Dawley rats were exposed to Cd via drinking water (5, 10 and 50 ppm) for 3 months. Cd 10 and 50 ppm exposure cause significant decreases in the sensitivity of vascular muscarinic receptors to ACh. However, Cd exposure did not alter the vascular relaxation induced by sodium nitroprusside which is a nitric oxide donor. Consistent with the reduction of ACh-induced relaxation, treatment with Cd decreased eNOS protein level in blood vessels. These results suggested that Cd suppressed ACh-induced vascular relaxation by interfering with muscarinic receptor function and its downstream signaling pathway may be one of the contributing factors for the development of hypertension.

In conclusion, this study provides evidence that subchronic Cd exposure suppressed endothelium-dependent vasorelaxation which may be due to muscarinic cholinergic and NO-dependent pathway dysfunction in vascular endothelium. The impairment of vascular relaxation may contribute to Cd-induced hypertension.

Source: Toxicology Letters, Vol. 176, January 2008.

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