Session 5 Human Health

Semen Quality in Relation to Exposure to Currently Used Pesticides

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Background: We previously reported reduced sperm concentration and motility in fertile men in agrarian Columbia, MO (MO) relative to men from urban centers in urban Minneapolis, MN (MN), Los Angeles, CA and New York, NY. The present study addresses the hypothesis that pesticides currently widely used in agriculture in the Midwest contributed to these differences in semen quality.

Methods: Subjects were partners of pregnant women seeking routine prenatal care in MO and MN. To eliminate confounding we selected cases and controls from men with no known risk factors for poor semen quality. We selected men in whom all semen parameters (concentration, % normal morphology and % motile) were low (cases) and men in whom all semen parameters were within normal limits (controls) within MO and MN (sample sizes 50 and 36 in MO and MN, respectively). We measured metabolites of eight non-persistent, current-use pesticides in urine samples provided at the time of semen collection. All pesticide analyses were conducted blind with respect to center and case-control status.

Results: Pesticide metabolite levels were elevated in MO cases compared to controls for the herbicides alachlor and atrazine, and for the insecticide diazinon (2-isopropoxy-4-methyl-pyrimidinol, or IMPY) (P-values for Wilcoxon rank test = 0.0007, 0.012, and 0.0004, for alachlor, atrazine and IMPY, respectively). MO men with high levels of alachlor or IMPY were significantly more likely to be cases than men with low levels (OR=30.0, 16.7 for alachlor and IMPY, respectively), as were men with atrazine over the LOD (OR=11.3). Men exposed to multiple pesticides at elevated levels were at particularly high risk. The herbicides 2,4-D and metolachlor were also associated with poor semen quality in some analyses, while acetochlor levels were lower in cases than controls (P=0.04). No significant associations were seen for any pesticides within MN, where levels of agricultural pesticides were low, or for the insect repellant DEET or the malathion metabolite MDA.

Conclusion: The associations observed between adult exposure to current-use pesticides and reduced semen quality suggests that agricultural chemicals may have contributed to the reduction in semen quality in fertile men from mid-Missouri we reported previously. Because these men were not exposed occupationally, drinking water appears to be a likely route of exposure.

A Focus on Children's Environmental Health

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Introduction

Protecting children's health is an important and necessary goal for all institutions, governments, businesses and individuals worldwide. Environmental factors pertinent to children's health include biological, chemical and physical factors, social factors, geographic characteristics and behavioral, cultural and family influences. Health indicators and chemical risk assessment methods will be discussed to examine the presumption of greater susceptibility or sensitivity of children to toxicants in the environment.

Children's Environmental Health Indicators

The use of health outcome indicators to track trends in children's environmental health will be discussed. In developed countries, children's health has improved considerably over the last 50 to 100 years and life expectancy has risen dramatically. Even over the last 50 years infant mortality, neonatal mortality, and post-neonatal mortality rates have all declined substantially, dropping by some 75% in the US. In many ways, the products of chemistry (e.g., water disinfection, medicinal products, safety materials, pesticides etc.) have played a substantial role in this improvement in health. However, the pattern in developing countries is quite different. Developing countries experience 10-, 20-, even 30-fold higher rates of neonatal mortality. The UN declares that 10-11 million children die each year due to preventable causes: besides malnutrition, the overwhelming causes of child mortality globally today are infectious diseases transmitted through unclean water, vaccine preventable diseases, malaria and respiratory infections. Real improvements clearly can be made in children's health if we can find the means to apply our knowledge and resources to provide water treatment systems, to prevent malaria, to prevent or treat infectious diseases and to continue to develop and disseminate innovative products to improve child nutrition, health, and safety.

Chemical Risk Assessment Methods Applicable to Children

The use of risk assessment to evaluate potential threats to children's health will be discussed. Chemicals in the environment may affect children in different ways than adults because of differences in the physiology of children as well as differences in diet and activity patterns. Risk assessment has evolved as the preferred scientific tool for product safety evaluation and regulatory decision-making. For children, where the potential exists for differences in exposure compared to adults, these differences are evaluated within the exposure assessment component of a child-focused risk assessment. Concerns associated with exposures during critical windows of developmental sensitivity are evaluated within the hazard evaluation component of the risk assessment. Pre-market and post-market laboratory animal toxicity testing and risk assessment paradigms for drugs, food, pesticides, and industrial chemicals provide important and useful information relevant to assessing risks to infants and children, as well as the developing fetus. Such evaluations specifically include exposures of the fetus during gestation and, in the reproduction toxicity tests, evaluation of the postnatal developing organism. These endpoints are particularly relevant for assessing potential hazards to children because the exposure periods evaluated are those associated with critical windows of developmental sensitivity. Recent studies of differential sensitivity of children to chemical toxicity will be discussed. After 6 months of age, children are not usually more sensitive to chemical toxicity than adults, and in many cases children are less sensitive than adults, thus categorical assertions that children are consistently more susceptible to environmental agents cannot be substantiated. Yet as knowledge about children's health, biological systems and environmental factors associated with disease continues to expand, there are many avenues for further research. New testing and evaluation paradigms, such as the High Production Volume Challenge Program, the Voluntary Children's Chemical Evaluation Program tiered approach which incorporates lifestage exposure information and is now being piloted in the US, and research conducted as part of the global chemical industry's Long-Range Research Initiative, further our industry's role in protecting children's health.

Polybrominated Diphenylethers (PBDEs) and Endocrine Disruption

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Brominated flame retardants (BFRs) are comprised of diverse classes of chemicals used in a variety of applications. They are widely used in polymers and textiles, and applied in construction materials, furniture and electronic and electric equipments. Research interest on BFRs has grown since the mid-1990s as emerging chemical classes of environmental concern, in particular the polybrominated diphenyl ethers (PBDEs) have been identified as contaminants of increasing environmental significance. BFRs with the highest production volume are the PBDEs, tetrabromobisphenol A (TBBP-A) and hexabromocyclododecane (HBCD). Total market demand in 2001 for the PBDEs (deca-BDE, octa-BDE and penta-BDE) is estimated at 67,000 tons (deca-BDE being the predominant compound), by region divided as 49% for the Americas, 37% for Asia an 12% for Europe. Because of their persistence and low biodegradation profile, several of the PBDE congeners accumulate in biota and are widely found in the aquatic food chain. This presents a global problem as their levels in tissues of humans and wildlife in Europe, North America and Japan have increased during the last decades, in contrast to *e.g.* PCBs and DDT. Levels of PBDEs in humans are in particular high in North America. Humans may be exposed to PBDEs mainly through consumption of fatty food from animal origin, *e.g.* fish, but exposure through skin contact with textiles protected with flame retardants or through inhalation of BFRs volatilized from electronic and electric equipment may also occur. Levels of PBDEs in Swedish human milk showed a doubling in concentration every five year over the period 1972 to 1997 (2,2',4,4'-tetraBDE being the predominant congener). The levels of penta- and hexa-BDEs increased at the same rate in ringed seals collected in the Canadian Arctic from 1981-2000. PBDEs are potential endocrine disrupters. Disruption of the thyroid hormone system seems to be a sensitive endpoint which may be explained by the structural resemblance to thyroxin. For example, decreased serum T4 levels were shown in mice exposed to a penta-BDE mixture as well as to the 2,2',4,4'-BDE congener (BDE-47). Also in rats, BDE-47 has been reported to reduce plasma level of free T4. In addition, it was found that PBDEs after metabolic activation compete with the thyroid hormone (T4) for binding to transthyretin, the T4 transporting protein. Following perinatal maternal exposure of rats to DE-71 (a commercial tetra- and penta-BDE mixture) reduced serum T4 was measured in the offspring showing its developmental toxicity. Neonatal exposure to PBDE has been found to induce in adult mice neurobehavioral effects, *i.e.*, altered spontaneous motor behavior and reduced habituation responses, disturbances that are induced during a defined critical period of neonatal brain development. In mice, the penta-BDE mixture appeared immunotoxic as shown by a decrease in the thymus/body weight ratio and in the antibody response to sheep red blood cells. Some PBDEs and their hydroxylated metabolites can act as oestrogenic compounds *in vitro*. In addition, *in vitro* data indicate that PBDE congeners show partial Ah-receptor agonist but mainly antagonist activity. PBDEs may be contaminated with polybrominated dibenzo-p-dioxins and dibenzofurans (PBDDs/PBDFs). These compounds as well as their brominated-chlorinated counterparts can also be formed during combustion processes, and can be regarded as equally toxic as the PCDDs/PCDFs. So far, the toxicological profile of PBDEs is too incomplete and insufficient to perform an adequate risk assessment and further information has to be collected (*e.g* in the EU-funded project FIRE; www.rivm.nl/fire) regarding their potential for endocrine disruption.

The Developmental Basis of Disease: Role of Endocrine Disrupting Chemicals

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It is recognized that between two-percent and five-percent of all live-born infants have a major developmental defect. Approximately 40-percent of these defects are thought to be due to the effect(s) of an adverse exposure of a genetically predisposed fetus to intra-uterine environmental factors. It is now clear that in many cases the fetus is more sensitive than the adult to the same environmental insults. Exposure to environmental agents during early development can result in death, structural malformation, and/or functional alteration of the embryo/fetus. While the focus of developmental toxicology has been on understanding the effects of toxicants on malformations, recently the focus has changed to an important and emerging area of developmental toxicology: the effects of *in utero* exposures that cause permanent functional changes that are not overtly, grossly teratogenic yet that result in increased susceptibility to disease/dysfunction later in the life span. This new area of developmental toxicololgy is termed the developmental basis of disease.

Most of the supporting studies in this area have concentrated on grossly altered nutrition *in utero* and its striking influence on multiple aspects of adult health and disease risk including increased susceptibility to heart disease, diabetes and cancers. There is, however, evidence that some environmental agents, especially those with endocrine agonist or antagonist activity, may cause functional deficits that do not become apparent until later in life. In the reproductive tract, the classic example of this phenomenon is the action of diethylstilbestrol (DES). In humans, *in utero* exposure to DES leads to an increase in vaginal adenocarcinoma around the time of puberty. In mice, neonatal DES exposure leads to an increase in uterine adenocarcinoma in adulthood. While the direct connection has not been made between *in utero* programming changes due to DES and later life disease, it is known that DES (in the animal studies) results in altered gene expression in the uterus that is irreversible without any noticeable gross alterations in uterine morphology. Other examples in the reproductive area include developmental exposures of the monkey to androgens that lead to polycystic ovary syndrome-like effects in the adult, data showing a link between *in utero* exposures such as DES, methoxychlor and bisphenol A, and alterations in gene expression in the rat prostate that are irreversible and are correlated with increased prostate cancer and data showing a link between *in utero* exposure to dioxin and endometriosis later in life in primates and rodents. There are increasing data that show a role for *in utero* exposure to environmental estrogens in the etiology of obesity, as well as alterations in the development of the immune system and the nervous system that may lead to increased susceptibility to disease.

Based on the epidemiology data that support the hypothesis of the developmental basis of disease and the preliminary data showing alterations in gene expression and imprinting due to *in utero* exposures to some environmental agents, it is proposed that exposure to certain environmental chemicals as well as altered nutrition, or in combination with altered nutrition, will in some situations, not lead to easily identifiable structural malformations, but instead to alterations in developmental programming expressed as a permanently altered gland, organ or system potential. These states of altered potential would be a result of changes in gene expression, due to altered imprinting, and the underlining methylation-related protein-DNA relationships associated with chromatin remodeling. These effects will occur in a time specific (i.e. vulnerable window) and tissue specific manner and such alterations may be irreversible. The end-result is an animal that is sensitized such that it will be more susceptible to diseases later in life. The environmental insult could act via a one hit or two/three hit scenario. That is, there could be an *in utero* exposure that would lead by itself to pathophysiology later in life or there could be *in utero* exposure combined with a neonatal exposure (same or different compound(s) or adult exposure that would trigger the pathophysiology. The pathophysiology or functional change that results from the exposures/insult could lead to: a) the occurrence of a disease that otherwise would not have happened, b) an increase in risk for a disease that would normally be of lower prevalence, or c) either an earlier onset of a disease that would normally have occurred or an exacerbation of the disease. Finally, the pathophysiology could have a variable latent period from onset in the neonatal period, to early childhood, to pubertal, to early adulthood to late adulthood depending on the toxicant, time of exposure and tissue/organ affected. The effects could potentially be transgenerational. This new approach to developmental toxicology and the study of endocrine disrupting chemicals could have a major impact on our understanding of gene-environment interactions in disease.

Fetal Exposure to Multiple Chemicals: Necessity to Develop a New Risk Assessment and Risk Reduction Method Based on Human Fetus

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Our previous studies analyzing umbilical cords show that human fetuses are exposed to multiple chemicals, including endocrine disruptors (EDs) in Japan (Mori 2001; Todaka and Mori 2002). In animal experiments, it has been shown that EDs have adverse effects on the development and/or the function of the reproductive system, particularly when the exposure occurs during the fetal or neonatal periods. Human fetuses and infants are thought to be significantly more sensitive to a variety of environmental toxicants than adults. It is urgently necessary to establish a new method of evaluating health risk factors to human fetuses of the possible delayed long-term effects caused by prenatal exposure to multiple chemicals. Moreover, it is necessary to develop a method of the risk reduction for future generations. In this presentation, I would like to introduce: 1) a summary of our analysis of human fetal exposure to multiple chemicals, 2) our preliminary toxicogenomic analysis of human umbilical cords for development of a new risk assessment based on human fetus, 3) our attempts of risk reduction by using risk communication to reduce exposure level, and 4) a clinical strategy to reduce accumulation level of persistent chemicals in human body.

Toxicogenomics has been rapidly developing in recent years. It is the study of the genes and their products, which shows the adaptive response to toxicants- and EDs-exposure. Recently, we introduced our attempts to apply toxicogenomic analysis of umbilical cords using DNA microarray to the future risk assessment (Mori et al., 2003; Komiyama & Mori, 2003 in press). Since the umbilical cord is a part of the fetal tissue, it is possible to estimate the effects of chemicals on the fetus by analyzing alteration of the gene expression. So far, we have analyzed the relationship between the concentration level of persistent chemicals (polychlorinated biphenyls [PCBs] and organochlorine pesticides) and the gene expression patterns in umbilical cords of 9 Japanese newborns. Our preliminarly results suggest that the gene expression profile of umbilical cord can be used for the evaluation of exposure levels during the fetal period. Moreover, it might be possibly to use toxicogenomic analysis to detect potential high risk group, because both of the actual high exposure and genetic high susceptibility to multiple chemicals should be regarded as higher health risk to the individual.

As mentioned above, toxicogenomic analysis could be used as a powerful and effective tool for developing a new risk assessment to understand and hopefully to prevent the long-term effects caused by fetal exposure to multiple chemicals. In addition to the establishment of the new risk assessment, it is necessary to develop a risk reduction method to avoid multiple chemical exposure and to reduce the concentration level of persistent chemicals in human body at the same time.

To reduce the risk for the future generations, worldwide cooperation is urgently required.