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INTERNATIONAL CENTRE FOR ENVIRONMENTAL AND INDUSTRIAL TOXICOLOGY (ICEIT)

CRI's ICEIT has been designated as a
"UNEP Centre of Excellence for Environmental and Industrial Toxicology".

HRH Princess Chulabhorn addresses the EUROTOX 2003 Conference

The 41st Congress of the European Societies of Toxicology, EUROTOX 2003, was held in Florence, Italy, from 28 September to 1 October, with the theme “Science for Safety”

In her opening speech to the conference, HRH Princess Chulabhorn detailed the growing concern over the spread of environmental toxicological problems in countries in Southeast Asia, where the rapid increase in the industrial sector in recent years has had harmful impacts on the quality of the environment.

Her Royal Highness told delegates that the most pressing need in Thailand, as in other developing countries, was to produce more qualified toxicologists. Currently, however, there are very few academic or research institutes in Southeast Asia that are in a position to offer training in environmental toxicology specifically tailored to regional needs.

In the scientific sessions of the EUROTOX meeting there were two plenary lectures, one by Prof. P.B. Farmer (Leicester, UK) on DNA and Protein Adducts as Markers of Genotoxicity, and one by Prof. C Bismuth (Paris, France) on Chemical Weapons, Documented Use and Compounds on the Horizon.

On the final day of the conference, there was a Round Table presentation on the topic: “Is Toxicology Protocol Adequate for Drug Evaluation?”

Dangers posed by neurotoxicants during pregnancy

Recent research findings show that mothers exposed to polybrominated diphenyl ethers (PBDEs) are passing the suspected neurotoxicants on to their unborn infants.

A recent study carried out in the United States found that infants not only had the same serum concentrations of these persistent organic pollutants as their mothers, but those concentrations were 20-106 times the levels found in mothers in Sweden, where concern over the chemicals has led to a ban.

PBDEs came into use over the last two decades as flame retardants. They are used in many household items including carpets, furniture cushions, and construction materials. PBDEs are of concern to the public health community because of their structural similarity to polychlorinated biphenyls (PCBs). Once widely used as electrical insulators, PCBs were banned in the United States more than 25 years ago after they were associated with memory and learning impairment, immune disorders, and possibly cancer in humans. Studies show that mice and rats exposed to PBDEs before and shortly after birth suffer permanent learning, memory, and behavior disorders. PBDEs hamper the rats' mental development by reducing thyroid hormone. Researchers believe the chemicals may have a similar effect in humans.

No one is sure just how widespread exposure to PBDEs is in the United States. But several recent studies of scattered populations, including this one in Indiana, suggest that U.S. exposure is among the highest in world. PBDEs have been found in meat, poultry, fish, and dairy products, and people are exposed by eating these foods. Researchers have also found evidence, described elsewhere in this issue, that people inhale PBDEs in household dust.

In the present study, researchers theorized that further PBDE exposure may occur *in utero*. To test this theory, they measured concentrations of PBDEs in 12 paired samples of maternal and umbilical cord blood. They recruited their subjects at two hospitals in Indianapolis. Maternal blood was taken upon admission to the labor and delivery unit; cord blood was taken after delivery.

Maternal blood PBDE concentrations ranged between 15 nanograms per gram (ng/g) lipid and 580 ng/g lipid, with a mean of 118 ng/g lipid. Concentrations in the cord blood ranged from 14 ng/g lipid to 460 ng/g lipid, with a mean of 105 ng/g lipid. The researchers found no statistical difference between the mothers' levels and those of their matching cord blood, indicating that the infants had essentially the same levels as their mothers. The correlation indicates that maternal PBDE levels can be used to predict exposure in neonates.

Source: Environmental Health Perspectives, Vol. 111, No. 9, July 2003.

AGRICULTURAL PESTICIDES AND PROSTATE CANCER RISK

A new study examined the relationship between 45 common agricultural pesticides and the risk of getting prostate cancer for the first time among individuals participating in the Agricultural Health Study (AHS). The AHS is a long-term project designed to evaluate the health experience of farmers, farmer's spouses, and pesticide applicators from Iowa and North Carolina. This report is based on information about all deaths and new cases of cancer that occurred among 55,332 licensed male pesticide applicators who had completed a questionnaire about pesticide use, health history, diet and lifestyle characteristics during the period from 1993-1997. This group included both farmers and commercial pesticide applicators. Information about new cases of prostate cancer came from the state cancer registries in Iowa and North Carolina. Doctors and hospitals in these states are required by law to report all cases of cancer to the registry. A total of 566 new prostate cancer cases were reported for the pesticide applicators in the AHS since they enrolled in the study. This was 14% more cases than expected based on rates of prostate cancer in Iowa and North Carolina.

Most pesticides investigated in this study were not associated with increased risk for prostate cancer. However, methyl bromide, a chemical used as an agricultural fumigant in Iowa, North Carolina and many other states, was associated with an increase in prostate cancer risk. The risk of prostate cancer rose as the total number of days of methyl bromide use increased. This same pattern was seen in Iowa and North Carolina, and among private and commercial applicators. A family history of prostate cancer is known to be a risk factor for prostate cancer. This was also the case in the AHS. Interestingly, it was found that there were six different pesticides that were associated with increased risk of prostate cancer among those who reported a brother or father with prostate cancer. The six chemicals were butylate, chlorpyrifos, coumaphos, fonofos, permethrin, and phorate. These pesticides were not associated with increased risk among applicators without a family history of prostate cancer.

This is the first long-term study with detailed information on pesticide use to evaluate the relationship between use of these chemicals and prostate cancer. Although these findings are intriguing, more work will have to be completed before firm conclusions can be made. The increased risk of prostate cancer from the combined effect of exposure to some specific pesticides and a family history of prostate cancer was somewhat unexpected and needs to be replicated before recommendations can be made. As the applicators in the study get older, researchers expect to see more cases of prostate cancer diagnosed.

Source: American Journal of Epidemiology, No. 157, 2003.

New approaches to the treatment of naturally occurring asbestos

Although the use of asbestos is now banned in buildings in most western countries, asbestos-containing minerals that occur naturally in the soil surrounding abandoned asbestos mines continue to pose an environmental problem.

However a team of Italian researches has found a new treatment for removing such deposits by using soil fungi.

Iron is known to contribute to asbestos toxicity, and its removal reduces *in vitro* cell damage caused by asbestos fibers. The researchers thus reasoned that, if iron could be continuously extracted by means of a natural process from the fibers dispersed in the environment, consequent modifications at the surface of the fibers could result in a decrease in their carcinogenic potential, or even in a full inactivation.

Several soil microorganisms are capable of scavenging iron for their own metabolism from recalcitrant substrates by releasing, in the surrounding soil environment, potent chelators with different metal specificity (for example, polycarboxylic acids and other siderophores). Some ericoid mycorrhizal fungi, which are able to colonize roots symbiotically, have been reported to produce several hydroxamate siderophores known to form very stable complexes with iron (III) ions. Fungi are of particular interest because they can extend through extensive volumes of soil thanks to their hyphal structures. Researchers thus investigated the ability of some soil fungi to grow in the presence of crocidolite (fibrous riebeckite or blue asbestos) fibers, release iron chelators that modify the surface of the fibers at the atomic level, and reduce the potential of the generation of free radicals . A number of fungi with different trophic characteristics were selected because of their ability to grow on silicates, produce siderophores, and tolerate different metals.

In cell-free tests, a 1-mM solution of the strong iron chelator desferrioxamine – a hydroxamate siderophore usually employed clinically in the treatment of hemochromatosis and other diseases related to iron-overload – extracted iron from crocidolite fibers. The amount of iron extracted greatly exceeded what was expected to be at the surface based on the crystal structure of crocidolite. Both iron and silicon were found in the supernatant but with an iron:silicon ratio greater than that in the solid. This suggests that the progressive removal of iron from the bulk of the fiber by desferrioxamine is followed by diffusion of bulk iron ions in the silicate matrix, which eventually leads to collapse of the silicate structure in the outmost layers. The newly formed surface is amorphous and is much less reactive in generating free radicals than the original surface. Also, the amount of iron extracted by the fungal siderophores was greater than what might have found at the surface.

On the basis of the results obtained, researchers believe that fungi might be possible agents for the decontamination of asbestos-polluted soils.

Not only may adhesion of the fibrils on the fungal mycelium limit their dispersal in the specific sites where the fungi grow, but also the action of the potent siderophores could modify the fiber surface, depriving it of the free radical generating sites crucial to triggering of the carcinogenic mechanisms.

Source: Angew. Chem. Int., Vol. 42, No. 2, 2003.

Mercury and the risk of cardiovascular disease

A commonly accepted belief is that a diet rich in fatty fish or fish-oil supplements reduces the risk of cardiovascular disease. However, the results of epidemiologic studies relating fish-oil levels to coronary events have been contradictory, since fish intake is a major source of exposure to mercury, and it has been suggested that mercury may counteract the beneficial cardiovascular effects of fatty acids in fish.

In order to evaluate the association of mercury with the risk of myocardial infarction and to test the hypothesis that high mercury levels may offset the benefits of dietary fish oil, a recent study has examined the joint association of mercury levels and docosahexaenic acid levels with the risk of a first myocardial infarction among a target population of men 70 years of age or younger, from eight European countries.

The subjects were recruited from the coronary care units of hospitals participating in the study. All patients were men with a first acute myocardial infarction confirmed by electrocardiographic changes and elevated enzyme levels.

The controls were men without a history of myocardial infarction, recruited from the population of the catchment areas from which the patients originated, and frequency-matched for age in five-year intervals. In Finland, Israel, Germany, Scotland, and Switzerland, random sampling from local population registers was used to select controls. In Russia and in the two Spanish centers, population registries could not be used, because of the lack of complete census data or because of legal restrictions. Therefore, controls were selected from among hospitalized patients with disorders not known to be associated with dietary factors.

Patients and controls were recruited concurrently during 1991 and 1992. The participation rates among potential subjects were 81 percent for patients and 64 percent for controls. Local institutional review boards approved the study, and written informed consent was obtained from study participants.

Information on smoking, hypertension, and diabetes was collected by standard questionnaires. A history of hypertension or diabetes was based on the patient's report of a physician's diagnosis. A family history of coronary heart disease was defined by a self-reported fatal or nonfatal myocardial infarction in a parent. Clippings from all 10 toenails were collected within eight weeks of enrollment. The mean (±SD) weight of the samples was 53.8± 39.0 mg. A subcutaneous specimen of

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Post-graduate Education, Training and Research Program in Environmental Science, Technology and Management (ESTM) enters into a collaborative research agreement with the Institute of Risk Assessment Science (IRAS) of Utrecht University

On 6 October 2003, at Utrecht University, The Netherlands, HRH Princess Chulaborn, as President of the Chulaborn Research Institute, accompanied by Professor Jean Louis Armand, President of the Asian Institute of Technology, and Professor Pornchai Matangkasombut, President of Mahidol University, took part in the signing ceremony of the Memorandum of Understanding between the inter-university post-graduate program of the three institutions represented and the University of Utrecht.

In her speech delivered to the assembly of dignitaries from the university, Her Royal Highness Princess Chulabhorn emphasized the importance of the occasion for the Thai inter-university post-graduate program since it enabled the program to extend its resources of expertise by working with the Institute of Risk Assessment Science of Utrecht University in collaborative research and facilitating exchange of faculty. Such co-operation was to the mutual benefit of all concerned, and particularly to the students engaged in the program. Her Royal Highness informed the assembly that the establishment of the tripartite program in Thailand was assisted initially by support from the United Nations Development Program which enabled the Chulabhorn Research Institute to spearhead the development of an innovative interdisciplinary curriculum, the design of which has received international acclaim. Indeed, the curriculum that has now been implemented is seen as a model interdisciplinary program that crosses traditional subject boundaries by combining the disciplines of toxicology, environmental technology and engineering. Through the effective pooling of the specialized resources of three leading teaching and research institutions in Thailand, AIT in Environmental Engineering, Mahidol University in Life Sciences and CRI in Environmental and Industrial Toxicology, the program has been able to contribute more effectively to the social and economic development of Thailand and of the Southeast Asian region.

Innovative Post-graduate Program:

Three consortium members (CRI, MU and AIT) have collaborated in launching a new inter-disciplinary program which combines toxicology, technology, environmental engineering and management –

The inter-university post-graduate program on Environmental Toxicology, Technology and Management.

This new post-graduate program is the creation of an innovative program cross-disciplinary in nature. Such a program requires the integration of at least 3 major disciplines, which are toxicology, biotechnology, and environmental engineering technology. Expertise and resources in each of these disciplines incorporate the strengths of the participating institutes.

Program Objectives

1. To develop a center of excellence in the area of environmental sciences, toxicology, technology, and management for human resources, and research development through 6 post-graduate programs and research.

2. To strengthen and enhance the endogenous capability of the paticipating institutes/universities through the staff development program and the short term capacity building programs designed and taught by a team of international and local experts.

Strategic Planning

(1) Provide research-led education in member programs with the Inter-University (joint CRI/MU/AIT) Program serving as a core to facilitate the transfer of knowledge and technology through distinguished visiting faculty.

(2) Strengthen and integrate the activities of member programs through cross-registration of courses and joint research.

(3) Maximize consortium resource utilization by sharing laboratory facilities and using the Inter-University Program for staff development.

(4) Achieve research focus by adhering to a comprehensive research plan.

(5) Strengthen research capability through collaboration with universities or research institutes in the developed world and with industry.

(6) Transfer knowledge and technology to participating institutes, and private and public-sector personnel through short-term training and distance learning.

Short-term Training Program

A short-term training program has been initiated and operated by the Chulabhorn Research Institute. This program is designed to increase the national capacity to manage environmental toxicological and health problems through the development of human resources in the government and private sectors.

The training program comprises a series of training courses and workshops, taught by world-renowned international experts. Training modules and teachers guide to assist trainers are also prepared.

Research Program

All members emphasize research-led education, and the center provides research grants to member programs for research conducted to satisfy thesis requirements.

Comprehensive research has been designed to integrate knowledge and expertise of members to address problems in many aspects.

In the first phase the center has selected 2 groups of chemicals: petrochemicals and heavy metals, to conduct in-depth study on the effects on health, the environment, and ecosystems; on technology (including biotechnology) for treatment detoxification and remediation; and on assessment of risk. Fundamental research at molecular and cellular level as well as applied research have been included.

The Center for Environmental Science, Technology and Management

The principal role of the Center is to contribute to sustainable national development through human resource development and research.

1. A major contribution to human resource development to support sustainable development at a national level will be made by both M.Sc. and Ph.D. programs which will produce the highest caliber of graduates with a level of expertise and training that will add significantly to the human resource capacity of the nation. It is envisaged that the center will produce at least 350 M.Sc. graduates and 95 Ph.D. graduates.

2. The level and nature of the research undertaken on the programs, strengthened by cooperation with government and private sectors, focuses on the needs of sustainable national development in all areas of the economy. Research projects already in progress aim to increase the country's economic potential and make an important contribution to Thailand's competitiveness at a global level.

The success of the Center for Environmental Science, Technology and Management and its graduate programs depends on the quality of research undertaken by the faculty and students which should not only fit with academic and individual research interest but should also serve the country's needs and development.

Research which addresses national problems and has national social and economic impacts will require a well defined objective and set directions which are normally multidisciplinary in approach.

The center recognizes that its members constitute a diverse group of graduate programs with a wide-ranging technical focus and expertise.

Comprehensive Research Project

Comprehensive research has therefore been designed to integrate knowledge and expertise of members to address environmental toxicological problems of the country resulting from the use of chemicals. These research programs cover in-depth study of the effects of chemicals on human health, the environment and the ecosystem, on technology (including biotechnology) for treatment, detoxification and remediation, and on assessment of environmental and health risks. Fundamental research at molecular level as well as applied research are carried out.

The first phase of comprehensive research has initial focuses on two major topics which have high impact on environmental quality and health. These are problems resulting from industrial chemicals, such as heavy metals and petrochemicals.

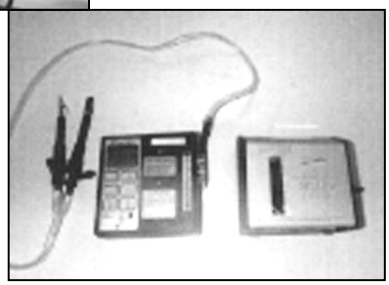
Heavy Metals

Heavy metal contamination and pollution of the environment are major environmental problems for Thailand. Heavy metals released from tanning and electrical industries have contaminated soil and water resources which in turn affect health and agricultural products. The mining industry also releases significant amounts of heavy metals into the environment. The comprehensive project on heavy metals aims at understanding the effects of environmental pollution by heavy metals on health and ecosystems. In addition, technologies to monitor environmental contamination, metals remediation, and waste reduction are covered in the comprehensive projects.

Petrochemicals

The second area of important environmental research deals with the effects of petrochemicals on the environment. Pollution from petrochemicals in urban areas is a major health hazard. In addition, the development of the petrochemical industry has resulted in increasing environmental pollution by organic compounds derived from oil. The comprehensive research project addresses the environmental health effects and remediation techniques of petrochemical pollution.

Monitoring of benzene in the atmosphere of a gas station



In the future, other important environmental problems could be added to those on the existing list of comprehensive projects. These topics might include pesticide usage and disposal, industrial chemical wastes, and industrial solvents. Research through these comprehensive research programs should have significant impacts on science and society and the sustainable economic development of Thailand.

EFFECTS OF THE OTOTOXICITY OF STYRENE IN THE WORKPLACE

Styrene is an aromatic hydrocarbon, commonly used in the manufacturing of polyester laminates, polymers, and copolymers in the yacht industry. The solvent is absorbed through the airways and the skin. Animal studies indicate that an exposure to styrene leads to damage of hair cells in the basal and lower middle turn of rat cochlea and to hearing loss within moderate frequencies (8 and 16 kHz), as assessed by auditory brainstem evoked potentials. Prolonged, simultaneous exposures of rats to both styrene and noise increase hair cell loss and severity of hearing loss when compared to isolated exposures. Moreover, the ototoxic effect is more severe than what would be expected from a theoretically calculated sum of isolated exposure effects, implying a synergistic action of noise and styrene on hearing. Enhanced ototoxicity of styrene also occurs in coexposures to other chemicals.

Studies on occupational styrene-related hearing loss in humans are scarce, and the results less clear than those obtained from animal studies.

Now, however, a Polish study has evaluated the risks of developing hearing loss and the severity of hearing impartment in workers in the marine craft building industry exposed to styrene alone or to the compounded effects of styrene and noise.

The study included employees of four yacht yards and one plastics factory, exposed at their workplaces to a mixture of organic solvents, having styrene as the main compound. A small subpopulation of workers was coexposed to styrene and toluene – another chemical of confirmed ototoxicity in animals. In addition, at some workplaces the subjects were exposed to noise that exceeded the Polish Occupational Exposure Limit.

All employees exposed to solvents for at least 6 months were interviewed according to a questionnaire, before undergoing ear, nose, and throat examinations and evaluations by impedance audiometry.

The questionnaire included detailed inquiries on present and previous employment exposure to solvents and noise, medical history, physical features, lifestyle, military service, and exposure to ototoxic factors beyond occupational environment. Medical history evaluated signs and symptoms of auditory and vestibular disorders, past middle ear diseases and surgery, hereditary disorders, chronic systemic diseases, cholesterol levels, arterial hypertension, head trauma, and current and past medications having an ototoxic potential. The occupational history emphasized the changes in the duties performed, place of work in given conditions, and application of individual protection on a regular basis (type, availability, and actual application) along with a detailed analysis of exposures to noxious factors in former places of work.

An individual dosimetry method was used to evaluate current occupational exposures to all toxic compounds in the work environment, including organic solvents.

Depending on the type of exposure, the entire population of 513 persons was divided into the 6 following subgroups: exposed to styrene only; exposed to noise only; exposed to styrene and toluene; exposed to styrene and noise; exposed to styrene, toluene, and noise; and unexposed to either noise or solvents.

The subgroup exposed to styrene and noise did not differ significantly from the noise-only exposed subgroup, except for the frequency of 8 kHz. At this particular frequency, mean hearing threshold was significantly higher in the noise and styrene subgroup.

Similar to the entire group, the subgroups that were exposed only to solvents (styrene and styrene and toluene) in the multiple linear regression analysis, (including age, past noise exposure, and styrene exposure as variables), showed a positive linear relationship, not only between age and hearing thresholds but also between the averaged working life exposure to styrene concentration and hearing thresholds.

The results the study show that the odds ratio of workers' developing hearing loss related to styrene exposure is almost four times higher than for unexposed subjects and that the resultant hearing losses cover a wide range of frequencies. Previous clinical and epidemiological investigations have focused on the extent of hearing loss in persons exposed to styrene in industry and the relationships between those exposures and the central auditory and vestibular disorders.

The synergistic, or at least additive, effect of the combined exposures to styrene and noise on the profoundness of hearing loss is supported by currently available experimental data in animals. Simultaneous exposure of rats to styrene and noise caused threshold elevation and hair cell loss. These exceeded the sum of damaging effects from noise and styrene alone and were within the range from 8 kHz to 16 kHz. The damage caused by noise and styrene seems to result from two parallel mechanisms (chemical and mechanical). The noise-induced hearing loss is mainly related to the mechanical injury of hair cell stereocilia, whereas the styrene-induced hearing loss is related to the toxic damage of outer hair cells.

This study indicates that combined exposures to two or more ototoxic agents – such as styrene and noise; or styrene, toluene, and noise – are more ototoxic than an exposure to noise alone. The quantification of the relationship between the exposure level and the severity of hearing loss in humans may be as challenging as it is difficult.

Source: Journal of Environmental Medicine, Vol. 45, No. 1, January 2003.

ADVERSE EFFECTS OF BENZENE

Benzene is used in fuels, as an industrial solvent, and in other manufacturing applications, and is also found in cigarette smoke. Human populations generally are exposed through polluted ambient air or contaminated water. Benzene is known to cause hemato-toxicity and blood tumors in humans and mice. Studies so far have focused on benzene's carcinogenic and genotoxic metabolites, which cause various types of tumors in a number of mouse organ systems. Hepatic enzymes convert inhaled benzene into genotoxic metabolites. A number of these benzene metabolites (primarily phenol, hydroquinone, catechol, and trans-trans muconic acid) actually intensify the chemical's toxic effect on an organ.

Past studies have suggested that benzene's toxic effects on bone marrow tissue – its major target organ – may be enacted through multiple pathways, including growth factor regulation, oxidative stress reduction, DNA damage repair, cell cycle regulation, and apoptosis. Also, genetic variations may upset the cellular-environmental homeostasis that protects bone marrow cells from toxic effects such as those caused by benzene, resulting in altered gene expression.

Arguing that only studying a few specific genes may be insufficient to thoroughly explain the complex molecular mechanisms of benzene-induced hematotoxicity and leukemogenicity, a group of researchers from Japan and Korea have now conducted broad cDNA microarray analyses using multiple gene expression profiling technologies.

The team analyzed mouse bone marrow tissue during and after exposure to 300 parts per million benzene over a 2-week period for 6 hours a day, 5 days a week. Two types of C57BL/6 mice were used – standard wild-type mice possessing the gene for p53 and p53-knockout mice. The mice were randomly grouped into control and benzene-exposed groups.

Twice during the exposure period and then 3 days after the full 2-week exposure, the researchers collected bone marrow from both femurs of each mouse in each group. RNA was extracted from this tissue and used to synthesize cDNA, which was then hybridized onto a microarray chip. The resulting array of gene fragments was scanned as a digital image and analy-zed using software that searched for clustering genes specifically expressed and/or suppressed in each group.

The researchers found that benzene caused DNA damage in cells during all phases of the cell cycle. In the benzene-exposed wild-type mice, DNA repair genes were activated, but they were suppressed in the p53-knockout mice. Mice in the latter group were therefore susceptible to benzene's direct genotoxic leukemogenicity, whereas those in the former still experienced epigenetic leukemogenicity via cell-cycle perturbations despite DNA repair.

Besides the p53-mediated pathway, the investigators identified other specific genes that may be involved in G1 cell cycle arrest and apoptosis following benzene exposure, and confirmed that certain repair genes – including the tuberous sclerosis gene and the metallothionein 1 gene – are also triggered by such exposure. They also found that, during benzene exposure, the production of blood cells was arrested due to alterations in the expression of cell cycle checkpoint genes in the wide-type mice. However, production continued in the p53-knockout mice, an important difference that the researchers say could point to mechanisms of benzene's hematotoxicity.

The researchers' cDNA microarray analyses supported the theory that the gene for p53 mediates the effect of benzene on bone marrow tissue by regulating specific genes instrumental in cell cycle arrest, apoptosis, and DNA repair. Because careful simultaneous screening of different expression patterns of many interrelated genes between the two groups is necessary, toxicogenomics should prove extremely useful for future investigations into the toxicity and leukemogenicity mechanisms of benzene.

Source: Environmental Health Perspectives, Vol. 111, No. 11, August 2003.

Mercury and the risk of cardiovascular disease

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adipose tissue was taken from the buttock by needle aspiration. The adipose-tissue sample was taken from patients within seven days after admission to the hospital. A nonfasting sample of venous blood was also obtained. Blood samples were drawn from patients within 24 hours after hospital admission.

Toenail mercury was measured by instrumental neutron-activation analysis. Toenail clippings were irradiated for four hours in a thermal flux of $5x10^{12}$ neutrons per second per square centimeter. After a decay time of 21 days, the gamma radiation of mercury was measured in a well-type Ge(Li) detector for one hour. Irradiation of study samples was conducted from April 1998 through June 1999. Samples from patients and controls were analyzed together, randomly distributed across batches, and masked with respect to case-control status.

Fatty acids in adipose tissue were assayed by gas chromatography. The portion of the fatty-acid peak area containing DHA, as determined by gas chromatography, was calculated and expressed as a fraction of the total fatty-acid peak area. Because the levels of eicosapentaenoic acid (C20:5n-3) in adipose tissue were below the detection limit of the chromatograph for most samples, fish-oil fatty acids were represented exclusively by DHA. The interassay coefficient of variation for DHA in adipose tissue was 25 percent. The serum total cholesterol levels were determined by standard methods.

The results showed that in comparison with the controls, the patients had significantly higher body-mass index and lower high-density lipoprotein cholesterol levels and were more likely to have hypertension, to have diabetes, to smoke, and to have a family history of myocardial infarction. The total cholesterol level was lower among patients than among controls, almost certainly reflecting the effect of acute myocardial infarction. Therefore, total cholesterol was not further considered in case-control comparisons.

Controls from Zeist, the Netherlands, and Berlin, Germany, had the lowest average levels of mercury among controls (0.14 and 0.17 µg per gram, respectively), whereas those from the two Spanish centers had the highest (0.57 µg per gram in Granada and 0.51 µg per gram in Malaga) – a 4.1-fold range of variation. The level of DHA in adipose tissue was strongly correlated with the toenail mercury level. The age- and center-adjusted correlation coefficient between the levels of DHA and mercury was 0.34 (P<0.001).

After adjustment for age, center, and DHA level, the patients had higher mercury levels than the controls (case-control ratio, 1.10; 95 percent confidence interval, 1.03 to 1.18). This association persisted after the exclusion of the two Spanish centers, which were the centers with the highest mercury levels (DHA-adjusted case – control ratio, 1.09;95 percent confidence interval, 1.02 to 1.17), and after adjustment for multiple cardiovascular risk factors (case–control ratio, 1.15; 95 percent confidence interval, 1.05 to 1.25).

Analysis with adjustment for age and center showed an increased risk of myocardial infarction at high mercury levels (P for trend = 0.01). Adjustment for DHA markedly increased the association and elicited a graded, positive dose–response pattern. This trend was further strengthened after adjustment for traditional risk factors and levels of antioxidants, resulting in an odds ratio of 2.16 for patients in the highest quintile of mercury level, as compared with the lowest (95 percent confidence interval, 1.09 to 4.29; P for trend = 0.006). When mercury was introduced as a continuous variable in the regression models, the multivariate odds ratio associated with a change from the 25th to the 75th percentile of the mercury distribution was 1.63 (95 percent confidence interval, 1.22 to 2.18; P = 0.001).

The dose–response curve for the relation between the mercury level and the risk of myocardial infarction was further examined by nonparametric logistic regression. There was a positive, monotonic increase in risk associated with mercury levels above 0.25 µg per gram, which was steeper after adjustment for DHA levels.

The average levels of DHA, expressed as a percent age of the total fatty-acid peak area, were 0.24+0.13 percent in patients and 0.25±0.13 percent in controls. In analyses adjusted for age and center, there was no consistent relation between increasing DHA levels and the risk of myocardial infarction. After adjustment for the mercury level as well, there was a significant trend toward a lower risk of myocardial infarction with higher DHA levels (P for trend = 0.01). This trend was confirmed in the nonparametric analyses. There was no interaction between mercury and DHA with respect to their associations with the risk of myocardial infarction (P for the interaction = 0.61).

In this international case–control study, an independent and graded association was found between toenail mercury levels and the risk of myocardial infarction. Furthermore, mercury masked an inverse association between DHA levels and the risk of myocardial infarction that became evident only after adjustment for the mercury level.

The study thus found that the risk of cardiovascular disease in a population may depend on the balance between n–3 fatty acids and methylmercury in the fish consumed. Exposure to methylmercury is already a concern in specific high-risk groups; the Food and Drug Administration has advised pregnant women and women who may become pregnant not to eat swordfish, king mackerel, tilefish, shark, or fish from locally contaminated areas. The results raise the possibility that this advice should be extended to the general adult population. However, the findings do not imply that people should stop eating fish. The mercury-adjusted analysis is consistent with a protective effect of dietary fish, provided it is not heavily contaminated.

Source: N Engl J Med, Vol. 347, No. 22, November 2002.