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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".

His Majesty King Bhumibol Presided over the Opening of The Fifth Princess Chulabhorn International Science Congress

August 16–20, 2004, Bangkok, Thailand

On August 16, 2004, His Majesty King Bhumibol Adulyadej accompanied by Her Majesty Queen Sirikit, Her Royal Highness Princess Maha Chakri Sirindhorn and Her Royal Highness Princess Chulabhorn presided over the opening of the Fifth Princess Chulabhorn International Science Congress (PC-V): Evolving Genetics and Its Global Impact, an event especially organized to commemorate the sixth cycle of the birth of Her Majesty Queen Sirikit of Thailand, and attended by scientists and eminent guest speakers from over thirty countries.

The 5 day scientific program of the congress included 8 plenary lectures and a closing lecture; 2 round table discussions; 13 symposia; a workshop and a poster session.

In addition to this main program, the congress also featured 3 satellite concurrent workshops and meetings.

These were:

1. The Judicial Institute on Bioscience and Biotechnology
2. Dialogue on Climate—Sustainable Development—Society: Implications and Response
3. IPCS Workshop to expand collaborative research networks among scientists in developing and developed countries in the area of gene-environment interactions among children

Highlights from congress are presented on pages 4 and 5.

NEW MERCURY STANDARDS: PROBLEMS IN DECIDING BY HOW MUCH EMISSIONS SHOULD BE CUT

Mercury can clearly damage the brain, and fetuses are particularly vulnerable. Children who were continually exposed in the womb tend to have developmental delays and learning deficits. The primary route of exposure is through eating fish, which bioaccumulate mercury from their prey. Between 1995 and 1997, the Environmental Projection Agency (EPA) in the U.S.A. ruled that all municipal and medical incinerators—major sources of the toxin entering the food chain—cut their emissions by 90% to 94%.

The net result is hard to quantify because of a lack of long-term monitoring. But findings released in November 2003 are encouraging. This 10-year study of the Florida Everglades showed that mercury levels have declined by as much as 75% in fish and wading birds at half the sample sites. Experts caution, however, that the unique hydrogeology of the Everglades raises questions about the relevance for other regions.

Left unregulated were power plants, which now account for some 40% of overall mercury emissions in the United States. As part of a legal settlement in 1994, EPA agreed to study the hazard of these emissions. In December 2000, the agency categorized mercury as "a hazardous air pollutant" and determined that power plants should be regulated. It also agreed to propose ways to do so by December 2003. That is a tricky task. Scientists are uncertain about important details, from the idiosyncratic chemistry of coal combustion to the myriad reactions that determine when mercury falls from the sky and how toxic it becomes.

Legally, because mercury is categorized as a toxic air pollutant, EPA must propose a rule that requires every power plant to meet a certain emissions standard, as it did with incinerators. Under one of EPA's new proposals, every coal-fired plant would be allowed to emit no more mercury than the cleanest 12% of plants do today. It has been calculated that this

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Effects of low dose exposure to inorganic mercury on disease and mortality in acquired murine lupus

Inorganic mercury (iHg) is known to induce autoimmune disease in susceptible rodent strains. Additionally, in inbred strains of mice prone to autoimmune disease, iHg can accelerate and exacerbate disease manifestations. Despite these well-known links between iHg and autoimmunity in animal models, no association between iHg alone and autoimmune disease in humans has been documented. However, it is possible that low-level iHg exposure can interact with disease triggers to enhance disease expression or susceptibility. In a recent study to address whether exposure to iHg can alter the course of subsequent acquired autoimmune disease, researchers used a murine model of acquired autoimmunity, lupus-like chronic graft-versus-host disease (GVHD), in which autoimmunity is induced using normal, nonautoimmune prone donor and F1 recipient mice resistant to Hg-induced autoimmunity.

In the new study, a relatively low donor cell inoculum (8×10^7), which is just above the threshold of disease induction, was used. As a result, autoimmune features such as serum anti-ssDNA levels and histologic evidence of glomerulonephritis were mild in GVHD mice in the absence of iHg pretreatment, and there was no mortality in this group at 4 months after GVHD induction. This treatment allowed the observation of inhibition or acceleration of disease by iHg. It was found that iHg pretreatment significantly worsened lupus-like disease, as evidenced by earlier onset of proteinuria, more severe histologic features of lupus-like glomerulonephritis, and premature mortality. It is important to note that these results were induced using doses of iHg and a duration of treatment that are substantially lower than those used by most other studies of iHg immunotoxicity.

The results indicate that mercury has very potent interactive effects with autoimmunity at doses considerably lower than those required to induce autoimmunity in susceptible mouse strains that do not develop disease in the absence of iHg.

It is becoming increasingly accepted that the development of autoimmune disease in humans involves a combination of factors, which include the appropriate genetic predisposition and encounter(s) with acquired risk factors in the environment, including infections and immunotoxic agents. The results of the present study support the hypothesis that low-level environmental exposure to Hg is one potential factor in the development of autoimmune disease. Specifically, low-level iHg exposure likely does not induce disease by itself; however, it may lower the threshold for disease development in susceptible individuals who later encounter the appropriate infectious or toxic triggers of disease.

Source: Environmental Health Perspectives, Vol. 111, No. 10, 2003.

A multicenter study of effects of particulate air pollution on subjects with cardiovascular disease

A recent European study to assess the effect of daily concentrations of air pollution and heart rate of 131 adults with coronary heart disease has been conducted in three centers: Amsterdam (The Netherlands), Erfurt (Germany), and Helsinki (Finland).

In each center, a panel of subjects with coronary heart disease was studied for 6 months during the winter of 1998-1999. The study protocol consisted of clinical visits once every 2 weeks, and daily recording of symptoms, and medication use. Subjects who were included in the study had to be free-dwelling nonsmokers and ≥ 50 years of age with doctor-diagnosed coronary heart disease. Subjects with a recent (< 3 months) cardiac event such as myocardial infarction, stroke, coronary artery bypass graft, or percutaneous transluminal coronary angioplasty (PTCA) were excluded from the panels. Other exclusion criteria were unstable angina pectoris and type 1 diabetes mellitus. Subjects were examined by a physician to exclude persons who were too ill, unable to perform the exercise challenge, or likely to have problems with the study for other reasons. The subjects were characterized by a questionnaire and a recording of a 12-lead standard resting electro-cardiogram (ECG).

Blood pressure was measured by a digital monitor, and heart rate was calculated as beats per minute from an electrocardiogram recording with the patient in supine position. Particle concentrations were measured at central measuring sites. Linear regression was used to model the association between 24-hr mean concentrations of particles and blood pressure and heart rate. Estimates were adjusted for trend, day of week, temperature, barometric pressure, relative humidity, and medication use. Pooled effect estimates showed a small significant decrease in diastolic and systolic blood pressure in association with particulate air pollution; a slight decrease in heart rate was found. Of the three centers, Erfurt revealed the most consistent particle effects. The results do not support findings from previous studies that had shown an increase in blood pressure and heart rate in healthy individuals in association with particles. However, particle effects might differ in cardiac patients because of medication intake and disease status, both affecting the autonomic control of the heart.

Researchers conclude that overall there is convincing evidence that particulate air pollution is associated with cardiovascular health. More studies in patients with cardiovascular disease need to be conducted to establish further evidence on how particles affect the control of blood pressure and heart rate in a diseased population and how these effects contribute to adverse health outcomes.

Source: Environmental Health Perspectives, Vol. 112, No. 3, March 2004.

NEW MERCURY STANDARDS: PROBLEMS IN DECIDING BY HOW MUCH EMISSIONS SHOULD BE CUT

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would reduce mercury emissions by 29% by 2007.

EPA prefers a second option, however, which cuts mercury further but takes longer to do so. This plan is a trading scheme, which sets a two-stage cap on overall emissions and cuts them 70% by 2018. Plants that emit less than their allocated amount of mercury may sell pollution credits to those releasing more.

The "cap and trade" rule is modeled on the successful reduction of acid rain. But many scientists say that mercury may behave differently from those air pollutants, most crucially in how far it travels from power plants. Models produce a wide range of results, and some predict that up to 50% of mercury emissions are deposited locally. That raises the concern that if particular plants do not reduce emissions, nearby communities will remain polluted. EPA acknowledges that these so-called hot spots could conceivably occur but notes that states can implement tighter restrictions.

Another worry is that the 15-year deadline would prolong exposure to mercury. Researchers had once assumed that regardless of emissions cuts, fish would remain contaminated for decades because soil and lake sediments contain mercury from 150 years' worth of pollution. Recent research, in addition to the Everglades sampling, suggests more immediate results. An effort called METAALICUS shows that mercury isotopes recently added to an experimental lake in Ontario are much more rapidly converted to a biologically active form—methylated by sulfate-reducing bacteria—than is mercury that has been in the sediment for years. That suggests that cutting emissions could clean up lake waters relatively quickly.

Even so, it Is difficult to establish with any precision what biological benefits will result from a particular cut in mercury emissions.

EPA did not calculate benefits to human health from cleaner fish when it proposed its rules. Instead, the agency looked at the better-understood health benefits from reducing sulfur dioxide, nitrogen oxides, and particulate matter, which would also drop with mercury emissions. EPA will collect comments until at least early March and hold a public meeting before deciding which rule to finalize in December 2004.

Source: Science, Vol. 303, January 2004.

Highlights from the Fifth Princess Chulabhorn International Science Congress (PC-V)

In her report to His Majesty the King at the Opening of PC-V, Her Royal Highness Princess Chulabhorn, President of the Chulabhorn Research Institute and of the Organizing Committee of the congress, set out the reasons for the choice of the theme of the congress: "Evolving Genetics and Its Global Impact".

The 21st Century has seen progress in the new genomic technologies and the unveiling of the entire human genome, which already have contributed greatly to developments in health sciences and biotechnology.

Information on the human genome provides opportunities for studying many diseases of genetic origin, which will revolutionize medical practice and biological research. Accurate diagnostics will be developed for many inherited diseases. In addition, animal models for human disease research will be more easily developed, thus facilitating the understanding of gene function in health and disease.

It is now known that a number of diseases are caused by changes in a single gene. A great number of diseases such as heart diseases, diabetes and several kinds of cancer, however, are even more complex. They are caused by several genes or by a gene interacting with environmental factors.

The mechanisms underlying the etiology of these diseases, hopefully, will soon be uncovered.

In the world of today, we are continuously exposed to a variety of chemicals and hazardous agents in our environment. As a result of such exposure, some individuals may develop diseases, while some may not. Individual susceptibility and risk are affected by polymorphisms of genes and in some instances, environmental factors.

The identification of the genes and their proteins, which are the functional molecules of the genes, will pave the way to more effective therapies and perhaps preventive measures.

In line with these developments, the program of the congress covers 3 main areas.

The first area is *Genetics in Health and Medicine* which focuses on the dramatic impact that genomic research has had on modern medicine and health. The symposia in this area include the following:

Genetics: Individual Risk and Susceptibility

Eukaryotic Cells Genetics and Gene Regulation

Liver Diseases: Epidemiology and Toxicology of Aflatoxin B_1

Gene Therapy

Integrating Genomics with Clinical Research and Therapy in Cancer

Proteomics and Genomics of Human Disease

The second area is *Gene and Environment Interaction.* This area covers the interaction of genes and the environment in the development of diseases. Symposia include *Molecular Biological Techniques in Environmental Health Research; Gene-Environment Interactions; New Directions in Microbial Research; Molecular Epidemiology – Gene Environment Interaction and The Use of Toxicogenomic Data in Risk Assessment*

The third area covers *Genes and Biotechnology,* and focuses on new technologies developed for genomic research. Such technology will also find myriad applications in industry such as in agricultural and medical biotechnology, as well as in providing new directions in microbial research. Symposia include *Post-Genomic Drug Development;* and *Plant Genetics: From Gene to Fields*

Princess Chulabhorn Gold Medal Award 2004

Professor Dr. Her Royal Highness Princess Chulabhorn has instituted the "Princess Chulabhorn Gold Medal Award" to honor and acclaim persons or organizations that are world renowned and have provided outstanding support for the activities of the Chulabhorn Research Institute. Recipients of this most prestigious award were, in 1995, Professor Frederick F. Becker (USA), Dr. Nay Htun (UNDP) and Professor Ronald C. Shank (USA) and in 1999, Professor Paul M. Newberne (USA). This year, 2004, at the closing ceremony of the congress, Her Royal Highness Princess Chulabhorn presented the Gold Medal Award to Professor John M. Essigmann (USA), Professor of Chemistry and Toxicology at the Massachusetts Institute of Technology (MIT). The citation states that Professor Essigmann has made great contributions to fields of molecular biology and environmental toxicology. It was Professor Essigmann and his colleagues at MIT who synthesized the first DNA adduct in

a defined-sequence oligonucleotide, inserted the oligonucleotide into the genome of a virus, and then replicated the virus in a living cell. The result of this work was a new technology for evaluating the mutagenic and lethal properties of defined DNA lesions.

As early as the mid-1980s, Professor Essigmann decided that the tools developed for the study of the genetic effects of environmental carcinogens also could be brought to bear to help develop an understanding of how cells respond to DNA-acting anticancer drugs. Insights from this work have become the platform for a novel class of candidate anticancer agents.

Professor Essigmann's contribution as an educator has been outstanding. At MIT, he has taught at both the graduate and undergraduate levels. In addition to his MIT teaching duties, in Thailand he teaches a course entitled Bioengineering and Environmental Health in the Inter-University Program of CRI, AIT and Mahidol University.

It is for his strong commitment and sustained support to the advancement of science in developing countries as well as for his selfless dedication to teaching and research at the very highest level, that Professor John Martin Essigmann fully merits the honor of the Princess Chulabhorn Gold Medal.

KEYNOTE LECTURE

The keynote lecture at the congress was given by Professor Harold Varmus, a Nobel Laureate and Former Director of the National Institutes of Health in the United States and since January 2000, President and Chief Executive Officer of Memorial Sloan-Kettering Cancer Center in New York City, where the focus of much of his current work is on the development of mouse models for human cancer.

Professor Varmus began his lecture by stating that we have learned a great deal about the genetic basis of cancer over the past 30 years, from a variety of experimental approaches.

A few hundred of our nearly 30,000 genes have been repeatedly implicated in the generation of cancer. We know many of the biochemical and physiological attributes of these genes and their protein products. Mutations affecting these genes—some inherited, most somatic; some acting in a dominant manner, some recessive; some in the form of simple base substitutions, others in the form of chromosomal translocations, amplifications, and deletions—contribute to oncogenesis in different combinations in different cell lineages. Several methods have been developed for detecting these mutations, including rapid sequencing and hybridization methods to detect changes in gene copy number and chromosomal organization. The consequences of the mutations can often be tracked to the cardinal attributes of a cancer cell, including loss of growth control, escape from cell death, instability of the genome, induction of blood vessel formation, and a propensity to invade and spread. Furthermore, recapitulation of human oncogenesis in mouse models has allowed identification of mutations that are required to maintain the tumor phenotype, thereby implicating the mutant genes and proteins as important targets for novel therapies.

The influence of this enormous new body of information has already begun to affect the way cancer is controlled in medical practice. An individual's risk of developing certain cancers can be assessed by seeking inherited mutations in a small number of genes. The classification of cancers can be refined by identifying the mutations promoting each patient's cancer or by examining patterns of gene expression in each tumor. Such subdivisions can lead to predictions about the severity of disease and to choices of therapies,

especially when new therapeutic tools—such as specific antibodies or small molecule inhibitors of oncogenic enzymes—are available.

It is even possible to envision how knowledge of the genotypes and molecular phenotypes of certain cancers might allow earlier detection of cancers and better monitoring of disease progression and response to therapy.

Professor Varmus went on to describe a number of experimental findings, using animal models, cultured cells, and human cancers, that illustrate how the research community has advanced our understanding of cancer and our efforts to control it.

The Judicial Institute on Bioscience and Biotechnology

In recognition of the importance of genetics in the judicial system, the Chulabhorn Research Institute organized this Judicial Institute with the Einstein Institute for Science, Health and the Courts (EINSHAC), U.S.A. in cooperation with the Ministry of Justice and the Court of Justice, Thailand as a satellite meeting in the Fifth Princess Chulabhorn International Science Congress on the theme of Evolving Genetics and Its Global Impact.

In her opening address at this meeting, Her Royal Highness Princess Chulabhorn stated that genetic evidence and various roles of DNA, the molecules of life, will have ever increasing importance in the courtroom. Genetic tests will be offered in evidence in criminal and in intellectual property disputes in virtually any courtroom. In criminal cases, genetic identification is now a commonplace technology, while genetic proof is routinely offered in paternity actions. These tests will soon provide a surfeit of evidence purporting to support medical and non medical cases alike. In criminal cases, when properly used, DNA evidence is a powerful tool in conclusively proving or disproving various arguments and assumptions.

Progress in molecular biology and biotechnology in the last decades has affected all areas of our life. These advances offer scientific, medical, environmental and societal opportunities unmatched in our time. Biotechnology makes it possible to transfer the results of scientific research into products broadly available to the general public. The ability to make useful therapeutic proteins in abundant quantity through recombinant DNA technology saves not only countless lives but also improves the quality of life for many more people. In medicine, advances in DNA technology have made possible presymptomatic and predisposition genetic tests for many diseases.

The huge potential for commercialization, in the saving of human suffering and life, can however create problems and disputes between individuals and for society overall. Differences between parties may well end up in a court of law. Today, judges have the task not only of understanding the complex technical issues involved in derivation of evidence but also of how to pass appropriate judgement.

The scientific processes which increasingly become more and more complex need to be understood by jurists. Thus, there is a clear need for ongoing dialogue between jurists and scientists.

HEALTH RISKS FROM FLAME RETARDANTS

Brominated flame retardants (BFRs) have routinely been added to consumer products for several decades in a successful effort to reduce fire-related injury and property damage. Recently, concern for this emerging class of chemicals has risen because of the occurrence of several classes of BFRs in the environment and in human biota. The widespread production and use of BFRs; strong evidence of increasing contamination of the environment, wildlife, and people; and limited knowledge of potential effects heighten the importance of identifying emerging issues associated with the use of BFRs.

New data presented at the Third International Workshop on Brominated Flame Retardants held at the University of Toronto, Canada, in June revealed that concentrations of a potentially toxic class of substances in flame retardants, polybrominated diphenyl ethers (PBDEs) are three times higher in women's breast milk in Canada than in the U.K. and Germany. However, the Canadian levels are still lower than those in the United States, where PBDE contamination levels have been seen to double every 5 years.

Although human health effects have not been demonstrated, PBDEs are persistent organic pollutants that bioaccumulate; they are known developmental toxins in animals. Use of some PBDEs, typically in electrical appliances and polyurethane foams for furniture, carpets, and insulation, will be banned in Europe this August.

New research results presented in Toronto also linked many BFRs with endocrine pathway disruption and one with thyroxine displacement.

If humans are as sensitive as animals to PBDE-induced developmental toxicity, the current margin of safety appears low for many individuals.

Source: BFR 2004, Toronto, 6-9 June 2004.

Health Effects of Particulate Air Pollution – A study of Heritable Mutation Rates in Laboratory Mice

Air pollution has the potential to affect millions of humans worldwide and has been associated with an increased risk of lung cancer and of genetic damage in other tissues. To investigate whether air pollution induces heritable DNA mutations, researchers have carried out a study exposing sentinel laboratory mice in situ to ambient air for 10 weeks at two field sites: one was located in an urban-industrial area near two integrated steel mills and a major highway on Hamilton Harbour (Ontario, Canada), and the other was in a rural location 30 km away. Comparison of germline mutation rates at expanded-simple-tandem-repeat (ESTR) DNA loci in mouse pedigrees from each site revealed a 1.5- to 2.0-fold increase in mutation rate at the urban-industrial site, providing evidence that air pollution can cause genetic damage in germ cells, inducing transgenerational effects.

However, the study did not identify causative agents or potential approaches for reducing the risk of mutation.

To address these issues, two new groups of sentinel laboratory mice were housed for 10 weeks at the earlier urban-industrial site.

The first group was exposed to ambient air, whereas the second group was housed inside a chamber equipped with a high-efficiency particulate-air (HEPA) filtration system. HEPA filtration removes at least 99.97% of particles 0.3 µm in diameter, and the system used in the study is rated by the manufacturer to remove up to 99.99% of particles down to 0.1 µm. Mice inside the HEPA filtration chamber were therefore protected from exposure to all airborne particulate matter, with the exception of the smallest ultra-fine particles. Simultaneously, third and fourth groups of mice were housed under identical treatment conditions at a rural location, 30 km away, for comparison. Nine weeks after concluding the exposure, germline mutation rates among groups, using pedigree DNA profiling at ESTR loci were compared.

Extensive polymorphism at ESTR loci *Ms6-hm and Hm-2* allowed researchers to determine the parental origin of all mutant bands. The offspring of mice exposed to ambient air at the urban-industrial site inherited ESTR mutations of paternal origin 1.9 to 2.1 times as frequently as the

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The pathophysiologic and molecular effects of arsenical exposure on mouse liver

Recent studies have demonstrated that arsenic acts as a co-promoter with 12-O-tetradecanoylphorbol-13-acetate (TPA) because together they enhance skin tumor development in transgenic (Tg.AC) mice, which overexpress the v-Ha-ras oncogene.

Because hepatic metabolism in Tg.AC mice is not compromised by this overexpression, researchers have hypothesized that organic and inorganic arsenicals produce similar yet distinct changes in Tg.AC liver gene expression that may be predictive of hepatoxicity.

This latter point is important because studies have shown that the liver is a major target organ of arsenic carcinogenicity after *in utero* exposure in mice and in humans exposed to environmental arsenic.

In a new study Tg.AC mice were provided drinking water containing As(III), sodium arsenate [As(V)], monomethylarsonic acid [(MMA(V)], and 1000 ppm dimethylarsinic acid [DMA(V)] at dosages of 150, 200, 1500, or 1000 ppm as arsenic, respectively, for 17 weeks. Control mice received unaltered water. Four weeks after initiation of arsenic treatment, TPA at a dose of 1.25 µg/200 µl acetone was applied twice a week for 2 weeks to the shaved dorsal skin of all mice, including the controls not receiving arsenic. In some cases arsenic exposure reduced body weight gain and caused mortality (including moribundity). Arsenical exposure resulted in a dose-dependent accumulation of arsenic in the liver that was unexpectedly independent of chemical species and produced hepatic global DNA hypomethylation. cDNA microarray and reverse transcriptase—polymerase chain reaction analysis revealed that all arsenicals altered the expression of numerous genes associated with toxicity and cancer. However, organic arsenicals [MMA(V) and DMA(V)] induced a pattern of gene expression dissimilar to that of inorganic arsenicals. In summary, subchronic exposure of Tg.AC mice to inorganic or organic arsenicals resulted in toxic manifestations, hepatic arsenic accumulation, global DNA hypomethylation, and numerous gene expression changes. These effects may play a role in arsenic-induced hepatotoxicity and carcinogenesis and may be of particular toxicologic relevance.

Source: Environmental Health Perspectives, Vol. 112, August 2004.

Health Effects of Particulate Air Pollution – A study of Heritable Mutation Rates in Laboratory Mice

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offspring in any of the other three treatment groups. Mice exposed to HEPA-filtered air at the urban-industrial site had paternal mutation rates that were 52% lower than those of mice exposed to ambient air at the same location. Exposure site and HEPA filtration treatment, as well as the interaction between these two variables, explained a significant proportion of the variance in paternal mutation rates. In contrast, maternal mutation rates were not significantly affected by either exposure site or HEPA filtration. When males exposed to ambient air at the urban-industrial site were mated to unexposed females, their offspring inherited ESTR mutations of paternal origin 2.8 times as frequently as those of rural males mated to unexposed females. In this case, the main effects of site and sex were significant, but there was no significant interaction between the two variables.

HEPA filtration of ambient air therefore reduced ESTR mutation rates at the urban-industrial site, indicating that airborne agents removed by the HEPA filter were necessary for mutation induction.

Human epidemiological studies have associated air pollution exposure with negative health consequences, including cardiovascular, respiratory, and developmental impairments and lung cancer. Identification of the most dangerous air pollutants and their mode of action in producing specific health effects remains uncertain. The present study identifies airborne particulate matter as a contributor to heritable mutation induction in mice; however, a direct link between ESTR mutations and health effects has not yet been established. In addition, although elevated germline mutation rates have been documented in both birds and mice near industrial areas, it is not clear whether these results can be extrapolated to humans. Nonetheless, structural changes in DNA have been detected in human sperm after air pollution exposure. Data from mouse studies suggest that a relationship may exist between mutation rates at ESTR loci and those in coding regions of the genome that affect phenotype. To reduce the potential risk of harmful heritable mutations for humans and wildlife, along with a suite of other health problems, steps should be taken to reduce levels of airborne particulate matter in urban environments.

Source: Science, Vol. 304, May 2004.

New Trends in Herbicide Resistance

Crops that can withstand herbicides have been a major economic success for genetic engineering in recent years. In the United States some 80% of the market in soybeans and cotton is now in plants that tolerate glyphosate, a safe, cheap and potent herbicide. Glyphosate inhibits a key enzyme that plants use to make amino acids and biotech companies have successfully engineered resistance by adding the gene for a similar microbial enzyme that isn't affected.

This technology however, although successful in several crops, has not been accepted by wheat farmers who are concerned that they may have problems marketing their product: flour.

In an attempt to find another way to protect plants, researchers have turned to another technology — one in which a microbial enzyme is used to modify a herbicide called glufosinate.

First, the researchers searched for an enzyme that would detoxify glyphosate. After growing several hundred strains of common microbes, they determined that the most effective was a soil microbe called *Bacillus licheniformis.* The team identified three related genes encoding the enzyme, called glyphosate *N*-acetyltransferase (GAT).

To speed the search for the best enzyme, the researchers fragmented the genes, shuffled the pieces, and added them back to bacteria. Then they selected those more effective at acetylating glyphosate. After 11 rounds of selection, the enzyme was nearly 10,000 times more efficient. In a test of its potential, corn plants were outfitted with the gene. They tolerated six times the concentration of glyphosate that farmers normally apply, with no apparent effect on health or reproduction. Preliminary studies suggest that the enzyme's byproduct is as nontoxic to mammals as is glyphosate.

If this new technology is accepted, it will encourage agbiotech companies to explore even more genetic traits that improve crop production.

Source: Science, Vol. 304, May 2004.

QUANTITATING GENE NETWORKS

Gene expression arrays (gene chips) have enabled researchers to roughly quantify the level of mRNA expression for a large number of genes in a single sample. Several methods have been developed for the analysis of gene array data including clustering, outlier detection, and correlation studies. Most of these analyses are aimed at a qualitative identification of what is different between two samples and/or the relationship between two genes.

Now a new study proposes a quantitative statistically sound methodology for the analysis of gene regulatory networks using gene expression data sets. This method is based on Bayesian networks using gene expression networks.

Many methods have been developed for the analysis of gene expression microarray data, but few methods exist for using these data to quantify the interrelated behavior of genes within gene interaction networks. Most network-based methods are focused on network identification, not quantification. Given a hypothesized gene interaction network, this approach develops and demonstrates the use of Bayesian network models as a tool for the analysis of a network using microarray data. The method allows for evaluating the strength of relationships within a hypothesized network and could also be used to test for additional linkages within the network.

The approach uses known scientific inferences and gene annotation to develop the initial tested network. It can also be applied to evaluating the likelihood of any hypothesized network developed by other approaches.

As such, it can be applied to networks developed using other types of analyses including Bayesian, Boolean, and informatics-based approaches, as well as other known networks in the scientific literature. The ability to test hypotheses in the context of the network and to build modules that can be quantitatively linked to toxicity are first steps in a true systems-biology approach to mechanism-based use of genomics in risk assessment. This analysis is unique in that it directly addresses these uses.

Source: Environmental Health Perspectives, Vol. 112, No. 12, August 2004.

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