



**CRI/ICEIT
NEWSLETTER**

VOL. 14 NO. 3 – July 2004
ISSN 0858-2793
BANGKOK, THAILAND

Chulabhorn Research Institute

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 7TH BIENNIAL SYMPOSIUM OF THE INTERNATIONAL SOCIETY OF ENVIRONMENTAL BIOTECHNOLOGY (ISEB)



This year the biennial symposium was held in Chicago, U.S.A. from 18 to 21 June. The ISEB conference serves as a forum for scientists, engineers, biotechnologists, industrialists and policy makers from various governmental, industrial, and academic organizations and foundations to discuss the progress of basic science of environmental biotechnology, its current and potential industrial applications and the impacts of various biotechnological processes on the environment. As well as the scientific program, the meeting also addressed issues pertaining to international collaboration, environmental justice and the impact of the progress of environmental biotechnology on our society and our legal systems.

In her speech at the opening of the conference, HRH Princess Chulabhorn

emphasized the importance of international collaboration to ensure that developments in science and technology are applied to the benefit of all countries in our global community in order to safeguard and protect our shared environment for all future generations.

Her Royal Highness stated that in applying new technologies we must draw on the expertise of a wide range of professional bodies and international institutions encompassing science, industry, government and the judiciary. Only by incorporating all these elements in the decision-making process can we hope to provide strategies to deal with current concerns and to plan for safe and globally beneficial applications of science and technology in the future.

ENVIRONMENTAL BIOTECHNOLOGY FOR DEVELOPING COUNTRIES: NEEDS AND PRIORITIES

In her main address to the ISEB, HRH Princess Chulabhorn described the environmental toxicological problems facing countries in Southeast Asia.

"Countries in Southeast Asia share many similarities in terms of life-style, diet, climate and geography, ecological systems as well as health and environmental problems. However, our patterns of development over the last twenty years have been somewhat different. Some countries in the region, and I would include Indonesia, Malaysia, Philippines, Singapore, Thailand and more recently Vietnam, have experienced very rapid industrial development. However, other countries in our region and I would include Laos, Cambodia and Myanmar in this category, have not experienced the same degree of industrial development and their economies are still largely agro-based. They are, however, in a good position to learn from the experience of the first group of countries with regard to the negative impacts of chemicals and industry on the environment.

There are clear differences between the two categories of countries in the nature and severity of environmental toxicological problems as well as in the infrastructure and capacity to recognize, solve and manage their problems.

In some countries, environmental management units or agencies are still in their infancy. Environmental standards, quality criteria and legislation are still being developed. Lack of laboratory facilities and trained/qualified personnel are serious problems.

The main environmental toxicological problems that are currently being addressed include water pollution caused by uncontrolled industrial effluents and uncontrolled use of pesticides, which results in toxic substances entering the food chain and causing groundwater contamination.

In the countries which have experienced rapid industrial development in the last twenty years, environmental

management systems are already in place. However, the situation with regard to environmental problems is more complex. The problems of chemical waste from agriculture, mainly pesticides, is now compounded by industrial waste. As a result of the increase in use of chemicals both in agriculture and industry, there is a concomitant increase in potentially hazardous exposure and related occupational and environmental injury.

Industrial wastewater discharged from industrial enterprises is at present inadequately treated. Large amounts of toxic chemicals are discharged without routine monitoring.

Clearly, in all countries in the region there exists an urgent need for appropriate mechanisms and systems for proper management of chemicals. In addition, corrective measures and remediation of environmental degradations are becoming increasingly necessary. Biotechnology can be employed as a tool for treatment and remediation. Application of new technology in waste clean up, waste minimization and remediation are areas that need to be promoted and strengthened. However, in developing countries in Southeast Asia as elsewhere in the world, inadequate infrastructure such as qualified and trained scientists and technological capability limit our possibilities for conducting necessary research.

Transfer of this technology from advanced, industrialized countries will facilitate the development in biotechnology in countries which already have both infrastructure and qualified manpower but it will not be feasible in less developed countries in which human resource development is still the highest priority. Without appropriate numbers of qualified scientists, technology transfer cannot be accomplished. There is an urgent need

for international cooperation among scientists worldwide to promote the development and application of biotechnology in developing countries."

Her Royal Highness went on to describe the research projects in Environmental Biotechnology currently being undertaken at the Chulabhorn Research Institute.

"Environmental biotechnology has for many years been an area of active research at CRI, with a range of research projects from bioremediation of toxic compounds to environmental effects of heavy metal pollutants on soil microbes. The most recalcitrant group of organic compounds is chlorinated benzene derivatives. The presence of chloride atoms slows down the natural biodegradation of these compounds significantly. However microbes have remarkable ability to acquire the necessary genes and pathways to completely degrade these chlorinated compounds. The aim of one project has been to isolate bacteria capable of complete degradation of chlorobenzoate and other chlorinated benzenes from the local environment. This would alleviate the need for extra steps required for optimization of bacterial growth rate and cell mass in local conditions. Furthermore, simple genetic manipulation could result in bacteria with enhanced ability to biodegrade chlorinate compounds, which can be used in the local in situ biodegradation of contaminated areas. Using a simple selection and an enrichment technique, we have isolated a number of *Pseudomonas* species from local soil near waste dumpsites that have the capacity to completely degrade chlorobenzoate and chlorobenzene compounds. The biochemical pathways for biodegradation of these compounds from these locally isolated bacteria are being worked out. We have also applied

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Consumer exposure to arsenic in poultry

Between 1966 and 2000, average annual chicken consumption in the United States jumped from 32.1 to 81.2 pounds per person. Earlier studies have shown that trace elements ingested by chickens such as iron, iodine, and zinc can end up in the chicken meat that humans eat. Scientists at the NIH, and at the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture report that American chicken consumers may be taking in more arsenic than previously suspected.

Arsenic occurs naturally in the Earth's crust, and people are exposed to it in drinking water, dust, and foods. Inorganic arsenic is more toxic than organic forms, and is classified as a carcinogen; studies have linked chronic exposures of 10-40 micrograms per kilogram per day ($\mu\text{g}/\text{kg}/\text{day}$) with skin, respiratory, and bladder cancers. Among foods, seafood, rice, mushrooms, and poultry contain some of the highest reported arsenic levels. Arsenic is an approved feed supplement that farmers use to control intestinal parasites in chickens – especially young chickens ("broilers"), which are more vulnerable to such parasites – provided they wait five days after dosing to slaughter, to allow time for the toxicant to pass through the birds' bodies. Current data suggest that 65% of the arsenic in poultry is inorganic.

Since 1970, the FSIS has monitored meat and poultry through its National Residue Program, mainly in order to determine chemical residue levels in food and prevent contaminated goods from reaching the public. Scientists analyzed the reported arsenic content of more than 20,000 meat samples taken by the FSIS between 1993 and 2000, including more than 5,000 chicken samples. They found that young chickens showed arsenic concentrations 3-4 times higher than those for mature chickens or other meat types: mean levels for young chickens were 0.33-0.43 parts per million. In 1997, broilers represented 99% of chickens consumed.

The researchers used data on chicken consumption from a Department of Agriculture survey to estimate the mean amounts of chicken consumed by the U.S. population at the 50th, 95th, and 99th percentiles. By multiplying the amount of chicken consumed by estimates of arsenic in

chicken muscle (the most popular form of the meat consumed), the researchers estimated the amount of arsenic ingested by the general population and various subgroups.

They calculated that a person consuming an average of 60 grams (about 2 ounces) of chicken per day may be getting 3.52-5.24 μg of inorganic arsenic daily. For a person weighing 70 kg (154 pounds), this breaks down to 0.05-0.07 $\text{fig}/\text{kg}/\text{day}$, well below the joint Food and Agriculture Organization of the United Nations/World Health Organization tolerable daily intake of 2 $\text{fig}/\text{kg}/\text{day}$ inorganic arsenic. But groups that tend to eat more chicken (including

children, people aged 55 and older, and African Americans) may face doses up to 10 times higher, constituting a sizable proportion of their tolerable daily intake.

The arsenic concentrations found in this study lead the authors to conclude that assumptions about the public's exposure to arsenic in food and water might need to be recalibrated by regulatory agencies. These initial reports on arsenic levels may be useful in risk assessments of arsenic exposure and its consequences.

Source: Environmental Health Perspectives, Vol. 112, No. 1, 2004.

Exposure to arsenic through chicken consumption

The ability of trace elements ingested by chickens to affect the dose delivered to humans through chicken consumption has been shown for iodine, iron, zinc, uranium, and potassium. It is thus reasonable to assume that arsenic ingested through chicken consumption may similarly affect the dose delivered to humans.

Indeed a recent study suggests that arsenic concentrations in young chickens may be approximately 3-fold greater than in other meat and poultry products. The higher arsenic concentrations observed in chickens compared with other poultry and meat products is consistent with the use of chicken feed containing additives including arsenic compounds. This prelimi-

nary data analysis can be refined with greater understanding of sources of variation in arsenic concentrations in meat products, for example, regional and seasonal variation. Samples were selected for analysis from plants with varying production levels, and future analyses may require plant estimates to be weighted by production volume in calculating a summary estimate. The present analysis is the first step in progressing from a categorical count of violations and positive tests to a quantitative measure of arsenic concentrations in meat and poultry.

Source: Environmental Health Perspectives, Vol. 112, No. 1, 2004.

Hyperactivity related to intolerance to food additives

Despite initial claims of the detrimental effect of artificial food additives on children's behaviour, there have been no population based studies examining the prevalence of hyperactivity related to intolerance of such additives. Now, however, a study has been carried out to determine the effects of a double blind, placebo controlled, artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children.

The present study used population based screening to identify children with or without hyperactivity and with and without atopy. Children were selected from this population for the dietary challenge phase of a within subject double blind placebo controlled study examining the impact of artificial colourings and benzoate preservatives on hyperactive behaviour. The study was designed to test the hypothesis that food additives have a pharmacological effect on behaviour irrespective of other characteristics of the child.

A sample of 1873 children were screened in their fourth year for the presence of hyperactivity at baseline (HA), of whom 1246 had skin prick tests to identify atopy (AT). Children were selected to form the following groups: HA/AT, not-HA/ AT, HA/not-AT, and not-HA/not-AT (n = 277). After baseline assessment, children were subjected to a diet eliminating artificial colourings and benzoate preservatives for one week; in the subsequent three weeks within subject double blind crossover study they received, in

random order, periods of dietary challenge with a drink containing artificial colourings (20 mg daily) and sodium benzoate (45 mg daily) (active period), or a placebo mixture, supplementary to their diet. Behaviour was assessed by a tester blind to dietary status and by parents' ratings.

The results showed that there were significant reductions in hyperactive behaviour during the withdrawal phase. Furthermore, there were significantly greater increases in hyperactive behaviour during the active than the placebo period based on parental reports. These effects were not influenced by the presence or absence of hyperactivity, nor by the presence or absence of atopy. There were no significant differences detected based on objective testing in the clinic.

These findings suggest that significant changes in children's hyperactive behaviour could be produced by the removal of artificial colourings and sodium benzoate from their diet. The results were obtained in a general population sample with only a modest degree of self-selection. A total of 397 families were invited to enter the double blind food challenge phase. Although approximately one sixth of families did not complete the challenge phase, the completers were no different from the non-completers on any of our baseline measures. Such losses from the study would be expected given the heavy demands placed on these general population families to modify their children's diet over a five week period.

The reduction in hyperactive behaviour that would arise from removal of the additives used in this study from the diet of preschool children are ones that are not related to initial levels of hyperactivity in the child. The child with more extreme hyperactivity showed changes no greater but also no less than other children. The potential long term public health benefit that might arise is indicated by the follow up studies which have shown that the young hyperactive child is at risk of continuing behavioural difficulties, including the transition to conduct disorder and educational difficulties.

This study has shown that the effect of food additives on behaviour occurs independently of pre-existing hyperactive behaviour or indeed atopic status. This is consistent with other studies which have tended to suggest that if food additives have an effect at all, it is via a pharmacological effect which is best exemplified by the non-IgE dependent histamine release. The researchers believe that this suggests that benefit would accrue for all children if artificial food colours and benzoate preservatives were removed from their diet. These findings are sufficiently strong to warrant attempts at replication in other general population samples and to examine whether similar benefits of the removal of artificial colourings and sodium benzoate from the diet could be identified in community samples at older ages.

Source: Arch Dis Child, 89, May 2004.

THE SALE OF POLLUTED WHALE, DOLPHIN AND PORPOISE MEAT IN JAPAN

Approximately 98% of an estimated 5000 tonnes of mercury in the atmosphere is in elemental form. Mercury can travel airborne for up to one and a half years on wind currents before accumulating in bodies of water. Bacteria in water cause mercury to transform to its organic and more toxic state - methylmercury - which is then accumulated by living organisms. Mercury and methylmercury concentrations

increase during the life of an organism (bioaccumulation) and also increase up the food chain (biomagnification). Fish appear to bind methylmercury particularly strongly, resulting in selective enrichment of methylmercury at each successive trophic level. Levels of mercury and methylmercury in large predatory fish and marine mammals can be thousands or millions of times greater than those in the surrounding water.

The ease with which mercury can be transported means that no part of the earth is free from the possibility of contamination. Even remote areas free of mercury related human activities such as the Arctic are contaminated. Due to the slow cycling of mercury between the atmosphere and the ocean, mercury from anthro-

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Health risks from organic contaminants in farmed salmon

The annual global production of farmed salmon has increased by a factor of 40 during the past twenty years. Salmon from farms in northern Europe, North America and Chile are now available at relatively low prices. Salmon farms have been criticised for their ecological impact but previously the potential human health risks from farmed salmon consumption have not been analyzed. Now, however, a new study has measured organochloride contaminants in approximately 700 farmed and wild salmon, totaling over 2 metric tons, collected from around the world.

Contaminant concentrations were compared by analysis of variance. In comparing wild and farmed salmon, farmed salmon were considered as a single group.

In addition, locations at which salmon were farmed were compared by analysis of variance with multiple comparisons of means to test for differences among locations in contaminant levels. In all analyses of variance, the replicate composites from each source were not assumed to be independent observations. Differences between farmed and wild salmon and differences among farming locations were consistently substantial and highly significant.

The data indicate that farmed salmon have significantly higher contaminant burdens than wild salmon and that farmed salmon from Europe are significantly more contaminated than farmed salmon from South and North America. Fish that is not contaminated is a healthy food, high in nutrients, such as omega-3 polyunsaturated fatty acids, that are

known to have a variety of beneficial human health effects. However, this study suggests that consumption of farmed salmon may result in exposure to a variety of persistent bioaccumulative contaminants with the potential for an elevation in attendant health risks. Although the risk/benefit computation is complicated, consumption of farmed Atlantic salmon may pose risks that detract from the beneficial effects of fish consumption. This study also demonstrates the importance of labeling salmon as farmed and identifying the country of origin. Further studies of contaminant sources, particularly in feeds used for farmed carnivorous species such as salmon, are needed.

Source: Science, Vol. 303, January 2004.

The sale of polluted whale, dolphin and porpoise meat in Japan

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pogenic emissions continues to cycle in the environment long after direct releases cease.

A high proportion of methyl mercury can be found in the muscle tissue of marine mammals and it readily accumulates in internal organs with the liver acting as the main repository. Due to their position at the top of the food chain, toothed cetaceans tend to accumulate higher pollutant loads than baleen whales.

Cetaceans have evolved a natural system of mercury detoxification through the binding of inorganic mercury to selenium in the liver. However, it is unclear whether this defence mechanism is able to cope with the greatly increased levels of mercury due to anthropogenic emissions. In a study of stranded dead porpoises on the coast of England and Wales, scientists found higher levels of methylmercury in the livers of those that had died from infectious disease than those that had died of physical trauma. The International Whaling Commission (IWC) has recognised the

threat that pollutants pose to cetaceans and has developed a long-term multi-national research programme to study the effects of pollutants on cetaceans.

Mercury-contaminated whale, dolphin and porpoise products are widely available in Japan's shops and fishmarkets, according to a new report by the Environmental Investigation Agency (EIA).

The report, released in the week preceding the 55th annual meeting of the International Whaling Commission (IWC), provides evidence that Japanese consumers face serious health risks when they eat whale and dolphin products on sale in Japan. Chemical analyses of 58 meat and blubber samples purchased from Japanese supermarkets revealed that government-permitted levels for mercury were exceeded in 62 per cent of products.

Cetaceans are susceptible to accumulating toxins like mercury, as they are long-lived and feed at high trophic levels. Mercury is a potent

neuro-toxin, and scientists have found that even low concentrations can cause damage to nervous systems. Developing foetuses and children are especially at risk.

According to Japanese news articles, the Government of Japan is currently considering warning the public over the potential health problems associated with mercury levels in migratory fish. Yet they've taken no action over the consumption of cetacean products, which can contain mercury levels ten to hundreds of times higher than those commonly found in migratory fish.

EIA is urging the Government of Japan to immediately ban the sale of whale, dolphin and porpoise meat; to issue public health warnings recommending that pregnant and breastfeeding women and children stop eating these products; and to implement a complete ban on the hunting of coastal cetaceans.

Source: <http://www.peopleandplanet.net>.

HEALTH EFFECTS OF AMBIENT PARTICULATE AIR POLLUTION ON A PANEL OF ELDERLY SUBJECTS

There is increasing evidence that particulate matter (PM) air pollution is associated with cardiopulmonary morbidity and mortality.

Epidemiological studies have found elevated PM exposures are associated with specific physiological

outcomes including reduced lung function, increased blood plasma viscosity, reduced heart rate variability and markers of inflammation. Such studies have suggested that certain groups, particularly the elderly, may be more at risk than others for the effects of PM exposure.

On the basis of these indications, a new study has been conducted to further examine the influence of PMs on cardiac autonomic function by studying a panel of elderly persons in three communities in Utah, U.S.

Sources of PM in the communities included substantial traffic and urban-related sources, an integrated steel mill, and local oil refineries. All three communities are located along the Wasatch Front, a relatively densely populated area running north and south along the western front of the Wasatch Mountains. During winter low-level temperature inversion episodes, PM concentrations become elevated as local emissions are trapped in a stagnant air mass near the valley floor. Thus, PM pollution levels in the winter are generally higher and have much greater variability than during other seasons.

Panels of elderly residents of the three communities were recruited to participate in 24-hr ambulatory electro-cardiographic (ECG) monitoring and blood tests. Potential participants were initially recruited by directly contacting persons living in the neighborhoods adjacent to the monitoring sites and asking for neighborhood referrals. Information about the study was given, and for those who indicated a willingness to participate, an eligibility questionnaire was completed. A total of 89 persons were initially enrolled in the study.

All subjects were nonsmokers living in homes with no smokers. Subjects were retired persons between 54 and 89 years of age, and 57% were female. All subjects lived in private homes or were residents of a retirement home without special air filtration systems and had no serious medical conditions that would preclude their participation. Medical conditions that precluded participation included diabetes, renal failure, Parkinson's disease, mental illness, chronic alcohol abuse, treatment with oxygen therapy, abnormal heart rhythm, pacemaker use, implanted defibrillator use, heart transplant, or heart failure within the

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Occupational causes of chronic beryllium disease

Chronic beryllium disease (CBD) is a seriously debilitating, life-threatening occupational disease that occurs primarily among workers engaged in beryllium (Be) extraction and purification and preparation of Be metal and its alloys. High-risk, high-exposure job categories include machining and ceramics production. In addition, certain downstream users are also known to be at risk, for example, dental technicians who file or grind prostheses containing Be alloy. In addition, Be delivered on clothing or contaminated motor vehicles could constitute a risk to family members not actually employed in the Be industry.

CBD is generally recognized to be preceded by immunologic sensitization to the metal, a phenomenon shared with conditions related to a number of metals like gold, silver, nickel, vanadium, cobalt, chromium, and copper.

Occupational or environmental exposures to metals may cause severe health disorders related to modulation of immune homeostasis. Metal immunomodulation has been implicated in chronic inflammatory processes, autoimmune diseases, and related adverse. Molecular mechanisms of immunological actions of metals are largely unknown. Now a recent study has explored a possible link between molecular properties of genetic products conferring susceptibility to Be and the health effects of Be exposures.

As far as the molecular mechanism of CBD is concerned, a previous study has indicated that Be²⁺ may bind in the vicinity of Glu β69, perhaps directly in the peptide-binding groove. In this connection the log-linear dependence of the odds ratios (essentially the probability expressed in the units of background) on the charge is curious because it resembles the Boltzmann distribution in a two-site charge-charge interaction model when all other parameters are fixed. At present, researchers are uncertain whether this is a coincidence or a fact reflecting a yet unknown peculiar mode of ion binding. This puzzle may be resolved in future studies, which will shed additional light on the molecular mechanisms of metal-dependent immunomodulation.

The results of the present study complement recent findings, which implicate positions β55, β56, β69, β84, and β85 of HLA-DPB1 in susceptibility to CBD. This approach may prove useful in predicting risk associated with previously unknown alleles and may help lead to the elucidation of mechanistic issues associated with CBD. Further studies will be directed at identifying the beryllium binding site on the HLA-DP protein.

Source: Environmental Health Perspectives, Vol. 111, No. 15, 2003.

Ex planta phytoremediation: A novel strategy for removal of toxins from soil

Plant roots release a range of enzymes capable of degrading chemical compounds in their immediate vicinity. In a recent study, researchers have developed a system of phytoremediation *ex planta* based on the overexpression of one such enzyme, a secretory laccase.

Laccases catalyze the oxidation of a broad range of phenolic compounds, including polychlorinated phenols such as 2,4,6-trichlorophenol (TCP), that are among the most hazardous and recalcitrant pollutants in the environment. In a recent study, researchers in China isolated a secretory laccase cDNA of LAC1, which is specifically expressed in the roots of *Gossypium arboreum* (cotton). Transgenic *Arabidopsis thaliana* plants overexpressing LAC1 exhibited enhanced resistance to several phenolic allelochemicals and TCP. The secretory laccase activity in these plants was responsible for the conversion of sinapic acid into a mono-lactone type dimer and for the transformation of TCP.

It is not known if the pathway of TCP transformation by LAC1 is similar to that reported for fungal laccases and thus further elucidation of the biodegradation pathway of TCP by this plant laccase will be needed.

For the purpose of phytoremediation, the LAC1 plants represent an alternative approach to existing systems. It has been reported that overexpression of a polyphenol oxidase in tomato plants increases the oxidation rate of soluble phenolics. Laccase also is involved in plant phenolic metabolism. The study found that the soluble phenolics content was slightly lower in LAC1 4-2 seedlings than in the wild type, but the difference was marginal (<5%). The HPLC profiles of soluble phenolics of the LAC1 4-2 and wild-type seedlings were otherwise similar but for sinapoylmalate, which was reduced by 77% in LAC1 seedlings. When exogenous sinapic acid (0.5 mM) was applied to the medium, wild-type seedlings contained two to three times more sinapate esters of both sinapoylglucose and sinapoylmalate, whereas the LAC1 4-2 showed a moderate increase only. Sinapate esters, though a substantial proportion of all phenolic compounds in *A. thaliana*, are dispensable for plant development. It is therefore not surprising that LAC1 plants with a reduced level of sinapoylmalate appear normal.

Laccases catalyze the oxidation of a broad range of phenolic compounds, including the well-studied allelopathic flavonoid catechin. The

role of secretory laccases is not limited to phenolic metabolism within plants. After its release into soil, it helps the plant detoxify the immediate growth environment. This ecological role is exploited here to develop a new phytoremediation system for transforming phenolic pollutants. By detoxifying the direct surroundings of the root system, *ex planta* detoxification provides an advantage over phytoremediation strategies based on plant uptake, accumulation and/or metabolism of pollutants, because the plant cells are not directly exposed to the pollutant. For a large-scale and more effective in phytoremediation, the laccase gene can be transferred into a larger plant, or plants with a high level of secreted laccase activity can be selected. Furthermore, phytoremediation *ex planta* by secretory enzymes can be extended to other types of pollutants in soil, whenever a suitable enzyme is available. The study concluded that engineering plant secretory enzymes provides a novel strategy not only for producing more competitive crops but also for removing toxins from soil.

Source: Nature Biotechnology; Advance Online Publication, June 2004.

HEALTH EFFECTS OF AMBIENT PARTICULATE AIR POLLUTION ON A PANEL OF ELDERLY SUBJECTS

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previous 6 months. Research protocols and consent forms were approved by the institutional review board for human subjects at Brigham Young University. Before entering the study, all participants read and signed consent forms and completed a questionnaire pertaining to background information, medical history, and prescription medications.

Repeated 24 hr. ambulatory ECG monitoring was conducted on the subjects during periods of both high and low air pollution, and immediately after each monitoring period, blood was drawn so that blood cell counts and differential white cell counts could be determined. C-reactive protein (CRP) and whole blood viscosity were

also measured. Regression analysis was then used to evaluate associations between fine particulate matter with an aerodynamic diameter of $\leq 2.5 \mu\text{m}$ ($\text{PM}_{2.5}$) and heart rate variability (HRV), CRP, blood cell counts and whole blood viscosity.

A $100\text{-}\mu\text{g}/\text{m}^3$ increase in $\text{PM}_{2.5}$ was associated with approximately a 35 (SE = 8)-msec decline in standard deviation of all normal R-R intervals (SDNN, a measure of overall HRV); a 42 (SE = 11)-msec decline in square root of the mean of the squared differences between adjacent normal R-R intervals (r-MSSD, an estimate of short-term components of HRV); and a 0.81 (SE = 0.17)-mg/dL increase in CRP. The $\text{PM}_{2.5}$ -HRV associations were

reasonably consistent and statistically robust, but the CRP association dropped to 0.19 (SE = 0.10) after excluding the most influential subject. $\text{PM}_{2.5}$ was not significantly associated with white or red blood cell counts, platelets, or whole-blood viscosity. Most short-term variability in temporal deviations of HRV and CRP was not explained by $\text{PM}_{2.5}$; however, the small statistically significant associations that were observed suggest that exposure to $\text{PM}_{2.5}$ may be one of multiple factors that influence HRV and CRP on thus relate to the harmful effects of PM air pollution on human health.

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

ENVIRONMENTAL BIOTECHNOLOGY FOR DEVELOPING COUNTRIES: NEEDS AND PRIORITIES

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non-recombinant genetic techniques to mutagenize and select from these initial isolates mutant strains with increased capacity to degrade these toxic chlorinated compounds. Research is being conducted to determine if these microbes can readily degrade chlorinate compounds in soil samples.

The second major environmental biotechnology related project involves isolation and characterization of soil microbes highly resistant to a commonly used herbicide, paraquat. Paraquat is one of the most widely used herbicides in Thailand due to its rapid action. However, paraquat often remains unchanged in the environment for long periods albeit in non-active form. Soil immobilization of the herbicide results in the loss of its toxicity. In this state, paraquat remains structurally unchanged. Flooding and water-logged soil could leach out paraquat resulting in contamination of water resources and nearby areas. The original objective of the project is to identify microbes that have the ability to degrade and are highly resistant to paraquat. Although we have not identified any microbe capable of complete paraquat degradation in the initial rounds of screening, three paraquat resistant bacteria isolates were identified. Paraquat toxicity arises from the compound's ability to generate superoxide anions resulting from redox cycling action of the pesticide. Understanding these protective mechanisms will provide insight into how non-targeted microbes tolerate herbicide toxicity. The mechanism could be adapted to protect other organisms from toxic effects of paraquat. Characterizations of these bacteria show that they have enhanced superoxide anion and hydrogen peroxide metabolizing enzyme systems that protect themselves from paraquat toxicity. The levels of superoxide dismutase and catalase, the enzymes that degrade superoxide anions to hydrogen peroxide and to water respectively, are much higher in these bacteria than in other soil microbes. Genes coding for these enzymes have been isolated and they are being characterized at molecular levels to determine the mechanism

responsible for their high levels of expression.

In another study, the effects of cadmium and arsenic on bacterial stress response are investigated. Heavy metals are recognized as hazardous environmental pollutants being released from both industry and agriculture sources. Intensive usage of high phosphate fertilizer in agriculture leads to increased accumulation of metal ions especially cadmium in the soil. Cadmium ions are highly toxic to ecosystems even at very low concentrations.

Our data show that low concentrations of both cadmium and arsenic alter bacterial plant pathogen oxidative stress response by making them more resistant to oxidants. Thus, exposure of the bacteria to low levels of heavy metals from the polluted environment could lead to alteration in bacterial pathogenicity and hence disease development and progression that would have significant economical impact.

Overall, these findings could have important environmental consequences. Reactive oxygen species production and accumulation are part of active plant defense response against microbial invasion.

In another project we have studied pollution of the aquatic environment by chemicals, which has become a critical environment problem worldwide. These pollutants have been shown to alter biochemical and physiological functions of aquatic organisms resulting in threatening the health of such species and eventually posing human health risk. A project at CRI addresses this problem by investigating the effect of cadmium exposure on the metallothionein induction and lipid peroxidation in tilapia fish. The aim of the study is to use fish biomarkers to evaluate the effect of cadmium exposure. The two biomarkers being monitored are the levels of the metal binding protein, Metallothionein (MT) and lipid peroxidation in Tilapia fish. This is also an attempt to evaluate the potential usefulness of these two parameters as a biomarker of exposure and a

biomarker of effect of exposure to cadmium in local fish. Both short and long term studies are being conducted."

In concluding her address, Her Royal Highness stated her firm belief that advances in environmental health science and technology can be exploited to prevent long term disaster and irreparable damage due to environmental negligence. The harmful effects of chemicals can be prevented or at least minimized by research, training and increased public awareness.

By establishing an efficient network of channels of communication among scientists, policy makers, researchers and industrialists, resources and expertise can be harnessed in order to ensure a transfer of knowledge and technology from developed to developing countries in an appropriate and mutually beneficial manner.

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