CRI/ICEIT
NEWSLETTER

VOL. 12 NO. 3 – July 2002
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".

Executive Seminar and Workshop on Environmental Toxicology for Sustainable Development

24 June – 5 July 2002

A program of seminars and workshops held in three countries: the Union of Myanmar, Cambodia and Lao PDR.

The event was organized by the Chulabhorn Research Institute in co-operation with Yangon University and Yangon Institute of Economics; the Ministry of Environment, Phnom Penh and the Science Technology and Environment Agency, Vientiane. This was part of CRI's Human Resource Development Project in environmental toxicology for new member countries and other Asian countries, which is supported by the ASEAN Foundation.

The seminars and workshops focused on the following areas: management of industrial and environmental toxicology for human and sustainable development; environmental and industrial toxicology; specific industrial hazards and their impacts on the environment and health; and risk assessment and management strategies.

For details on the faculty of international experts and their presentations see pages 4 and 5.

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Arsenic in Ground Water

With the rapid increase in the world's population, the demand for water cannot be met only by surface water supplies. This has led to increased dependence on ground water resources in many parts of the world. In India and Bangladesh, the ultilization of ground water has caused serious health problems with an estimated 36 million people in the Bengal Delta at risk from drinking arsenic-contaminated water.

A small number of source materials are now recognized as significant contributors to arsenic in water supplies: organic-rich or black shales, Holocene alluvial sediments with slow flushing rates, mineralized and mined areas (most often gold deposits), volcanogenic sources, and thermal springs. The relationship between high arsenic concentrations and geothermal waters is not a simple one. Arsenic concentrations are high in the thermal waters of Kamchatka, New Zealand, Japan, Alaska, California, and Wyoming, where black shales are common, but they are low in thermal waters from Hawaii and Iceland, where most of the rocks are geologically young basalts. Aquifers with carbonaceous shales and without obvious thermal gradients, such as in Taiwan, also can lead to high dissolved arsenic concentrations.

Two other environments can lead to high arsenic: (i) closed basins in arid to semiarid climates (especially in volcanogenic provinces) and (ii) strongly reducing aquifers, often composed of alluvial sediments but with low sulfate concentrations. Young sediments in low lying regions of low hydraulic gradient are characteristic of many arsenic-rich aquifers. Ordinary sediments containing 1 to 20 mg/kg (near crustal abundance) of arsenic can give rise to high dissolved arsenic (>50 µg/liter) if initiated by one or both of two possible "triggers"---an increase in pH above 8.5 or the onset of reductive iron dissolution. Potentially important, additional factors promoting arsenic solubility are high concentrations of phosphate, bicarbonate, silicate, and/or organic matter in the ground waters. These solutes can decrease or prevent the adsorption of arsenate and arsenite ions onto fine-grained clays, especially iron oxides. Arsenite tends to adsorb less strongly than arsenate often causing arsenite to be present at higher concentrations. Unfortunately, these generalities do not allow prediction of high or low dissolved arsenic concentrations in any particular well because of heterogeneous distributions in the aquifers. Furthermore, arsenic concentrations can change in any given well over the course of a few years so that regular monitoring is required in high-risk areas.

The key to minimizing risk is to incorporate hydrogeological, geochemical, and microbiological expertise into the decision making process of water managers, remediation specialists, and policy makers. The geologic and ground water conditions that promote high arsenic concentrations are known and can help identify high-risk areas. The western United States has many ground waters where arsenic is found in concentrations >10 µg/liter, and treating them will be expensive but may be trivial compared with potential health-care costs. In the search for adequate water supplies and in the absence of adequate information, it is prudent to test selected wells before opening the tap.

Source: Science, Vol. 296, June 2002.

EVIDENCE FOR CAUSAL RELATIONSHIP BETWEEN OXIDATIVE STRESS AND CHRONIC EXPOSURE TO ARSENIC IN DRINKING WATER

Laboratory studies of cells and animals have shown that exposure to arsenic causes oxidative stress, a condition that damages cellular health leading to the development of a number of diseases, but, to date, no examination of this phenomenon in humans has been reported.

Now, however, an international team of scientists, in a research initiative coordinated by the University of Tsukuba in Japan, has come up with evidence that exposure to arsenic in drinking water results in oxidative stress in humans.

The team studied 43 residents in Wuyuan, Inner Mongolia, China. The study subjects, all members of the Han nationality, received their main arsenic exposure through groundwater, having drunk tube-well water for a mean duration of about 18 years.

The high-exposure group included 33 residents of Yindingtu and Shiba, two villages where tests of tube-well water showed that, at 0.41 mg/L, mean concentrations of inorganic arsenic were about 8.2 times higher than China's regulatory limit for drinking water and 41 times higher than the World Health Organization's guidelines for drinking water. These subjects showed various common symptoms of arsenic poisoning, including unusual loss or overgrowth of pigmentation, hyperkeratosis (a thickening of the outer skin), higher rates of peripheral vascular disorder, peripheral neuropathy, and liver swelling. The low-exposure comparison group included 10 residents from the village of Shahe, 35 miles away, who drank tube-well water with much lower arsenic concentrations (0.02 mg/L).

The team found that participants in the high-exposure group had significantly higher levels of lipid peroxide (LPO) in serum---24.0% higher---compared to the low-exposure group. Lipid peroxide is a product of lipid oxidized by free radicals, chemicals that rapidly react with other chemicals, damaging cellular DNA and playing an important role in processes such as mutagenesis, carcinogenesis, and aging. An elevated lipid peroxide profile indicates oxidative stress.

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EVIDENCE FOR CAUSAL RELATIONSHIP BETWEEN OXIDATIVE STRESS AND CHRONIC EXPOSURE TO ARSENIC IN DRINKING WATER

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Another indicator of oxidative stress in the high-exposure group was the subjects' non-protein sulfhydryl (NPSH) mean concentration in whole blood. NPSH plays an important role in protecting against damage by free radicals---approximately 95% of NPSH is the antioxidant glutathione, which can scavenge free radicals, or bring about enzymatic and chemical changes that trap free radicals before they damage DNA and RNA. The high-exposure group showed mean blood NPSH concentrations that were 57.3% of those in the low-exposure group.

The data of this study provide evidence that chronic exposure to arsenic from drinking water results in the induction of oxidative stress in humans, as indicated by the increase of serum LPO. The oxidative stress included by chronic arsenic exposure was associated with high levels of inorganic arsenic (iAs) and its methylated metabolites in blood, as well as with NPSH depletion caused by iAs and its methylated metabolites via different mechanisms.

Source: Environmental Health Perspectives, Vol. 110, No. 4, April 2002.

Association of Blood Arsenic Levels with Increased Reactive Oxidants and Decreased Antioxidant Capacity in Humans

To investigate the effect of arsenic exposure on oxidative stress in humans, a research study was recently conducted on a population in Northeastern Taiwan to determine the relationships of blood arsenic to reactive oxidants and antioxidant capacity at the individual level.

In Taiwan, well water with high arsenic levels is clustered in the Lanyang Basin and in the so-called BFD-endemic (Black Foot Disease) area in southwestern Taiwan. The Lanyang Basin of Ilan County is located on the northeastern coast of Taiwan. The arsenic concentration in well water from the Lanyang Basin area ranges from undetectable to over 3,000 µg/L, and over 50% of surveyed wells contained a level of arsenic below 50 µg/L. Although most of the residents in the Lanyang Basin use household-owned well water as their primary drinking source, arsenic-associated cancers observed in the BFD-endemic area of southwestern Taiwan have not yet been found extensively in the Lanyang Basin. However, a high prevalence of cerebrovascular diseases associated with long-term arsenic exposure has recently been reported in the Lanyang Basin.

The study focused on two villages---Meicheng and Meifu in Chuangwei Township, Ilan County---where residents currently use household-owned water supply wells as their main drinking source. The total population aged 40 years or over in the two villages was approximately 1,000 in 1995. Since then, the population has been regularly followed up for health status. The entire population has spent most of their lives in their respective villages. The subjects for the present study were recruited from this population.

They were first classified into four groups according to the arsenic level in their household well water:< 10 µg/L, 10.1-50 µg/L, 50.1-300 µg/L, and 300 µg/L. Eighty study subjects were grouped by exposure level, each group containing 20 individuals about the same age and equal in sex distribution to those in the other groups. Each subject was scheduled for a health examination in a local hospital. During the hospital visit, each was first asked for consent to join this study. Only study subjects who gave their consent were recruited for blood collection and were given a questionnaire-interview by a nurse in the hospital. All subjects recruited for this study were free of any clinical symptoms such as inflammatory diseases. For each study subject, a 10-mL blood sample was collected into a heparinized and aluminum foil-wrapped blood tube under fasting condition.

Only current users of household-owned well water were included in the study. All subjects were enrolled between November 1997 and May 1998. Arsenic content in whole blood was determined for each study subject.

The data show that arsenic concentration in whole blood of individuals is positively associated with the level of reactive oxidants and negatively associated with the antioxidant capacity level in plasma. The present results, consistent with what was observed in *in vitro* studies, provide evidence that drinking arsenic-contaminated well water may increase the levels of oxidative stress in peripheral blood in humans. The increased level of reactive oxygen radicals in plasma may represent the net result of increased radical production and decreased antioxidant activity. However, in this study, the positive association between arsenic content in blood and oxygen free radicals in plasma was only partially attributable to the inverse correlation of blood arsenic to plasma antioxidants. These results suggest that arsenic digestion in study subjects enhances the formation of oxygen free radicals in plasma and reduces the antioxidant capacity of arsenic-ingesting subjects.

The positive association of reactive oxygen radicals with arsenic content in blood found in this study may explain why arsenic induces both cancers and atherosclerotic lesions at several anatomic sites, as observed previously among residents of the

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Resource persons and program of the executive seminar and workshop on Environmental Toxicology for Sustainable Development which was organized with the support of the ASEAN Foundation in the Union of Myanmar, Cambodia and Lao Peoples' Democratic Republic from 24 June to 5 July 2002 as part of the project in human resource development in Environmental Toxicology for New Member Countries and other Asian Countries by the Chulabhorn Research Institute under the co-ordination of Dr. *Khunying* Mathuros Ruchirawat, Vice President for Research and Head of the Laboratory of Environmental Toxicology at CRI.

Program Coordinator: *Dr. Khunying Mathuros Ruchirawat*

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Day 1

EXECUTIVE SEMINAR: MANAGEMENT OF INDUSTRIAL AND ENVIRONMENTAL TOXICOLOGY FOR HUMAN AND SUSTAINABLE DEVELOPMENT

Impact of chemicals

- Chemicals and development: the critical role of toxicology
- Impact of toxic effects of chemicals: environmental and health effects:-
  1. A case study of negligence: kepone
  2. A case study of accident: dioxin
- Cost of exposure to chemical hazards
- Toxicology and management of industrial chemicals and wastes

WORKSHOP: ENVIRONMENTAL AND INDUSTRIAL TOXICOLOGY

Concepts and principles of toxicology

- Fate of chemicals in the environment
- Fate of chemicals in the body
- Factors influencing toxicity
- The use of toxicology to set safe levels of exposure (standards and criteria setting)
- General discussion

Day 2

Specific industrial hazards and their impacts on the environment / health

- Agro-industrial impact on the environment
- Toxicology of agrochemicals
- Specific industrial hazards – mining (Cu, Sn, Pb, As), textiles, dyeing, metal plating, dry cleaning and wood work (with focus on solvents)
- Specific industrial hazards (continued)
- Chemicals that cause cancer

Risk assessment and management strategies

- Review of approaches to risk assessment
- Managing occupational risk
- Managing environmental risk
- Integrating environmental solutions with industrial development and capacity building to promote sustainable development

Plenary Lecture: The Central Role of Environmental Toxicology in Sustained Economic and Human Development

Dr. Nay Htun
Professor and Executive Director for Asia and the Pacific,
University for Peace New York / Costa Rica

Dr. Nay Htun introduced his keynote lecture as follows:

Economic development has been a priority of every country since the beginning of human organization and civilization.

The process and implementation of economic development brings many benefits to a country and its people.

Jobs and employment are created providing wages and increased financial income for the people; trade is promoted; technical cooperation and technology transfer are fostered; standards of living and quality of life are improved and, importantly, poverty is reduced.

There are many ways and means to attain economic development.

The objective of all countries now is to ensure that the policies and practices of economic development are sustained and the benefits are available to all its people equitably.

Agriculture and agroindustry; forestry and forest products; horticulture; marineculture such as fish and prawn farming; power generation; mining; metal plating; leather tanning; textile and weaving are some examples of industrial processes that contribute to economic development.

Business and commerce also promote economic development, for example: dry cleaning, printing, furniture making, gold and silver smith.

All industrial processes and business practices have the potential to produce pollutants, some in larger quantities than others; some highly toxic, others less so.

For example: agriculture and agro-industry use pesticides, herbicides, fungicides, hormones and antibiotics; metal plating uses schromium; textiles and weaving use dyes; dry cleaning emits solvents.

Hence, all human activities have the potential to form, produce, emit and discharge toxic by-products and hazardous wastes.

Every substance is potentially poisonous. Drinking too much water at one time can be fatal. The size of the dose is one of the major determinants --- a critical factor. The concentration of toxic substances in air, water, soil, food and the ingestion into the human body, depositing in the tissues, blood, lungs, kidneys, for example, have a critical effect on health and well being.

Concerning the importance of human resource development and capacity building in the area of Environmental Toxicology, Dr. Nay Htun stated the following:

Whereas 30 years ago, at the time of the 1972 UN Conference on the Human Environment, held in Stockholm, Sweden, there were around 7 or 8 countries which had offices and departments of the environment, now there is not one country which does not have a ministry or department responsible for the environment.

This evolution is equally true for academia, business, industry and the NGO movement.

Furthermore, whereas 30 years ago there were very few articles on the environment reported in the media, now almost daily in all newspapers of every country there is some report on the environment.

It is now timely to increase attention to the linkages of environmental quality with health, human and economic development.

Capacity Building is a broad generic term that can imply many things.

Increasing awareness, facilitating public involvement and participation, education and training are some of the major means to increase capacities and capabilities.

This Executive Seminar is a means to contribute to capacity building, which should be a continuous process, since knowledge and experience are constantly increasing.

HEALTH EFFECTS OF LONG-TERM EXPOSURE TO FINE PARTICULATE AIR POLLUTION

The convergence of data from a number of studies on particulate matter in air pollution carried out since the 1970s has prompted serious reconsideration of health guidelines and has led to a long-term research program on health related effects of particulate pollution.

Effect of Motor Vehicle Emissions on Respiratory Health

Over the last decade, a number of epidemiologic studies have attempted to examine the relationship between exposure to motor vehicle emissions and respiratory health. These studies are methodologically diverse, using case-control, cross-sectional, and ecologic designs. A variety of health end points have been measured, and a wide range of exposure assessment methods employed. Most studies support a relationship between some measure of respiratory health and some type of modeled exposure. However, few studies find an association for all respiratory health measures studied, and exposure assessment generally limits evidence of association. As a proxy for exposure, studies tend to model either traffic volume on the nearest road or distance to the nearest road.

Now a study has been reported by researchers from the University of Toronto, Canada, on the relationship between proximity to vehicle emissions and respiratory health using a geographic information system (GIS) to model the relationship between chronic exposure to particulate matter < 2.5 μm in diameter ($PM_{2.5}$) from motor vehicle emissions in an urban area and hospital admission rates for respiratory and other conditions.

The results of this study identify an ecologic effect of modeled $PM_{2.5}$ exposure from motor vehicle emissions on the rate of hospitalization for selected respiratory diagnoses. The possibility that this is a causal association is supported by a weaker effect of $PM_{2.5}$ exposure on hospitalization for all respiratory conditions, and by the lack of a similar effect of exposure on hospitalization for non-respiratory (i.e., genitourinary) conditions.

The strength of estimated effect in this study is similar to estimates from individual-level case-control and cross-sectional studies that note an association. Studies that do not find an association tend to use methods of exposure estimation that result in considerable misclassification, although this is not always so.

The researchers found that exposure to $PM_{2.5}$ at the enumeration area level had a significant positive correlation with hospital admission rates for the subset of respiratory diagnoses. They also found a weaker correlation between $PM_{2.5}$ exposure and hospitalization for all respiratory conditions, and no such correlation with hospitalization for genitourinary conditions.

While the use of hospital admission rates is a valid measure of health effects, the researchers point out that these rates probably give a conservative estimate of the health impact of exposure compared with other markers such as disease prevalence estimates or self-reported health status data. Given that most of the respiratory conditions they found to be associated with $PM_{2.5}$ exposure are typically chronic and often ambulatory in nature, and thus do not necessarily involve admission to the hospital, it seems likely that the link may be even stronger than that documented in this study.

Source: Environmental Health Perspectives, Vol. 110, No. 3, March 2002.

In 1997, the US Environmental Protection Agency adopted new ambient air quality standards that would impose regulatory limits on fine particles measuring less than 2.5 μm in diameter ($PM_{2.5}$). These new standards were challenged by industry groups, blocked by a federal appeals court, but ultimately upheld by the US Supreme Court.

Although most of the recent epidemiological research has focused on effects of short-term exposures, several studies suggest that long-term exposure may be more important in terms of overall public health. The new standards for long-term exposure to $PM_{2.5}$ were originally based primarily on 2 prospective cohort studies, which evaluated the effects of long-term pollution exposure on mortality. Both of these studies have been subjected to much scrutiny, including an extensive independent audit and reanalysis of the original data. The larger of these 2 studies linked individual risk factor and vital status data with national ambient air pollution data.

An extensive analysis has now been carried out using the data from the larger study but introducing a number of refinements.

The analysis is based on data collected by the American Cancer Society (ACS) as part of the Cancer Prevention Study II (CPS-II), an ongoing prospective mortality study of approximately 1.2 million adults. Individual participants were enrolled by ACS volunteers in the fall of 1982.

Vital status of study participants was ascertained by ACS volunteers in September of the following years: 1984, 1986, and 1988. Reported deaths were verified with death certificates. Subsequently, through December 31, 1998, vital status was ascertained through automated linkage of the CPS-II study population with the National Death Index. Ascertainment of deaths was more than 98% complete for the period of 1982-1988 and 93% complete after 1988. Death certificates or codes for cause of death were obtained for more than 98% of all known deaths. Cause of death was coded according to the *International Classification of Diseases, Ninth Revision (ICD-9)*. Although the CPS-II cohort included approximately 1.2

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Delays in clinical trials of botulinum vaccine

Recently much attention has been given to the development of new vaccines against possible biowarfare attacks using smallpox and anthrax. However, biodefense researchers have long claimed that botulinum toxin is potentially the deadliest of bioweapons. Botulinum is produced by an anaerobic bacterium that attacks the cholinergic nervous system, causing death by paralysis. Ingesting less than a millionth of a gram can be fatal.

Botulinum vaccine experts in the United States are eager to begin clinical trials on a new botulinum vaccine resulting from research into the use of recombinant technology to genetically engineer yeast factories that produce four partly dismantled botulinum toxins of serotypes A, B, C and F, with serotype E to be added soon. This resulting combination could replace the old vaccine that used modified versions of five toxoids to stimulate antibody protection against five serotypes, and is difficult and dangerous to mass produce.

Although the new vaccine can be manufactured more safely and inexpensively, there have been long delays in getting FDA approval to begin clinical trials. Researchers have been in consultation with FDA for two years planning safety and efficacy tests for all four serotypes; however, permission to conduct the trials has so far been withheld. FDA recognizes the urgency of the situation and in 1999 issued a proposed rule that would allow them to license vaccines and drugs against bioweapons without human efficacy studies.

FDA would base approval instead on substantial evidence from studies in two animal species but would pull such a product from the market if evidence surfaced that it did not work in humans. FDA says it does not know when it will make a final decision on the proposed rule.

Given all the uncertainty about bringing new biodefense vaccines to market and the myriad of federal agencies involved, a radical overhaul of the system seems necessary.

One option, that was discussed a decade ago at the end of the Gulf War but on which no action has yet been taken, is the creation of a dedicated government facility for military and civilian vaccines. Given the present preoccupation with national security in the United States, a decision on this is expected soon.

Source: Science, Vol. 294, October 2001.

Association of Blood Arsenic Levels with Increased Reactive Oxidants and Decreased Antioxidant Capacity in Humans

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arseniasis-endemic area. Arsenic also reduces antioxidant levels in plasma, which may accelerate disease development at target sites. This contention is consistent with observations of previous studies that levels of β-carotene were lower in patients with arsenic-induced skin cancer as well as in patients with ischemic heart disease than in healthy controls.

The study presents evidence that arsenic in blood is not only associated with an increased level of reactive oxygen radicals but is also inversely related to the antioxidant capacity in plasma of humans. The results of this study indicate that arsenic is a significant environmental toxicant that increases the risk of oxidative stress in exposed persons. Persistent high levels of oxidative stress may be a mechanism underlying the carcinogenesis and atherosclerosis induced by long-term arsenic exposure.

Source: Environmental Health Perspectives, Vol. 109, No., 10 October 2001.

HEALTH EFFECTS OF LONG-TERM EXPOSURE TO FINE PARTICULATE AIR POLLUTION

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million participants with adequate questionnaire and cause-of-death data, the present analysis was restricted to those participants who resided in US metropolitan areas with available pollution data. The actual size of the analytic cohort varied depending on the number of metropolitan areas for which pollution data were available.

The findings of this study provide the strongest evidence to date that long-term exposure to fine particulate air pollution common to many metropolitan areas is an important risk factor for cardiopulmonary mortality. In addition, the large cohort and extended follow-up have provided an unprecedented opportunity to evaluate associations between air pollution and lung cancer mortality. Elevated fine particulate air pollution exposures were associated with significant increases in lung cancer mortality. Although potential effects of other unaccounted for factors cannot be excluded with certainty, the associations between fine particulate air pollution and lung cancer mortality, as well as cardiopulmonary mortality, are observed even after controlling for cigarette smoking, BMI, diet, occupational exposure, other individual risk factors, and after controlling for regional and other spatial differences.

Source: JAMA, Vol. 287, No. 9, March 2002.

APPLICATION FOR SCHOLARSHIPS AND FELLOWSHIPS FOR 2002 – 2003 (Post-graduate Education and Training Program)

International Centre for Environmental and Industrial Toxicology (ICEIT), Chulabhorn Research Institute (CRI) is offering the scholarships / fellowships in the following programs:

1) **Post-graduate degree program** in Environmental Toxicology, Technology and Management (M.Sc. and Ph.D. levels) – an Interuniversity Program of Asian Institute of Technology (AIT), Chulabhorn Research Institute (CRI), Mahidol University (MU). This innovative, multidisciplinary program is a combination of health sciences (toxicology), biotechnology and environmental engineering designed to train human resources capable of undertaking control and management of toxic chemicals as well as research and development in the areas of toxicology, technology (including biotechnology) and environmental management.

   Scholarships are available for candidates from selected countries e.g. Bangladesh, East Timor, India, Lao, Mongolia, Myanmar, Nepal, Uzbekistan, Vietnam.

   Applicants should have: (a) an excellent academic record; (b) a very good command of the English language.

2) **Short-term training program** with a series of training courses designed to provide knowledge in 3 major areas, i.e. Fundamental Principles of Toxicology; Application of Toxicology in Regulatory Decision and Practical Methods and Techniques in Analysis of Toxic Chemicals / Pollutants and Testing of Toxicities.

   Forthcoming training course (Bangkok, Thailand):

   - *Training Course on Toxicology of Pesticides and Industrial Chemicals; Occupational Health and Safety* (February 17-21, 2003), course fee US$400, deadline for submission of written application and full Curriculum Vitae (CV) – **December 15, 2002.**

   Fellowships: are available for trainees from ASEAN countries*. Candidates must hold a bachelor degree in biological / medical sciences, chemistry or related areas.

Application: Please send written application along with full CV to:

Chulabhorn Research Institute (CRI)
Office of Academic Affairs
(APPLICATION FOR SCHOLARSHIPS / FELLOWSHIPS)
Vibhavadee-Rangsit Highway
Bangkok 10210, THAILAND
Fax: (66 2) 574 0616

* ASEAN or the Association of Southeast Asian Nations consists of:-
Brunei Darussalam, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam

International Conference on Malaria: Current Status and Future Trends

Date: **February 16-19, 2003**
Venue: **The Convention Centre**
Chulabhorn Research Institute (CRI)
Bangkok, THAILAND

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The ICEIT NEWSLETTER is published quarterly by the International Centre for Environmental and Industrial Toxicology of the Chulabhorn Research Institute. It is intended to be a source of information to create awareness of the problems caused by chemicals. However, the contents and views expressed in this newsletter do not necessarily represent the policies of ICEIT.

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