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IN THIS ISSUE

EDITORIAL

The problem of Arsenic toxicity has acquired great importance in India and Bangladesh due to wide spread exposure of human populations through drinking water. An authoritative review on this subject by Prof. Gebel provides information on the relationship between the chemical form of As and toxicity; methylation and detoxification. Besides pointing out to the limitations of epidemiological studies carried out on chronically exposed populations, the author has also suggested the possibility of arriving at a practical threshold at which the effects may be negligible or insignificant. He has highlighted the need to arrive at practical limits rather than adopting putatively overprotective standards. A brief article by Kaur and Kumar describes in detail the elegant method used for separation of various component of a medicinal plant Terminalia arjuna. This technique may be useful for many others planning to work on plant metabolites. As usual, the first quarter of the year 2001 was the time for the organization of scientific meetings. This issue embodies the report of the XXVI Annual Conference of EMSI held in JNU and the highlights of the IARP International Conference held in Mumbai. Dr. Krishnaja, the Indian participant in the International HUMN project has reviewed the progress made by the group.

It is heartening for me to hear our members appreciation of the Newsletter. Quite a few also want to contribute to the Newsletter. I once again want to stress that we would like to see that the Newsletter will continue to be a media for brief reviews related to mutagenesis and carcinogenesis, announcements of scientific meetings,

(Contd. on Page 2)

two steps via glutathione conjugation to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA).

The assumption that As methylation is a detoxification process was based on the observation that the acute toxicity of MMA and DMA is much lower than it is for inorganic arsenicals (for review see Vahter 1999). Moreover, MMA and DMA are less genotoxic than inorganic arsenicals by two to three orders of magnitude (for review see Gebel 2001). However, there is controversial data as well. DMA, the main metabolite, has been shown to act carcinogenic in animal experiments in higher concentrations (25-400 ppm in drinking water). Most of the studies found a tumor promotor-like action of DMA. In contrast, the evidence to classify inorganic arsenic as carcinogenic in animals is equivocal up to now. However, in a critical analysis of the studies available it was concluded that the so-called lack of animal carcinogenicity of inorganic As may have been mainly caused by inadequately performed long-term studies (for review see Huff et al. 2000).

In the methylated, stable metabolites of As [i.e. MMA (V) and DMA(V)] excreted in human urine, As predominates in pentavalent form. However, there is recent data showing that their trivalent analogues, MMA(III) and DMA(III), are as cytotoxic as is arsenite. MMA(III) (monomethylarsonous acid) appears as intermediary metabolite before the second methylation step to DMA(V). This indicates that trivalent As is highly toxic when methylated as well and that trivalency is the feature linked to high toxicity and that methylation is not relevant in this respect. Thus, it seems likely that the only important reason as to why As methylation is a detoxification process is that it leads to accelerated excretion of As.

Besides, there is a world-wide general population exposure to As compounds via the intake of seafood. Seafood, but not freshwater fish, contains comparatively high amounts of As. However, a high portion of the seafood-borne As is bound to proteins or sugars or it can be found as arsenocholine and arsenobetaine. The latter compound is the major source of As in seafood. All of these As compounds leave the human body metabolically unchanged and have been assumed to have only minor toxicological significance. However, few percent of the seafood-borne As is DMA which has a low, yet not irrelevant toxicological significance.

The highly toxic inorganic arsenicals, i.e. As(V) and As(III), are prevailing in even minor portions in fish and shellfish.

Mechanism of action

Arsenic, especially in its trivalent chemical species, is a highly bioreactive metalloid covalently binding to sulfhydryl moieties, most preferentially to vicinal dithiols. As a consequence, in laboratory experiments As was shown to react with and sometimes even inactivate a great number of enzymes and proteins containing thiol moieties. Because of this high reactivity, a wide variety of different biological effects mediated by As have been reported (for review , see de Wolff and Edelbroek 1994).

Unfortunately, it has not been elucidated up to now how As mechanistically leads to the induction of neoplasia. Besides its tumorigenic potential, As acts as chromosomal mutagen in vitro and in vivo (for review see Gebel 2001). It mainly acts clastogenic but also has a certain aneugenic potential. On the other hand, As is a very weak inducer of point mutations. Like it is the case for As carcinogenicity, it is not known through which mechanism the genotoxicity of As is mediated, although the data available

indicate that As may act indirectly on DNA, i.e. via mechanisms like interference of regulation of DNA repair or integrity.

Dose-response of chronic toxicity

Currently, it is hardly possible to assess the level of exposure at which the chronic toxicity of As becomes epidemiologically relevant because of several gaps in knowledge. One reason is that in spite of numerous efforts and studies the biochemical key mechanisms of arsenic's long-term toxicity have not been elucidated.

Because of the indirect mode of action, it has been discussed that As's genotoxicity and carcinogenicity may underlie a sublinear dose-response relationship or that its long-term toxicity may have a threshold-like dose-response. However, there are methodical problems which make it hardly possible to prove such statement. If studies do not show an effect of As in a certain dose-range, it is not possible to prove whether in fact there may be a toxic effect below the limit of detection of the respective setup used. This is the case for experimental and epidemiological studies and true as well, when assuming that As acts as indirect genotoxicant or carcinogen. Thus, from the scientifical point of view, it is not possible to prove a threshold-like action of As. However, as exposures to As are ubiquitous, it should be aimed at improving the knowledge of long-term toxicity of As in drinking water exposures in the range up to 300 µg As/L. This would help in establishing safe levels and not lead to putatively overprotective standards because of uncertainty in knowledge. This is likely to be the case for the 1993 WHO evaluation in which a drinking water standard of 10 µg As/L was recommended.

Conclusion

Unfortunately, a toxicologically precise risk assessment and standard setting, especially for long-term and low-dose exposures to arsenic, is not possible. The situation is even more complicated when taking into consideration that there are several compounds suspected to modulate the chronic environmental toxicity of arsenic, variables that may either enhance or suppress the in vivo genotoxicity and carcinogenicity of the semimetal. Among them are nutritional factors like selenium and zinc as well as putative drinking water co-contaminants like antimony. Moreover, malnutrition may lead to an increased susceptibility to As's long term toxicity.

Epidemiological data suggest that there may be no elevated tumor risk detectable consuming drinking water with As levels below 100µg As/l. However, the daily consumption of drinking water varies much in different countries which has to be included in risk assessment. With the help of additional studies, it is quite possible that a toxicological re-evaluation in the future may define an exposure level below which the carcinogenic action of As is not relevant. This would mean that toxicological based safe levels could be defined although As is classified as human carcinogen. However, before such re-evaluation can be performed, it will be necessary to carry out additional experimental and epidemiological studies.

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Isolation of some secondary plant metabolites from *Terminalia arjuna*

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Terminalia arjuna is one of the important medicinal plants widely used in the preparation of important Ayurvedic formulations used against several ailments. It is popularly used as cardiac tonic. The decoction of the bark of arjuna, cane sugar and boiled cow's milk is highly advocated in heart disease complicated with endocarditis, pericarditis and angina. Bark is also prescribed for biliousness, sores and as an antidote to poisons and is believed to have the ability to cure hepatic, congenital, venereal and viral diseases. An attempt was made to isolate and characterize the antimutagenic potential of various secondary plant metabolites harboured by *Terminalia arjuna*. Some tannin fractions and a triterpene diglycoside isolated, exhibited remarkable antimutagenicity in Ames assay.

The bark of *T. arjuna* was made free of dust and other impurities and then immediately dried in an oven at 40-60°C. The bark was finely powdered and the bark powder was sequentially extracted with different organic solvents in their increasing order of polarity using Soxhlet apparatus. The bark powder was first extracted with benzene in Soxhlet apparatus for 48-72 hours to remove lipophilic components. The remaining bark powder was extracted with chloroform in Soxhlet apparatus for 48-72 hours. Chloroform was distilled off to get chloroform fraction. The remaining residue duly dried was extracted with acetone for the same duration, i.e. 48-72 hours, to get an acetone extract (Ac). The remaining bark powder was further extracted with methanol in Soxhlet apparatus for 72 hours. Methanol fraction was obtained after distilling off methanol. Finally, the residue was extracted for 72 hours with methanol-HCl (99:1) mixture using Soxhlet apparatus. The fraction obtained was named as acidic methanol fraction. Various constituents were further isolated from the crude acetone extract (Ac). Acetone extract (Ac) was then treated by two methods.

Method A: Acetone extract in dried powder form was extracted with diethyl ether is Soxhlet

apparatus for 48 hours. Ether was distilled off to get ether fraction A (ET-A). The remaining acetone extract (Ac-1) was further extracted with ethyl acetate using Soxhlet apparatus to get ethyl acetate fraction (EA). Brown-coloured precipitate separated out in ethyl acetate extract. This was named as PB fraction.

Method B: In this method, acetone extract (Ac) in concentrated solution form was first made aqueous by adding distilled water. It was then extracted with diethyl ether using separating funnel. White solid separated out from ether extract which was named as ET-1 fraction. On separating ET-1 by filtration, the remaining ether extract was dried and then partitioned into acetone-soluble fraction (ET-Ac) and acetone-insoluble fraction (ET-2). ET-Ac was columnchromatographed over silica-gel which resulted in fractionation of the extract into ET-3 and ET-4 fractions.

The remaining acetone extract (aqueous) was extracted with ethyl acetate to get ethyl acetate fraction. The remaining acetone extract was then allowed to remain in refrigerator for 2-3 days. White coloured solid separated out which was filtered and washed with distilled water. This white solid was sparingly soluble in water. This was named as TW fraction. The tentative chemical nature of the extracted materials was inferred using various spectroscopic techniques, viz. ^{1}H-NMR, normal ^{13}C-NMR, distortionless enhancement by polarization transfer (DEPT-90 and DEPT-135), UV and IR.

Table1: Important secondary plant metabolitesfrom Terminalia arjuna

Fraction	Nature
ET-1	Triterpene diglycoside
ET-2	Ellagic acid
ET-3	Triterpene
ET-4	Triterpene
PB	Tannin
TW	Tannin

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HUMN.The International Collaborative Project on Micronucleus frequency in Human Populations

The idea of the HUMN project was conceived by Dr. Michael Fenech (CSIRO, Adelaide Australia) and Dr. Stefano Bonassi (National Cancer Institute, Genova, Italy). An invitation letter was sent in early 1997, by the steering committee of the HUMN project to 130 laboratories that had published studies on CBMN assay in human lymphocytes. The letter included a questionnaire for some basic information about the data available in each laboratory. Thus following the direct approach to scientists, an announcement and workshop held during the ICEM in Toulose, in September 1997, 42 scientists initially expressed their interest to participate in the HUMN project. Based on this information received from the preliminary questionnaire, a so called "information package" was prepared and sent to the interested laboratories. This time, the participating laboratories received a more detailed questionnaire requesting information about laboratory protocol, scoring criteria, individual data of subjects in the study and references of published studies, if any and an Excel file, to be used as template for submitting original data to the coordinating centre (NRC, Genova, Italy). Finally, primary data from historical records were submitted by 25 laboratories distributed in 16 countries, mostly in Europe, but also in Asia, America, Australia and New Zealand. (Bonassi et. al. 2001).

In the first phase of the project, a database of 6583 subjects, from 25 different laboratories, representative of many countries and populations has been compiled and analysed. The large variability observed in absolute frequencies in case of micronucleated cell (MNC) frequency, makes it difficult to understand the extent and relevance of the MNC frequencies associated with the exposures or conditions under study. Thus lack of reliable information on the natural background levels has always been a lacuna in interpretation of studies using it as a biomarker. Therefore one of the priorities of the HUMN project was to provide information on the baseline frequencies of MNC in human lymphocytes. The first results of the analysis of pooled data from laboratories participating in the HUMN (Human MicroNucleus project) international collaborative study have just been published. The individual data base sizes range from 11 to 1637 subjects, with a mean size of 263 subjects and each contained the results of one to seven different studies. Most of the studies included in the HUMN database were designed as occupational or environmental surveys of subjects exposed to genotoxic agents such as ionizing radiation, aromatic hydrocarbons, pesticides, cytostatic drugs, metals, air pollution or studies designed to evaluate the effects of genotoxic agents on the MNC frequency. Subjects exposed to known genotoxic agents, individuals exposed to atomic bomb radiation in Hiroshima, or living in high radiation background areas, or carriers of genetic alterations were not considered in the evaluation of baseline MNCs, because these conditions may have led to a certain genomic instability. The effect of laboratory protocol, scoring criteria and host factors on baseline MNC frequency are evaluated and reference range of "normal" values against which future studies may be compared is provided. The availability of such a large amount of data representative of different human populations

provided a valuable opportunity to quantitatively establish the effects of gender and age on MNC frequencies. Comparison of the results accumulated for baseline MN frequencies from various laboratories had provided information on the extent of variation in normal range values for different laboratories and countries. (Bonassi et.al.2001).

The second phase of this project ie. Method comparison Stage I. has commenced in September 2000. 34 laboratories from 21 countries are participating in the second phase of the project. The HUMN project has created an international net work of scientists working with CBMN to ensure appropriate quality control and for the development of standard experimental and documentation protocols.

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Report of the XXVI Annual Conference of Environmental Mutagen Society of India

The XXVI Annual Conference of Environmental Mutagen Society of India and "International Symposium on Environmental Health Sciences in the 21st Century" was held at School of Life Sciences, Jawaharlal Nehru University, New Delhi between March 5-7, 2001. In the inaugural session, Prof. Asis Datta, Vice Chancellor, JNU gave a brief description of the history of JNU, its achievements and its activities. This was followed by a talk by Dr. P.S. Chauhan, President, EMSI. Prof. R.N.K. Bamezai, Organizing Secretary XXVI EMSI Conference gave the welcome speech. The inauguration address was delivered by Padma Shree Dr. C.P. Thakur, Hon'ble Cabinet Minister of Health and Family Welfare, Government of India. The conference was formally inaugurated by lighting a lamp by dignitaries like Dr. Thakur, Prof. Datta, Prof. R.K. Saxena, Chairman, Organizing Committee, Dr. P.S. Chauhan, President, EMSI.

About 200 participants attended the conference. Among them there were many scientists from abroad viz., Prof. A. T. Natarajan and Dr. P. de Boer (The Netherlands), Prof. C. Streffer and Dr. Tom W. Gebel (Germany), Dr. Awadhesh Jha (U.K.), Dr. Pawan Kumar Dhar and Dr. Y. Ishikawa (Japan), Dr. Ronaldo Benigni (Italy), Dr. M. Torniqvist and Dr. Robert Nilsson (Sweden), Dr. S.G. Grant (USA), Dr. F. Cortes and Dr. E. Gocke (Switzerland).

Prof. A.T. Natarajan delivered a talk on "Formation of Chromosomal Aberrations Induced by Ionizing Radiation and Chemical Mutagens: Recent Developments." He stated that ionizing radiation (IR) and most of the chemical mutagens both induce chromosomal aberrations (CA) in somatic cells and germ cells leading to human neoplasia and congenital abnormalities respectively. While IR induced CA are formed by mis-repair of DNA double strand breaks (DSBs), chemically induced aberrations are formed during DNA synthesis due to misreplication of DNA strand containing the adducts. With the recent introduction of FISH (Fluorescent in situ Hybridization) technique, aberrations that cannot be visualized by solid staining eg., complex exchanges and inversions can be detected using FISH. Some of the important observations of these studies are as follows: (1) Although there is a 1:1 correlation between dicentrics and reciprocal translocations, if dicentric frequency is compared with total translocation frequency (which include reciprocal, terminal, interstitial, insertion and telomeric translocations), the dic: transl frequency ratio is higher than 1:1 (2) Chromosome intrachanges are induced more frequently than interchanges. For chromosome # 1, the ratio of intra to interchanges was 6.14 whereas for #3, the ratio was 8.75 (3) There is heterogeneity in induction of aberrations between the chromosomes, chromosome arms and regions. Chromosome#1 was involved less frequently in translocations whereas #4 was involved more frequently. While chromosome 1 has a mixture of early and late replicating regions, #4 has only late replicating regions. Chromosome 18 too has late replicating regions. On the basis of DNA content also, #18 should show less translocation frequency but it shows high frequency. Chromosome 19 has been predicted to show high translocation frequency but it shows less frequency.

Dr. C. Streffer presented a talk on "The Micronucleus (MN) Test as an Indicator of Radiation Exposures and Prognostic Factor of Damage." Higher frequency is found in females than males and it is also found to increase with age. MN frequency is not more in leukemia patients but it is more in Fanconi's anemia cases. Also, B-lymphocytes show more frequency compared to T-lymphocytes. MN frequency shows variability with volunteers. CSC and alcohol also increase MN frequency. The sensitivity of MN test is however lower than other test systems like CAA for detecting radiation exposures. This can be increased, if not only number of MN but also the number of MN with centromeres is counted. Then variability in the volunteers is found to decrease. This can be achieved by using DNA probe for centromeres and carrying out FISH. It was observed that in lymphocytes of unexposed persons, MN with centromeres are 70-80% indicating that in these cases a high number of MN are formed from loss of whole chromosomes. Following radiation exposure, acentric fragments increase and number of MN with centromeres decrease. Subsequent to this refinement in the technique, the sensitivity of this assay has increased and for low LET radiation, 0.1 Gy of dose can be detected. A good dose response is observed in the dose range of 0.1-2.0 Gy of low LET radiation. It has been further observed that measurement of MN with centromere is a good indicator of genomic instability. In cancer patients, frequency of MN with centromere is found to decrease. Also, in the case of uranium miners, the frequency of MN with centromeres decreases especially in persons suffering from bronchial carcinoma. This observation can be of prognostic value in detecting radiation damage, which has occurred many years ago.

Dr. Awadhesh Jha presented an invited talk on "Detection of Genotoxicity in the Marine Environment and Evaluation of an Integrated Approach." He stated that exposure to genotoxic contaminants to aquatic biota could pose a threat to human health via the food chain. In addition, in ecological context, the survival and reproductive output of the biota can lead to potential adverse changes at higher levels of biological organizations, which is of utmost importance. In order to expand our knowledge and also to respond to environmental health concern, it is possible to use natural biota or wild species as sentinel or surrogate species for the evaluation of genotoxic effects. For this, a range of molecular (RAPD), biochemical (DNA strand break measurements or comet assay) and cytogenetical (SCE, CA) methods have been developed to evaluate the genotoxic potential of environmental contaminants in different life stages of marine invertebrates. There is also a concern about the potential of an environmental chemical to disrupt the normal functioning of the endocrine systems in both humans and wild life. In this context, the effects of Tributayltin (TBT), an ingredient of antifouling paints has been carried out on certain marine gastropods, wherein population declines have resulted following exposure to this endocrine-disrupting chemical. Presence of this extremely toxic, anthropogenic chemical has also been found in human tissues. The genotoxic and developmental effects of TBT was evaluated in two ecologically relevant marine invertebrates: *Mytilus edulis* (edible mussels) and *Plytinereis dumerilii* (ragworm). TBT was found to be both toxic and genotoxic at comparable concentrations, for developmental and genotoxic effects. The non-target species (*P. dumerilii*) was found to be more sensitive. TBT has also been reported to be immunotoxic.

Dr. P. de Boer presented an invited lecture on "Mammalian Gamete Interaction at the Zygote Stage and the Transmission of Male Genetic Damage." He stated that the preferred treatment of human male sterility in western society is intracytoplasmic sperm injection (ICSI) for the 5 years. In the children thus conceived, an increase in sex chromosome aneuploidy was reported in addition to an increase in *de novo* structural chromosome aberrations. In parallel, other studies correlated the assessment of single strand breaks (SSBs) and double strand breaks (DSBs) in mature human with azoo and oligospermia. Present research studies analyzed the relation between spermatogenic efficiency and cytogenetic constitution of spermatozoa after fertilization in mouse model that allows a wide range of sperm count to be recovered. Results indicated that epididymal storage in an oligospermic environment is responsible for chromosomal breaks. Heavily fragmented male chromosome complements are indicative for activation failure of the oocyte or can lead to zygotic cell cycle delay and arrest. The use of intracytoplasmic sperm injection allows human sperm to be analysed in the mouse system as well.

Dr. Ronaldo Benigni presented a talk in which he stated that environmental factors are responsible for about 75-80% of all cancers in developed countries. Out of these, 50% of cancer causes could be åvoided by applying existing etiology knowledge. The model system used was Ames' Salmonella test systems. It was further stated that overall accuracy was found to be 50-65% for approaches that relied solely on chemical structure information. About 75% accuracy was observed for approaches, which were complex.

Dr. M. Torniqvist presented talk on "Methods for Cancer Risk Estimation of Environmental Chemicals." The purpose of this study was the development of a mechanism-based model to be used for estimation of cancer risks from genotoxic chemicals, using adducts to macromolecules for the determination of *in vivo* dose. On the simplified assumption that cancer is caused by an increased mutation frequency in tissues in interaction with inherited or acquired growth promoting factors, a model has been developed for estimation of risks from chemically reactive (mutagenic) agents. In the multiplicative model, $\Delta P = \beta D P^{\circ}$, the risk increment ΔP is proportional to the background incidence (P°) and is linearly dependent on dose at low to intermediate doses (D); β is the risk coefficient which is approximately the same for different tumour sites and different species. In human and test animals the dose can be derived from measured protein adducts and the rate of adduct formation. This approach has been applied to various exposures e.g. air pollutants in occupational settings, carcinogens in foods and in tobacco smoke. Also, previous unknown exposures to mutagens/ carcinogens can be detected.

Dr. Y. Ishikawa presented a talk entitled "Alpha-Particle Carcinogenesis in Patients Injected with Thorotrast: Epidemiology, Dosimetry, Pathology and Molecular Analysis." Thorotrast, an X-ray contrast medium is composed of 25% thorium dioxide and was used in the western countries and Japan in 1930-55. Since Th-232 (an alpha-emitter) has a 14 billion year half-life, injected thorotrast irradiates surrounding tissues during remaining whole life. It is a colloidal solution and therefore gets deposited in the liver, spleen and bone marrow after injection. Irradiation resulted in cancers and premalignant lesions after a long latent period typically of 40 years. Epidemiological studies revealed that increased liver cancer (O/E=18.2), liver cirrhosis (5.4) and leukemia and MDS

(8.0). Cancers of extra hepatic bile ducts were also significantly increased. Although thorotrast patients exhale Radon-220, a progeny of Th-232, no significant increase in lung cancer incidence was reported in Japan, Germany and Denmark. However, percentage of small cell lung carcinoma was significantly increased in Japan and Denmark. They have examined mutation status of the p53 gene in the 37 liver tumours. Loss of heterozygosity (LOH) at 17p13 locus was also examined for carcinoma cases. Small mutations such as point mutations of the p53 gene were observed and LOH at 17p13 locus was not frequent in carcinomas and angiosarcomas related to Thorotrast. Most of them were transitions and `hot spots' found in angiosarcomas and were similar to those seen in cancers of the general population. These facts imply that genetic changes of thorotrast related cancers are mainly "delayed mutations," not results of direct effects of radiation.

Dr. S.G. Grant delivered a talk on "Molecular Epidemiology of Human cancer: Biomarkers of Genotoxic Exposure and Susceptibility." "Molecular Epidemiology" attempts to investigate the link between toxic exposure and an associated health effect by defining presumptive intermediate stages in the development of the disease state based on known mechanisms. These processes can be monitored through biomarkers specific to each to the steps in the disease progression. Dr. Grant and Dr. Latimer have used two such biomarkers to the development of breast cancer. One such biomarker is the measurement of somatic mutational burden using GPA assay, which is a blood-based analysis of functional allele-loss at the heterozygous, autosomal gene that determines the MN blood group. This test has been used previously to demonstrate the genotoxic effects of chemical and radiation exposure and susceptibility to such injury is demonstrated by individuals with DNA repair deficiency diseases associated with high cancer incidence and/or premature ageing. The somatic mutational burden of newly diagnosed cancer patients, including breast cancer patients was found to be significantly higher than that of a population of age-matched controls. Moreover, the cancer patient population was found to be enriched in individuals with mutation frequency greater than 5 standard deviation from the mean. This population included approximately 40% of all breast cancer patients suggesting that individuals in combined population were at 5 fold higher risk of malignancy. Increased mutational frequency can be due to greater overall exposure, or to a greater susceptibility or both. To understand the underlying mechanism, the DNA repair capacity of the tumour itself was assessed using unscheduled DNA synthesis (UDS). All tumours but particularly early stage tumours found to have significant reductions in their ability to repair DNA damage. These results suggest that it may be possible to screen the population for their susceptibility to certain carcinogenic agents and develop chemopreventive measures to reverse/inhibit the development of the disease in those at risk.

Dr. F. Cortes delivered a talk on "DNA Damage in Birds after the Mining Waste Spill in South-Western Spain: A Comet Assay Evaluation." He reported that in April 1998, an ecological disaster occurred as a consequence of a massive toxic spillage of mining acid waste rich in heavy metals that posed a serious threat to Donana National Park in South-Western Spain. This protected area is the nesting and breeding site for many endangered bird species. Among many other bird species, white storks (*Ciconia ciconia*) and kites (*Milvus migrans*) are considered as the representative ones. Since comet assay has been validated

as a biomarker for genotoxic analysis in environmental biomonitoring using fish, amphibians, rodent and mollusks, birds preying on a variety of invertebrate and vertebrate species were chosen to evaluate the potential deleterious effects of the toxic spill on wild life of the Donana area. Blood samples from white storks and kites collected in the neighbourhood of Donana National Park 14 months after the mine waste spillage as well as from control birds sampled at reference areas for comparison were examined by fluorescence image analysis after isolation of lymphocytes and subsequent alkaline Single Cell Gel Electrophoresis (Comet Assay). Results indicated that exposed birds show a significantly increased level of genotoxic damage as compared with control birds from non-contaminated locations.

Dr. Gocke delivered a talk on "Photochemical Mutagenesis: Examples and Toxicological Relevance." He stated that induction of DNA damage as a consequence of UV exposures has been the major cause of skin cancer. Alternatively, endogenous or exogenous chemicals (sensitizers) may absorb light, with the potential of subsequent energy or electron transfer leading indirectly to DNA-damage. A few light absorbing pharmaceuticals eg., psoralene and chlorpromazine and more recently fluoroquinolone antibodies have been shown to be photomutagenic. In this routine genotoxicity studies, photomutagenic activity of a compound under development was detected in Ames tester strain at normal laboratory illumination conditions. This observation led to termination of its development. Several structural analogues have been tested for which structure activity relationships for $^1O_2$ generation, phototoxicity, photomutagenicity and photoclastogenicity have been studied. The risk/benefit assessment for the described compounds has to take into account the human exposure situation e.g. the ability to avoid light exposure during the treatment period.

On the last day in the last session, three best poster paper awards were declared followed by 10 minutes presentation of each paper by authors. The 1st prize was given to the poster entitled "Induction of Micronuclei in the Peripheral Blood Erythrocytes of the Catfish ***Clarias batrachus*** by 2,4-Dichlorophenoxyacetic acid and Butachlor" by **Abul Farah,** Bushra, M. Niamat and Waseem. The second prize was given to the poster entitled "Modulatory Effects of Spirulina fusiformis on chemically induced genotoxicity and oxidative stress in swiss albino mice" by **K. Premkumar,** C. Thirunavukarasu, V. Umashankar, D. Aarthi Sharon, A. Pachiappan, Abraham S.K., S.T. Santhiya and A. Ramesh. The third prize was awarded to the poster entitled "In vitro Assay for the Modulation of the Mutagenicity by Acacia auriculiformis A. cunn" by **A. Saroj, Kamaljit K.** and **Suboth K.**

A rapporteur session was held in the concluding session of this conference during which the rapporteurs summarized the presentations carried out in their sessions in about 10 minutes durations. The rapporteurs who presented their reports were Dr. R. C. Chaubey, Dr. Anupam Chatterjee, Prof. R.K. Kale, Dr. (Ms) S. Nagini, Dr. B.B. Panda, (Ms.) K.B. Anjaria, Dr. (Ms.) T.K. Shetty and Dr. R.C. Chaudhury.

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Panel Discussion

On the evening of March 5th, a panel discussion on "Environment & Health Concerns and Challenges" was held. The panelists were Prof. P.N. Srivastava, Prof. A.T. Natarajan, Prof Kasturi Dutta, Dr. P.S. Chauhan, Dr. B.B. Panda and Dr. A.N. Jha.

Prof Natarajan talked about one of the serious environmental health concerns affecting parts of West Bengal namely arsenic contamination in water. Arsenic induces skin lesions, cancer especially that of kidney and sores in feet. One of the remedies suggested was provision of safe drinking water, which can be supplied by Government agencies. In general, providing safe drinking water to all people can reduce environmental health concerns by 40%. The concerns of developing countries are different from that of developed countries due to more heterogeneity in the former. However, databases of the later can be effectively used to prevent serious environmental problems in the developing countries like India. If importance is given to the quality of air, food, water etc. the health problems can be significantly reduced. Dr. Natarajan also mentioned or rather lamented that environmental health is not taken seriously in India and this should be corrected, probably by giving more importance to the study of 'Environmental Science'. This may also help in preventing late onset disease.

Dr. B.B. Panda stressed the need for giving importance to 'ecosystem' especially in relation to evolution. He specially mentioned some environmental problems in Orissa due to effect of heavy metal toxicity on the ecosystem.

Dr. A.N. Jha, who is an expert in aquatic ecosystems mentioned that 'Man' should be considered as the dominant species in the ecosystem and: that to protect man other species also should be protected. Among the various species in the ecosystem, the most sensitive species should be given prominence. There is an urgent need to develop interdisciplinary areas of research. In developing countries like India environmental issues are compromised and this attitude should change.

Prof Kasturi Dutta stressed that issues related to health are important and that all disciplines should combine and work in a unified manner. Areas related to metabolism, biochemistry and genetics should combine together on environmental issues. EMSI can be one of the fora to promote such collaborative efforts:. In India 'population' is a major 'pollutant' and that traditional health care system also is important in approaches for environmental health.

Dr. Bamezai, who took part in the discussion, stressed the need for good database as well as interdisciplinary 'linkages'. Effective management of nature in relation to human life style is important. Prof Streffer mentioned that in developing countries feeding people may be more important than environmental issues, hence this also should be taken into account in planning strategies to counter environmental concerns. So economically and environmentally sustainable development is of importance in such situations.

Prof Srivastava, who concluded the session asked, to whom these issues should be addressed?. Providing safe drinking water is important and that our standards may be quite different from that of the western world. This is mainly due to 'adaptability' that is part of our system. Good education is also important, since it plays a major role in solving the population problem.

General Body Meeting

General body meeting of the EMSI was held on the evening of 6" March. Dr· P.S. Chauhan, President of the EMSI welcomed the members and also thanked Dr. Bamezai and his team including Prof. Asis Dutta, Prof R.K. Kale, Dr. Jadav and others who had done an excellent job in organizing this conference. The quality of work presented also was of high caliber. Prof A.T. Natarajan and his colleagues and friends who came from abroad were thanked. Dr. C.P. Thakur, Union Minister of Health who came to inaugurate the conference and this remarks were appreciated. It was mentioned that the society's byelaws required revision since it was framed 25 years back. Publishing of newsletter should be given more prominence and members are requested to contribute especially on issued related to current environmental concerns. More interaction between various institutes also should be encouraged. An effort should be made to increase membership.

In the absence of the election officer, President of EMSI announced the election results for the EMSI executive committee and the following were elected unopposed or by ballot. Dr. P.S. Chauhan (President), Dr. P.K. Sareen (Vice President), Dr T.P.A. Devasagayam (Secretary), Dr. G.B. Maru (Joint secretary), Dr. (Mrs.) K.B. Anjaria (Treasurer), Dr. A.N. Bhisey (Member), Dr. P.K. Seth (Member), Dr. (Mrs.) R.A. Bhisey (Member), Dr. B.S. Rao (Member), Dr. A.B. Vaidya (Member) and Dr. R.P. Saharan (Member). Two vacancies, one of the 'Vice-President' and one of Member' remained vacant, due to shortage of nominations.

In the discussion that ensued the following issues were discussed: Increase in travel grants for participants of EMSI conferences; Raising of money for inter-laboratory trials and workshops; To publish the 'decisions' that comes out of our society in 'Mutation Research Forum'; To develop 'linkages' with Prof A.T. Natarajan's group; To induct new members; To make EMSI economically viable by raising more funds, To have more focused programmes; To organize workshops along with conferences; To create databases with known expertise in environmental mutagenesis.

The timing of the conference: the general body members suggested that the timing of the conference should preferably be March 16 - 30 in the northern part of India and February 1 - 15 in the southern part of India.

The meeting ended with thanks to the chair.

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Highlights of the 25th IARP Conference - International Conference on Radiation Protection Measurement and Dosimetry : Current Practices and FutureTrends, February 20-23, 2001, Mumbai

Dr. V. Venkat Raj, Director, HS&E Group, BARC and his colleagues, organized the 25th conference of IARP, in Mumbai during February 20-23,2001. Among the distinguished delegates from abroad were Prof. A.T. Natarajan and Prof. K. Sankaranarayanan from the Netherlands; Prof. Guenther Stephan and Prof. C. Streffer from Germany. Dr. Anil Kakodkar delivered the inaugural address. During the scientific sessions that followed many topics of current interest related to the mechanism of induction of radiation damage, biodosimetry, radiation risk of interventional radiological procedures and genetic risk evaluation, featured during this meeting.

During the first scientific session on Biological Dosimetry and Biological effects Prof. Natarajan reviewed the recent advances in molecular biology that have contributed significantly to the area of radiation protection. Availability of chromosome specific probes and the recent introduction of fluorescence *in situ* hybridization technique (FISH) has enabled us to visualize a variety of radiation induced chromosomal damage. A quantum leap in the understanding of the mechanism of induction of cytogenetic damage could be achieved during last one decade. Since the stable chromosomal exchanges such as translocations can be scored with ease and rapidity by using the FISH technique, this has been introduced as a new method for the assessment of absorbed radiation dose in human beings. Since the stable aberrations can last for decades in human blood lymphocytes, this technique has a great potential to serve as a retrospective biological dosimeter. Further this technique can detect residual damage following chronic accumulated radiation exposure. Prof. Guenther Stephan presented some of his experiences in the area of retrospective dosimetry of human beings exposed to radiation.

Prof. K. Sankaranarayanan from the Netherlands, a pioneer in the field of genetic risk evaluation provided an update of this area. Since many years the problem of genetic risk evaluation was plagued for want of human data. Genetic risk estimates were entirely based on the doubling dose estimate derived from animal experiments and often fraught with uncertainties. Enormous efforts in the recent past to arrive at the spontaneous frequencies of various Mendelian diseases have resulted in the upward revision of the estimates from 1.25% to 2.4%. This has resulted in the refinement of risk valuation. At present doubling dose based on human data on spontaneous mutation rates and mouse data on induced mutation rates are clubbed to revise the risk estimates. Concept of the mutational component (MC) has been revised and applied to predict the responsiveness of Mendelian and chronic multifactorial diseases. Introduction of the concept of potential recoverability correction factor (PRCF) has to some extent bridged the gap between studies in mice and the risk in humans. Revised estimate suggests a total risk of ~3000-4700 cases per million per Gy (autosomal and X-linked diseases, ~750-1,500 cases; autosomal recessive, negligible; chronic multifactorial diseases, ~250 to 1200 cases and congenital abnormalities, ~2000 cases). This implies that at low doses of radiation, as in occupational exposure, the risk of adverse hereditary effects is small compared to the spontaneous background frequencies of genetic diseases in the

populations. The absence of excess genetic disorders among the progeny of the A-bomb survivors is consistent with a low risk associated with radiation exposure.

Prof. Christian Streffer from Germany discussed the recent progress in understanding the interindividual variation in radiosensitivity and its implications in radiation protection and radiotherapy. A number of genetic diseases seem to predispose human beings to the radiation effects. Extensive radiobiological studies with different cells suggest that x-ray resistant cell lines show a high sensitivity to neutrons. The experimental results suggest that individuals with hypersensitivity against sparsely ionizing radiation will probably have lower RBE for high LET radiation than individuals with normal radiation response. In the near future, it should be possible to clone genes predisposing human beings to the effect of radiation. Advances in molecular biological research can develop methods to easily identify such individuals. This may have important implications in radiation protection.

Some of the papers summarized the efforts done during the last decade for handling radiation accidents effectively, if they ever occur. Enormous effort has been made to ensure emergency preparedness, such as creating the necessary infrastructure, public education, emergency drills and methods to reduce the public exposures in the event of an accident. During the last decade three has been an ongoing programme to train medical personnel for handling radiation injuries such as localized exposures and acute radiation syndrome (ARS). In addition, a number of Personal Decontamination Centres, First Aid Post at Plant sites, Site Hospitals and Specialized Central Facilities, have been evolved.

Dr. D.N. Pahuja, of Radiation Medicine Centre presented the progress made in decorporation of radiocesium, one of the most important radionuclides released during criticality accidents. Among many chemicals screened in model animal system, potassium and iron based compounds, developed in RMC, at a dose equivalent to 3.5 g per 70 kg adult/day can decorporate radiocesium more efficiently than Prussian blue. Further work on many other promising nontoxic agents is in progress.

Ever since the ability of radiation to cause lethal effect in human beings became known, there has been an incessant search for a chemopreventive drug that can be used in an emergency. Many effective chemoprotectors such as - SH compounds were found to be very toxic. Hence, the search began with non-toxic radioprotectors. Prof. P.K. Goyal presented work done in India during the last decade at the University Rajasthan. These studies suggest that calcium channel blocker diltiazem and β carotene, a dietary ingredient can provide protection against lethal doses of radiation in model animal systems. Among the micronutrients, vitamin E and Vitamin C have been shown to afford protection against radiation damage to liver, bone marrow and testes. The aqueous extract of *Mentha piperita* (Linn) protects against lethal radiation effects by alleviating the haematopoietic injury, mainly due to the presence of eugenol, caffeic acid, rosmarinic acid and α tocopherol in Linn. This work also holds promise that the chronic oxidative stress suffered by human beings due to a number of environmental agents can be reduced by the use of dietary ingredients such as vitamins and food additives.

In addition, the work from many laboratories indicates the efforts to reduce the doses involved in medical and industrial application of radiation. This is being achieved by the development of a new generation of instruments,

improvements in the techniques, personal monitoring devices and environmental monitoring systems. Medical radiation exposure (~0.4 mSv/year) is the second most important source of population exposure (background radiation being the first, leading to an effective dose of ~2.5mSv/year). Prof. M.M. Rehani pointed out that in spite of the great technical improvements which have significantly reduced the patient dose, introduction of many new procedures like RF cardiac catheter ablation, coronary angioplasty, transjugular intrahepatic portosystemic shunt, therapeutic cardiac angiography, arrhythmia ablation procedure, multiple hepatic biliary procedures, neurological procedures such as carotid procedures, cerebral angiograms, nerve block procedures, lumber procedures and embolisation procedures, have resulted in steep increase in patient doses. Further, clinicians are performing many interventional procedures involving extended fluoroscopy without formal training in radiation protection. He expressed concern and suggested remedial measures to reduce the human exposures by appropriate up gradation of diagnostic facilities (such as changeover to rare earth screens), evolving strategies to avoid unnecessary exposures and by imparting adequate training to the personnel using specialized procedures such as interventional procedures. Efforts are made to monitor the radiation levels around patients who go through nuclear medicine procedures to ensure to adequate protection to relatives and personnel. All efforts are on to reduce both the personnel and public exposures to a reasonably low level.

In short, this Conference was a great success in providing the participants an update on the progress achieved on many fronts of radiation protection.

B.S. Rao
RP&AD, BARC, Mumbai

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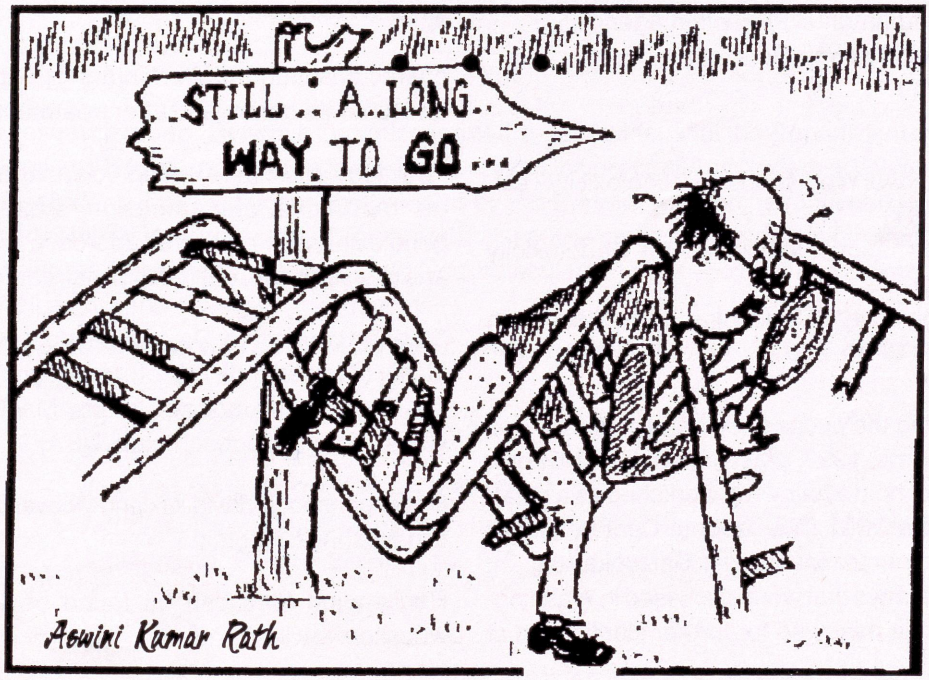

NEWS

Dear colleagues,

3rd IWGT Workshop

The 3rd in the series of International Workshops on Genotoxicity Testing (IWGT) will take place in Shizouka immediately prior to the ICEM. Details are given below and in the attached Word document. If you would like to attend, please complete the registration form which can be found on the IAEMS website (www.iaems.nl).

David Kirkland

INTERNATIONAL ASSOCIATION OF ENVIRONMENTAL MUTAGEN SOCIETIES

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International Workshops on Genotoxicity Testing (IWGT)
3rd International Workshop on Genotoxicity Testing
The University of Shizuoka, Shizuoka, Japan
October 19-20, 2001

Following the success of 2 earlier workshops (Melbourne, 1993; Washington, 1999) it is proposed to hold a follow-up workshop as a satellite of the 2001 International Conference on Environmental Mutagens, Shizuoka, Japan. Three assays that were discussed in Washington will be reviewed for further clarification of recommendations and 2 new groups will be established.

Working groups and topics to be addressed:

- Mouse lymphoma assay - measures and levels of cytotoxicity, use of conditioned medium, cleansing of cells, statistics, use of microwell vs agar techniques.

- In vitro micronucleus assay - use of cytochalasin B, primary cells vs established cell lines, measures and levels of cytotoxicity, discrimination of aneugens and clastogens, equivalence of different cell types.

- Transgenic genotoxicity models - treatment schedule, sampling times, size of experiment, data evaluation.

-P53 and Hras2 transgenic tumour models - genetic/molecular

characterisation required in animals, tissues and tumours before and after treatment.

- Strategy and classification - development of an IARC-like classification system for genotoxins, a decision-tree approach to testing and classification, definition of risk categories.

Steering committee: David Kirkland (UK), Lutz Muller (Switzerland), Makoto Hayashi (Japan), Toshio Sofuni (Japan), James MacGregor (USA), Leonard Schechtman (USA)

Correspondence: david.kirkland@covance.com or hayashi@nihs.go.jp

Registration form can be found on IAEMS website www.iaems.nl

Current Office-bearers of EMSI Executive Committee

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Vice President	:	Dr. P.K. Sareen, Dr. P.K. Seth
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Joint Secretary	:	Dr. T.B. Maru
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New Editorial Board of the EMSI Newsletter

Editor:

Dr. B.S. Rao

Members :

Dr. (Mrs.) S. Nagini
Dr. G.C. Jagetia
Dr. (Mrs.) K. Pasupathy
Dr. P. Harikumar

Dear friend

The research group "Dynamic Organization of the Cell Nucleus" at the Department of Cell Biology and Histology, Academic Medical Center, University of Amsterdam is calling for a Ph.D. student. The present project, funded by the European Union, is to investigate the motion of the DNA double strand breaks and consecutive processes leading to chromosome exchanges. The experimental work involves molecular genetics and biochemical methods as well as 3D and 4D digital microscopy.

For applications, as well as for more information, please contact (or just send a letter and CV to):

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Greetings
Xiao

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