ISSN : 0970-437X

TOXICOLOGY RESEARCH BULLETIN

Volume 29 Number 3 2009

INSIDE

Indian Institute of Toxicology Research, Lucknow, India

67th CSIR FOUNDATION DAY CELEBRATED

CSIR Foundation Day function was held in the auditorium of Indian Institute of Toxicology Research (IITR) on September 26, 2009. Dr DK Saxena, Director-in-charge, IITR while welcoming the members of IITR family said that CSIR with its 38 laboratories spread across the country is catering not only to the problems of the country in diversified areas by way of excellent R&D outputs but also competing internationally with its peers. He further said that he takes this opportunity to appreciate the efforts and foresightedness of our national leaders and stalwarts who laid the foundation of CSIR in 1942.

On this occasion officials of IITR who had completed 25 years of service and also those who had superannuated during the last one year were felicitated by Dr Saxena. The names of the officials who had completed 25 years are: Dr Ashwani Kumar, Dr Rakesh Kumar, Shri Mohd Javed, Smt. Shyamala Das, Shri Khalil Ahmed, Shri Mohan Lal, Shri Vikas Barua and Smt. Shanti Devi. The names of the officials who had superannuated are: Dr Jai Raj Behari, Dr DN Kachru, Dr SC Srivastava, Dr FN Jaffery, Smt Sushma Sharma, Shri VK Jain, Shri RP Singh, Shri MA Khan, Shri RS Bharti and Shri Devta Deen.

Earlier an essay competition was held for the children of CSIR employees. The titles of essay competition were "Unite to protect the environment" and "Urbanisation and environmental problems". Dr Mukul Das, Scientist, IITR gave away the prizes to the winners of the essay competition. The winners were (Junior Group) Mr Ambuja Srivastava, Km. Priya Awasthi and Km. Divisha Chaturvedi; (Senior Group) Mr Rahul S Naryani, Mr Aviral Prakash and Km. Shanya Das Rastogi.

Mr Mohit Srivastava son of Dr MK Srivastava, Tech. Officer, IITR was awarded a cash prize of Rs. 2000/- for securing over 90% marks in the three science subjects in the class 12 examination. Dr RK Upreti, organizing Secretary, CSIR Foundation Day programme proposed the vote of thanks.

CSIR Foundation day function

Dr DK Saxena honouring Dr Jai Raj Behari

RESEARCH HIGHLIGHTS OF IITR

Skin tumor promotion by argemone oil/alkaloid in mice: Evidence for enhanced cell proliferation, ornithine decarboxylase, cyclooxygenase-2 and activation of MAPK/NF-kappaB pathway

[Ansari KM, Das M. Food Chem Toxicol. 2009 Sep 29. (Epub ahead of print)]

Consumption of argemone oil (AO) contaminated edible oil causes "Epidemic Dropsy". Previously, we have shown that AO and isolated sanguinarine possess genotoxicity and skin tumor initiating activity. Here, we evaluate tumor-promoting potential of AO/sanguinarine alkaloid and investigate the molecular mechanisms involved therein. Single topical application of AO (50-400mul/mouse) or sanguinarine alkaloid (1.5-12.0mumol/mouse) afforded significant increase in (i) ornithine decarboxylase (ODC) activity, (ii) uptake of [(3)H]-thymidine in DNA, (iii) cyclooxygenase-2 (COX-2), proliferating cell nuclear antigen (PCNA) and ODC protein expressions, (iv) phosphorylation

of extracellular signal-regulated kinase (ERK)1/2, c-jun-N-terminal kinase (JNK)1/2 and p38 mitogen-activated protein (MAP) kinases, (v) increased NF-kappaB activation and (vi) no significant increase in dark basal keratinocytes. Subsequently, when AO and sanguinarine alkaloid were tested either as complete or stage I or stage II tumor promoter in 7, 12-dimethyl benz(a)anthracene (DMBA)-initiated mice, there was enhanced tumor incidence, tumor body burden and higher percentage of mice with tumors, when AO (0.1ml) or isolated sanguinarine (1.5mumol) was tested as stage II tumor promoter. However, no tumors were found when AO or sanguinarine alkaloid was tested either as complete or stage I tumor promoter. These results indicate that AO/sanguinarine alkaloid possesses tumor-promoting potential at stage II level involving MAPK/NF-kappaB pathway.

Involvement of mitochondria mediated pathways in hepatoprotection conferred by *Fumaria parviflora* Lam. extract against nimesulide induced apoptosis *in vitro*

[Tripathi M, Singh BK, Mishra C, Raisuddin S, Kakkar P. Toxicol In Vitro. 2009 Sep 20. (Epub ahead of print)]

Nimesulide, a popular nonsteroidal anti-inflammatory drug, has been associated with serious hepatotoxicity. Reactive oxygen species (ROS) and mitochondrial perturbations have been implicated in drug induced hepatotoxicity, although their role in the pathway needs exploration. The study was undertaken to elucidate the effect of *Fumaria parviflora* Lam. (Fp) on nimesulide induced cell death in primary rat hepatocyte cultures. Fp extract treated cells showed increased viability as compared to nimesulide stressed cells as assessed by MTT assay. LDH leakage increased significantly at 500muM nimesulide, and the data suggested that apoptosis was the predominant mechanism responsible for cell death. Nimesulide induced apoptosis was further confirmed by DNA fragmentation and chromatin condensation. Nimesulide exposure increased intracellular ROS, translocation of Bax and Bcl2 followed by mitochondrial depolarization and cytochrome c (Cyt c) release along with caspase-9/-3 activity confirming involvement of mitochondria in nimesulide induced apoptosis. Events like membrane depolarization of mitochondria, expression of Bax, Bcl2, externalization of phosphatidyl serine are substantially reversed by the pre-treatment with Fp extract. Thus, the study indicates that Fp extract modulates critical events regulating pro and anti-apoptotic proteins in mitochondria dependent apoptosis induced by nimesulide.

Characterization of sucrose-glutamic acid maillard products (SGMPs) degrading bacteria and their metabolites

[Chandra R, Bharagava RN, Rai V, Singh SK. Bioresour Technol. 2009 Dec;100(24):6665-8. Epub 2009 Aug 8]

Two aerobic bacteria RNBS1 and RNBS3 which were able to degrade and utilize sucrose-glutamic acid maillard products (SGMPs) as carbon, nitrogen and energy source were isolated and characterized as *Alcaligenes faecalis* (DQ659619) and *Bacillus cereus* (DQ659620) respectively by 16S rRNA gene sequence analysis. In the present study, mixed bacterial culture was found to be more effective as compared to axenic culture RNBS1 and RNBS3 decolourizing 73.79%, 66.80% and 62.56% SGMPs, respectively. The SGMPs catabolizing enzyme was characterized as manganese dependent peroxidase (MnP) by SDS-PAGE yielding a single band of 43 KDa. Further, the LC-MS-MS and other spectrophotometric analysis have revealed that most of the SGMPs detected in control were diminished from bacteria treated samples. The disappearance of SGMPs from bacteria treated samples could be related to the degradation of SGMPs.

Evidence for increased cytochrome P450 1A1 expression in blood lymphocytes of lung cancer patients

[Shah PP, Saurabh K, Pant MC, Mathur N, Parmar D. Mutat Res. 2009 Nov 2;670(1-2):74-8. Epub 2009 Jul 24]

To develop blood lymphocyte cytochrome P450 1A1 (CYP1A1) expression as a surrogate for monitoring tissue expression for polycyclic aromatic hydrocarbon (PAH) induced toxicity, the present study attempted to characterize CYP1A1 mRNA expression and its associated catalytic activity in freshly prepared blood lymphocytes isolated from healthy controls and patients suffering from tobacco induced lung cancer. Human blood lymphocytes were found to express CYP1A1 mRNA and significant activity of 7-ethoxyresorufin-O-deethylase (EROD). Significant increase in the activity of EROD and CYP1A1 mRNA was observed in blood lymphocytes isolated from patients suffering from lung cancer. Further, controls with variant genotypes of CYP1A1 (Msp1 or Ile/Val polymorphism) exhibited significant increase in the enzyme activity associated with an increase in CYP1A1 mRNA expression when compared to the controls with wild type genotype. Patients with variant genotypes of CYP1A1 also exhibited much greater increase in the blood lymphocyte CYP1A1 mRNA expression and EROD activity when compared to controls or patients with wild type genotype. Our data thus provides evidence of CYP1A1 expression in freshly isolated blood lymphocytes and differences in reactivity in individuals with variant genotypes of CYP1A1, suggesting that blood lymphocyte CYP1A1 expression profile could help in identifying individuals at risk to environment induced lung cancer.

Acute toxicity of metals and reference toxicants to a freshwater ostracod, *Cypris subglobosa* Sowerby, 1840 and correlation to EC(50) values of other test models

[Khangarot BS, Das S. J Hazard Mater. 2009 Dec 30;172(2-3):641-9. Epub 2009 Jul 17]

The ostracod *Cypris subglobosa* Sowerby, 1840 static

bioassay test on the basis of a 48h of 50% of immobilization (EC(50)) has been used to measure the toxicity of 36 metals and metalloids and 12 reference toxicants. Among the 36 metals and metalloids, osmium (Os) was found to be the most toxic in the test while boron (B), the least toxic. The EC(50) values of this study revealed positive linear relationship with the established test models of cladoceran (*Daphnia magna*), sludge worm (*Tubifex tubifex*), chironomid larvae (*Chironomus tentans*), protozoan (*Tetrahymena pyriformis*), fathead minnow (*Pimephales promelas*), bluegill sunfish (*Lepomis macrochirus*), and aquatic macrophyte duckweed (*Lemna minor*). Correlation coefficients (r(2)) for 17 physicochemical properties of metals or metal ions and EC(50)s (as pM) were examined by linear regression analysis. The electronegativity, ionization potential, melting point, solubility product of metal sulfides (pK(sp)), softness parameter and some other physicochemical characteristics were significantly correlated with EC(50)s of metals to *C. subglobosa*. The reproducibility of toxicity test was determined using 12 reference toxicants. The coefficient of variability of the EC(50)s ranged from 6.95% to 55.37% and variability was comparable to that observed for *D. magna* and other aquatic test models. The study demonstrated the need to include crustacean ostracods in a battery of bio tests to detect the presence of hazardous chemicals in soils, sewage sludges, sediments and aquatic systems.

Identification of polycyclic aromatic hydrocarbons in unleaded petrol and diesel exhaust emission

[Yadav VK, Prasad S, Patel DK, Khan AH, Tripathi M, Shukla Y. Environ Monit Assess. 2009 Jul 23. (Epub ahead of print)]

Inhalation of emissions from petrol and diesel exhaust particulates is associated with potentially severe biological effects. In the present study, polycyclic aromatic hydrocarbons (PAHs) were identified from smoke released from the automobile exhaust from petrol and diesel. Intensive sampling of unleaded petrol and diesel exhaust were done by using 800-$cm^{(3)}$ motor car and 3,455-$cm^{(3)}$ vehicle, respectively. The particulate phase of exhaust was collected on Whatman filter paper. Particulate matter was extracted from filter paper by using Soxhlet. PAHs were identified from particulate matter by reverse phase high performance liquid chromatography using $C^{(18)}$ column. Fourteen PAHs were identified in petrol and 13 in diesel samples after comparing with standard samples for PAH estimation. These inhalable PAHs released from diesel and petrol exhaust are known to possess mutagenic and carcinogenic properties, which may pose a potential risk for the health of inhabitants.

Induction of apoptosis by [6]-gingerol associated with the modulation of p53 and involvement of mitochondrial signaling pathway in B[a]P-induced mouse skin tumorigenesis

[Nigam N, George J, Srivastava S, Roy P, Bhui K, Singh M, Shukla Y. Cancer Chemother Pharmacol. 2009 Jul 24. (Epub ahead of print)]

To unravel the molecular mechanisms underlying the chemopreventive potential of [6]-gingerol, a pungent ingredient of ginger rhizome (*Zingiber officinale* Roscoe, Zingiberaceae), against benzo[a]pyrene (B[a]P)-induced mouse skin tumorigenesis, after [6]-gingerol (2.5 muM/animal) was given topically to the animals 30 min prior and post to B[a]P (5 mug/animal) for 32 weeks. At the end of the study period, the skin tumors/tissues were dissected out and examined histopathologically. Flow cytometry was employed for cell cycle analysis. Further immunohistochemical localization of p53 and regulation of related apoptogenic proteins were determined by Western blotting. Chemopreventive properties of [6]-gingerol were reflected by delay in onset of tumorigenesis, reduced cumulative number of tumors, and reduction in tumor volume. Cell cycle analysis revealed that the appearance of sub-G1 peak was significantly elevated in [6]-gingerol treated animals with post treatment showing higher efficacy in preventing tumorigenesis induced by B[a]P. Moreover, elevated apoptotic propensity was observed in tumor tissues than the corresponding non-tumor tissues. Western blot analysis also showed the same pattern of chemoprevention with [6]-gingerol treatment increasing the B[a]P suppressed p53 levels, also evident by immunohistochemistry, and Bax while decreasing the expression of Bcl-2 and Survivin. Further, [6]-gingerol treatment resulted in release of Cytochrome C, Caspases activation, increase in apoptotic protease-activating factor-1 (Apaf-1) as a mechanism of apoptosis induction. On the basis of the results we conclude that [6]-gingerol has chemopreventive potential against apoptosis induced in mouse skin tumors hence needs further investigation.

Anti-apoptotic role of omega-3-fatty acids in developing brain: perinatal hypothyroid rat cerebellum as apoptotic model

[Sinha RA, Khare P, Rai A, Maurya SK, Pathak A, Mohan V, Nagar GK, Mudiam MK, Godbole MM, Bandyopadhyay S] Int J Dev Neurosci. 2009 Jun; 27(4):377-83]

Inadequate maternal intake of omega-3-fatty acids (w3 FAs) causes adverse neurodevelopmental outcome in the progeny; however, their molecular mechanism of action is obscure. Since w3 FAs are known to inhibit neuronal apoptosis during neuro-degeneration, we investigated their possible contribution in regulating neuronal apoptosis during brain development. Using rat model of hypothyroidism-induced neuronal apoptosis, we provide evidence for anti-apoptotic role of w3 FAs during cerebellar development. w3 FAs were supplemented as a mixture of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) to pregnant and lactating rats, and primary hypothyroidism was induced by administering methimazole. The cerebella from postnatal

day 16 (d16) pups were isolated, and studies on apoptosis were conducted. We observed that w3 FA-supplementation significantly reduced DNA fragmentation and caspase-3 activation in developing cerebellum of hypothyroid pups. The protection provided by w3 FAs was associated with their ability to prevent increases in the level of pro-apoptotic basal cell lymphoma protein-2 (Bcl2)- associated X protein (Bax) in the cerebellum during thyroid hormone (TH) deficiency. w3 FAs increased the levels of anti-apoptotic proteins like Bcl2 and Bcl-extra large (Bcl-xL), known to be repressed in hypothyroidism. w3 FAs also restored levels of cerebellar phospho (p)-AKT, phospho-extracellular regulated kinase (p-ERK) and phospho-c-Jun N-terminal kinase (p-JNK), which were altered by hypothyroid insults, without interfering with the expression of TH responsive gene, myelin basic protein (mbp). Taken together, these results gave an insight into the molecular mechanism of action of w3 FAs in developing brain that involves regulation of apoptotic signaling pathways under stress.

Induction of apoptosis by lupeol in human epidermoid carcinoma A431 cells through regulation of mitochondrial, Akt/PKB and NFkappaB signaling pathways

[Prasad S, Madan E, Nigam N, Roy P, George J, Shukla Y. Cancer Biol Ther. 2009 Sep;8(17):1632-9]

There has been a rising incidence of skin cancer in humans which necessitates the need for better understanding and development of novel treatment and preventive approaches for skin cancer. Fruits and other plant derived products have gained considerable attention as they can reduce the risk of several cancer types. Lupeol, a triterpene, present in many fruits and medicinal plants, has been shown to possess many pharmacological properties including anti-cancer effect in both *in vitro* and *in vivo* assay systems. In the present study, apoptosis inducing effects of lupeol were studied in human epidermoid carcinoma A431 cells. Cell cycle analysis showed that lupeol treatment induces apoptosis (14-37%) in a dose-dependent manner as evident by an increased sub G(1) cell population. The RT-PCR and Western blot analysis showed that lupeol-induced apoptosis was associated with caspase dependent mitochondrial cell death pathway through activation of Bax, caspases, Apaf1, decrease in Bcl-2 expression and subsequent cleavage of PARP. Lupeol treatment also inhibited Akt/PKB signaling pathway by inhibition of Bad (Ser136) phosphorylation and 14-3-3 expression. In addition, lupeol treatment inhibited cell survival by inactivation of NFkappaB through upregulation of its inhibitor Ikappabetaalpha. The Caspase mediated apoptosis was noticed by decrease in lupeol induced apoptosis by Caspase inhibitors as well as increase in reactive oxygen species generation and loss of mitochondrial membrane potential. These results suggest that lupeol could be an effective anti-cancer agent and merits further investigation.

Hazardous effect of tannery solid waste leachates on development and reproduction in *Drosophila melanogaster*: 70kDa heat shock protein as a marker of cellular damage

[Siddique HR, Mitra K, Bajpai VK, Ravi Ram K, Saxena DK, Chowdhuri DK. Ecotoxicol Environ Saf. 2009 Sep;72(6):1652-62. Epub 2009 Jul 2]

Rapid industrialization has increased the burden of chemicals in the environment. These chemicals may be harmful to development and reproduction of any organism. We, therefore studied the adverse effects of leachates from a tannery solid waste on development and reproduction using Drosophila. We show a significant delay in mean emergence of flies observed at the higher concentrations of the leachates, indicating their effect on the organism's development. Significant leachate-induced effect on reproduction of the organism was also observed. Sub-organismal analyses revealed Hsp70 expression and tissue damage in a sex-specific manner. Refractoriness of Hsp70 expression in accessory glands of male flies and ovaries of females was concurrent with tissue damage. Genes encoding certain seminal proteins (Acp70A and Acp36DE) from accessory glands were significantly down-regulated at higher concentrations of the leachates. The study suggests that (i) sub-organismal adverse responses are reflected at organismal level, (ii) tannery waste leachates cause adverse effects on the expression of genes encoding seminal proteins that facilitate normal reproduction and (iii) Hsp70 may be used as a marker of cellular damage for reproductive organs.

Induction of apoptosis by tea polyphenols mediated through mitochondrial cell death pathway in mouse skin tumors

[Roy P, Nigam N, George J, Srivastava S, Shukla Y. Cancer Biol Ther. 2009 Jul;8(13):1281-7]

Many naturally occurring phytochemicals have shown cancer chemopreventive potential in a variety of bioassay systems. One such compound is tea, *Camellia sinensis*, which is the most consumed beverage in the world after water. The most abundant and active constituents of tea are polyphenols (epigallocatechin gallate and theaflavins). In the present study, cancer chemopreventive properties of both black tea polyphenols (BTP) and green tea polyphenols (GTP) on 7,12-dimethylbenz[a]anthracene (DMBA) induced mouse skin carcinogenesis were studied. BTP and GTP treatment showed delay in onset of tumorigenesis, reduction in cumulative number of tumors and increased tumor free survival. Both BTP and GTP were found to modulate the expression of proteins involved in apoptotic pathway. Tea polyphenols treatment along with DMBA exposure resulted in upregulation of p53, and proapoptotic protein Bax, whereas enhanced expression of antiapoptotic proteins, Bcl-2 and survivin by DMBA were down regulated. Further, we showed

that tea polyphenols supplementation resulted in release of cytochrome C, caspases activation, and increase in apoptotic protease activating factor and poly (ADP-ribose) polymerase cleavage as mechanism of apoptosis induction. The results also provide strong evidence that BTP is a better chemopreventive agent against skin tumorigenesis as compared to GTP at the tested dose levels. Thus, we can conclude that the polyphenolic constituents present in black tea and green tea induce mitochondrial pathway of apoptosis and hence can be used as a potential chemopreventive agents against skin cancer.

Altered platelet monoamine oxidase-B activity in idiopathic Parkinson's disease

[Husain M, Shukla R, Dikshit M, Maheshwari PK, Nag D, Srimal RC, Seth PK, Khanna VK. Neurochem Res. 2009 Aug;34(8):1427-32]

A case-control study was undertaken to investigate the status of platelet monoamine oxidase-B (MAO-B) activity in Indian cases of idiopathic Parkinson's disease. A significant increase in the activity of platelet MAO-B was observed in Parkinson's cases (n = 26) as compared to controls (n = 26). No significant change in the activity of the enzyme was observed while the data was analysed with respect to age, sex and duration of disease. A trend of decrease in platelet MAO-B activity was observed in Parkinson's cases with respect to stage although the change was not significant. No correlation in platelet MAO-B activity was observed with respect to age and sex in the control subjects. Parkinson's cases treated with L-DOPA and MAO-B inhibitor exhibited decreased platelet MAO-B activity as compared to drug naive cases and those treated with L-DOPA alone. Interestingly, Parkinson's cases treated with L-DOPA and amantadine also had lower platelet MAO-B activity as compared to drug naive cases and those treated with L-DOPA alone. Activity of platelet MAO-B in Parkinson's patients was increased in naive cases and those treated with L-DOPA alone or in combination with other drugs compared to controls. The results of the present study indicate that phenotypic activity of platelet MAO-B is high in Indian Parkinson's cases. Further, action mechanism of drugs used in the treatment of Parkinson's disease could be understood by assay of platelet MAO-B activity. It is an interesting observation and may be looked further in large number of cases.

Reduction of pollutants in pulp paper mill effluent treated by PCP-degrading bacterial strains

[Chandra R, Raj A, Yadav S, Patel DK. Environ Monit Assess. 2009 Aug;155(1-4):1-11. Epub 2008 Jul 12]

Two PCP-degrading bacterial strains, *Bacillus cereus* (ITRC-S6) and *Serratia marcescens* (ITRC-S7) were used for the treatment of pulp and paper mill effluent at conditions; 1.0% glucose and 0.5% peptone at 30 +/- 1 °C at 120 rpm for 168 h of incubation. These two bacterial strains effectively reduced colour (45-52%), lignin (30-42%), BOD (40-70%), COD (50-60%), total phenol (32-40%) and PCP (85-90%) within 168 h of incubation. However, the highest reduction in colour (62%), lignin (54%), BOD (70%), COD (90%), total phenol (90%) and PCP (100%) was recorded by mixed culture treatment. The mechanism for the degradation of pulp and paper mill effluent may be explained by an increase in the cells biomass using added co-substrates resulting in liberation of significant amount of chloride due to bacterial dechlorination of chlorolignins and chlorophenols and reduction in colour, lignin and toxicity in the effluent. Further, GC-MS analysis of ethyl acetate-extractable compounds from treated pulp paper mill effluent reinforces the bacterium capability for the degradation of lignin and pentachlorophenol, as many aromatic compounds such as 2-chlorophenol, 2, 4, 6-trichlorophenol and tetrachlorohydroquinone, 6-chlorohydroxyquinol and tetrachlorohydroquinone detected which were not present in the untreated effluent.

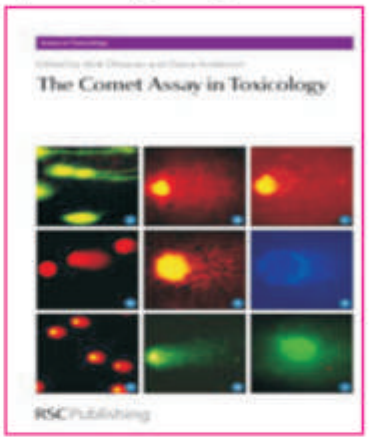
BOOK RELEASED - THE COMET ASSAY IN TOXICOLOGY

Dr Alok Dhawan, Scientist, Indian Institute of Toxicology Research, Lucknow, edited a book with Professor Diana Anderson, University of Bradford, U.K. entitled "The Comet Assay in Toxicology. This has been published by The Royal Society of Chemistry, U.K. under its Series "Issues in Toxicology" in September 2009.

The vast number of chemicals existing or being added into the environment, have globally aroused great concern regarding their adverse effects in human population. Development and validation of sensitive and better test systems which can assess the adverse effects of chemicals at an early stage for intervention strategies to be implemented in time is currently in progress. This book documents the latest research and showcases the versatile, state-of-the-art technique - the Comet assay - in the field of modern toxicology. The assay is a simple, sensitive, rapid and visual technique for the quantitative and qualitative assessment of DNA damage in single cells. It can be used to assess DNA damage in all single cells from prokaryotes and eukaryotes, in plants and animals including humans, involving both somatic and germ cells. It is also a relatively inexpensive assay to perform.

This book is the first of its kind devoted exclusively to the Comet assay and its applications as an important

tool in current toxicology. This multi-author book will serve both as a reference and a guide for investigators in the biomedical, biochemical and pharmaceutical sciences. Specialists from the fields of genetic toxicology and human epidemiology, with first-hand knowledge of their chosen sub-specialities, have contributed to this peer-reviewed scientific venture. Thirty eight authors from 11 countries have contributed to the 17 chapters in this book. The book is divided into four sections, reflecting the range of interest in the exploitation of this assay. It begins with an introductory section reviewing the genesis of the assay for those new to the technique, and details the various fields in which it finds wide acceptance. This sets the scene by explaining why the assay has become the most sensitive and sought after methods in modern toxicology. Section II describes the protocols being followed to assess various types of DNA damage in different cell types. The third section brings together the specific applications of the assay in diverse areas ranging from genetic toxicity testing to human monitoring, and environmental toxicology. The last section considers strategies for the conduct of the assay using *in vitro* and *in vivo* systems, based on internationally accepted guidelines. The book draws to a close with an assessment of image-analysis principles and the statistics used for evaluating the data generated by the assay. The book is aimed at students as well as scientists in the area of molecular epidemiology and genetic toxicology.



VISITS ABROAD

- Dr D. Kar Chowdhuri, Scientist deputed to U.K. under UK-IERI Major Research Award Project from June 27 to July 7, 2009.
- Dr Poonam Kakkar, Scientist deputed to Malaysia from July 9-12, 2009 to delivere a lecture during the 7th COSTAM/SFRR International Concerence on Chemoprevention and Translational Research held at Langkawi, Malaysia.
- Dr Alok Dhawan, Scientist deputed to Daejeon, South Korea from August 20-23, 2009 to deliver a lecture during the Nanobiotechnology workshop at the International Conference for the Integtration of Science and Technology into Society 2009-KAIST (ICISTS-KAIST 2009).
- Dr D. Kar Chowdhuri, Scientist deputed to Florence, Itlay from August 20-25, 2009 for attending 10th International Conference on Environmental mutagens.
- Dr Alok Dhawan, Scientist visited University of Bradford, U.K. under UKIERI Project and also visited Astana, Kazakhstan for delivering an invited lecture from September 6-27, 2009.

RESEARCH DIGEST

A Novel Two-Step Hierarchical Quantitative Structure–Activity Relationship Modeling Work Flow for Predicting Acute Toxicity of Chemicals in Rodents

[Environ Health Perspect 117:1257–1264 (2009)]

Accurate prediction of *in vivo* toxicity from *in vitro* testing is a challenging problem. Large public–private consortia have been formed with the goal of improving chemical safety assessment by means of high-throughput screening. A wealth of available biological data requires new computational approaches to link chemical structure, *in vitro* data, and potential adverse health effects. A database containing experimental cytotoxicity values for *in vitro* half-maximal inhibitory concentration (IC_{50}) and *in vivo* rodent median lethal dose (LD_{50}) for more than 300 chemicals was compiled by Zentralstelle zur Erfassung und Bewertung von Ersatz- und Ergaenzungsmethoden zum Tierversuch (ZEBET ; National Center for Documentation and Evaluation of Alternative Methods to Animal Experiments) . The application of conventional quantitative structure–activity

relationship (QSAR) modeling approaches to predict mouse or rat acute LD_{50} values from chemical descriptors of ZEBET compounds yielded no statistically significant models. The analysis of these data showed no significant correlation between IC_{50} and LD_{50}. However, a linear IC_{50} versus LD_{50} correlation could be established for a fraction of compounds. A novel two-step modeling approach has been developed. First, all chemicals are partitioned into two groups based on the relationship between IC_{50} and LD_{50} values: One group comprises compounds with linear IC_{50} versus LD_{50} relationships, and another group comprises the remaining compounds. Second, conventional binary classification QSAR models to predict the group affiliation based on chemical descriptors only. Third, *k*-nearest neighbour continuous QSAR models for each subclass to predict LD_{50} values from chemical descriptors. All models were extensively validated using special protocols. The novelty of this modeling approach is that it uses the relationships between *in vivo* and *in vitro* data only to inform the initial construction of the hierarchical two-step QSAR models. Models resulting from this approach employ chemical descriptors only for external prediction of acute rodent toxicity.

A Breathalyzer for Cancer

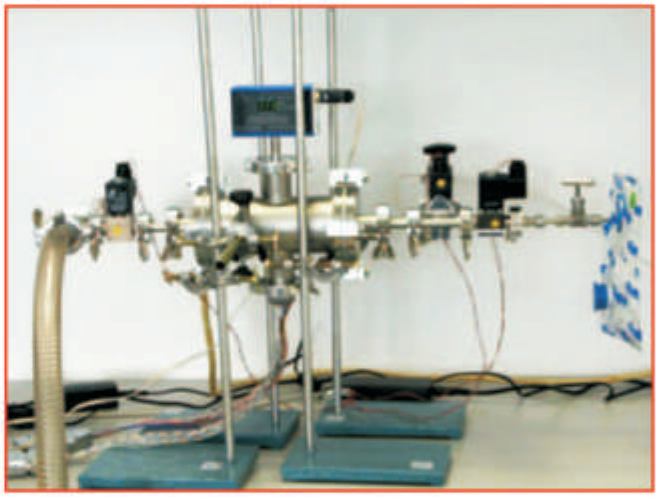
[Nature, doi:10.1038/news.2009.858]

A team of researchers may have come up with a golden idea for diagnosing lung cancer. By coating tiny nuggets of gold with a thin layer of organic material, the researchers have developed an "electronic nose" that, with some additional work, could spot lung cancer instantly by analyzing someone's breath. Hossam Haick and colleagues at the Technion-Israel Institute of Technology in Haifa embedded the 5-nanometer-long gold nanoparticles in a silicon wafer and then collected exhaled air from 40 cancer patients and 56 people with healthy lungs. All the subjects had to breathe deeply through a purifying filter for 5 minutes. After this "lung washout," they filled five 750-milliliter Mylar bags with air. A machine blew this air over the silicon-gold circuit, and the electrical resistance of the gold nanoparticles rose or fell depending on the presence or absence of certain compounds.

Cancer cells exude different compounds than healthy cells do, Haick explains, and the circuit picks up this difference. Tumor growth causes stress in cells, leading to a build up of free radical molecules that attack the lipids in cell walls, tearing out molecules with long chains of carbon atoms. The team identified 42 such molecules and settled on four to track with the nanoparticles: decane, trimethylbenzene, ethylbenzene, and heptanol. These four molecules appear at relatively high concentrations and, after binding to the organic coat on the nanoparticles, cause the resistance to electric current in the circuit to fluctuate in a predictable way. The sensors respond rapidly and are completely reusable, the team reported it online on 30 August in Nature Nanotechnology.

The gold circuit, now patented, is a big improvement over Haick's previous electronic nose, which used carbon nanotubes. The resistance of the nanotubes was too sensitive to water vapor, a major component of breath, whereas gold particles are unperturbed by water. After finishing the Nature Nanotechnology paper, Haick's team discovered a bonus: With gold, patients don't have to avoid alcohol, coffee, tobacco, or food before tests, all of which had confounded previous devices. "This paper is the best one I've seen on the sensor technology," says David Smith, a physical chemist at Keele University in the United Kingdom. He says the breath diagnosis is especially promising for detecting lung cancers early, before they show up on x-rays. However, Smith says he has seen similar research unravel when scientists tried to develop tools for doctors, which ideally must be small, portable, and inexpensive. He also cautions that blinded clinical trials will be necessary to determine if the pricey nanoparticles can complement or replace existing tools, such as biopsies and x-rays.

Golden idea. Gold nanoparticles in this sensor can detect lung cancer by analyzing people's breath.



Ozone Threat is no Laughing Matter

[Science advance online publication doi:10.1126/science.1176985 (2009)]

Nitrous oxide (N_2O) has become the greatest threat to the ozone layer, a new analysis suggests. The ozone-destroying abilities of the gas have been largely ignored by policy-makers and atmospheric scientists alike, who have focused on the more potent chlorofluorocarbons (CFCs) — historically the dominant ozone-depleting substances in the atmosphere.

But CFC levels have been falling since the 1989 adoption of the Montreal Protocol on Substances that Deplete the Ozone Layer, an international agreement that mandated the phasing out of CFCs, and more recently hydrochlorofluorocarbons (HCFCs). Meanwhile, nitrous oxide levels have been climbing as a result of increased emissions from agricultural fertilizers, biomass burning and

animal waste. Atmospheric chemist A. R. Ravishankara and his colleagues at the National Oceanic and Atmospheric Administration (NOAA) in Boulder, Colorado, have now used a chemical model of stratospheric ozone to calculate the ozone-depleting potential (ODP) of nitrous oxide. That provides a measure of how much ozone is depleted by a particular gas, relative to that destroyed by the same amount of trichlorofluoromethane (CCl_3F, also known as CFC-11), one of the most significant ozone-depleting substances. "We wanted to see how nitrous oxide stacked up as an ozone-depleting gas," says Ravishankara. "People haven't looked at it this way before."

They computed the ODP of nitrous oxide at 0.017, or about one-sixtieth of that of CFC-11. This seems like a pretty feeble punch, but when the authors took into account the large scale of human-related emissions of nitrous oxide — as estimated in the latest report from the Intergovernmental Panel on Climate Change — they found that nitrous oxide has the greatest impact of the ozone-depleting substances emitted by human-related activities today. Nitrous oxide is also a major greenhouse gas which is controlled under the Kyoto Protocol on climate change, although emissions are not currently expected to fall significantly in the coming century. The authors project that if nitrous oxide emissions are not reduced, they could be 30% more destructive to ozone in 2050 than the combined CFC emissions from 1987, when these were at their peak. The team's results are published online by the journal Science. "This is the first time someone has dealt with nitrous oxide in isolation like this," says atmospheric chemist Susan Solomon of the NOAA, who was not involved in the study. "It's one of those things that has simply been overlooked." Atmospheric scientists have known since the 1970s that nitrous oxide depletes the ozone layer, but did not group it with other ozone-depleting substances because it seemed to be impotent relative to CFCs. Atmospheric scientist Don Wuebbles at the University of Illinois in Urbana-Champaign agrees that nitrous oxide deserves more attention. "In a sense, nitrous oxide is almost a forgotten gas. When we talk about ozone, we talk about halocarbons. When we talk about climate, we talk about carbon dioxide and methane. We forget that nitrous oxide is the third largest-growing gas in the atmosphere."

The findings won't come as a surprise to most atmospheric scientists, says Ravishankara. "Everyone's going to say they knew it. But that's not the same as showing it." That distinction has important implications for policy-makers, who use the ODP to make quantitative comparisons between ozone-depleting substances. "Without this information, decision-makers do not have the tools to evaluate the role of nitrous oxide in ozone-layer depletion. In that sense, we have bridged the gap between policy relevance and atmospheric science," says Ravishankara.

But not everyone is concerned about nitrous oxide's impact on the ozone layer. "Nitrous oxide sort of died out as a problem [for the ozone layer] in the 1970s, because we knew it was increasing at such a slow rate," says atmospheric chemist Richard Stolarski at NASA's Goddard Space Flight Center in Greenbelt, Maryland. "In our chemical climate models, where nitrous oxide increases by 15 or 20 per cent by 2100, we still end up with more ozone than we had in 1960 [before mass production of CFCs]." Ravishankara notes that ozone-depleting gases should still be a cause for concern. "Now it's up to the decision-makers on how they're going to deal with this," he says. "This is just one piece of information to feed into the discussion."

Nitrous oxide is damaging the Earth's protective ozone layer. NASA

Climate will Cost Much More than UN Thinks

[New Scientist, 04 September 2009]

A key number in the struggle to tackle global warming is flawed. The cost of adapting to the effects of climate change is two to three times the figure quoted by the UN climate change convention, a new study claims. The UN has estimated that meeting health needs, adapting farming and infrastructure, and so on will cost $70 to 100 billion a year by 2030. Now Martin Parry of Imperial College London says this "back of a metro ticket calculation" ignores major sectors, including energy generation and manufacturing, as well as the costs of protecting people from inland flooding and coastal storms, and of maintaining ecosystems. It badly underestimates other areas, too. "If you read the small print, the health figure covers the cost of preventing increases in just three diseases - malaria, diarrhoea and malnutrition," says Parry. "The real figure will be far higher." Parry fears that the UN figure could be damaging if it is when politicians meet in December to agree on future emissions targets. They might conclude it is cheaper to adapt to climate change than to prevent it, he says.

Fat Reprograms Genes Linked to Diabetes

[Cell Metabolism, DOI: 10.1016/j.cmet.2009.07.011]

A gene that helps muscle cells burn fat can be radically altered and switched off if the cells carrying it are exposed

to fat. The finding suggests that the same process may occur when people eat too much fat-rich junk food, resulting in drastic changes to this "fat burning" gene. "Somehow, the environment plays on the genes we have," says lead researcher, Juleen Zierath of the Karolinska Institute in Stockholm, Sweden. She says her team's findings provide new clues to how this happens, and may help explain how type II diabetes develops in adulthood. One possibility, she says, is that the altered cells become so engorged with unburnt fat, they become "diabetic", no longer accepting signals from the hormone insulin, which normally triggers the absorption of glucose from the bloodstream. But proof that components in the diet can permanently alter genes is itself a breakthrough, providing the first evidence that the food we eat may change the function of our DNA. This is a process known as "epigenetics".

In this study the DNA itself remained unchanged, except for a masking process called methylation which can permanently mothball a gene by capping individual chemical units, or bases. Earlier in the same set of experiments, the researchers discovered that muscle cells from people with type II diabetes already showed these telltale epigenetic alterations to their DNA, particularly in the PGC-1 gene, which orchestrates metabolic programmes critical to the burning of fat in mitochondria, the chambers in cells that generate energy. By contrast, the healthy muscle cells from non-diabetics functioned normally. The most significant result came when one team member, Romain Barrés, exposed the healthy muscle cells to the edible fatty acid, palmitic acid. He found that the PGC-1 gene became methylated, just as it is in people with diabetes. "The palmitic acid essentially switches off the gene," says Zierath. The same thing happened on exposure to tumour necrosis factor-alpha, a substance produced by white blood cells to help fight infection.

But the fact fat produced the effect is highly significant, because it means that over-consumption of junk food could produce the same response. "It suggests that if you eat a fat-rich diet, something in that - either the fat itself or the build up of metabolites - triggers the methylation of genes. The net effect is that it switches off the gene," says Zierath. This, in turn, could lead to the gradual shutdown of mitochondria, an effect already observed in muscle cells from type II diabetics. The team's analyses also reveal that the shutdown of PGC-1 led to inactivation of other genes vital for burning or transporting fat, such as those that produce the enzymes citrate synthase and carnitine palmitoyltransferase-2. The next step, says Zierath, is to find out how different diets affect the methylation status of PGC-1 and other genes vital for burning energy. In one study, she hopes to take muscle biopsies from obese patients before and after they undergo bariatric surgery to cut appetite by reducing the size of their stomachs. Through this and other experiments to probe the effects of diet on gene function, Zierath and her colleagues hope to tease out a potential mechanism by which type II diabetes develops. One intriguing unknown is whether methylation of genes triggered by exposure to fat is inheritable. If it is, it means that the "disease" would be handed down from parents to their children and could explain previous research indicating that what you eat could affect all your descendents.

Toxic Chemicals Found in Laundry Soaps and Air Fresheners

[http://www.livescience.com/health/080724-toxic-laundry.html]

A study of top-selling laundry products and air fresheners found the products emitted dozens of different chemicals. All six products tested gave off at least one chemical regulated as toxic or hazardous under federal laws, but none of those chemicals was listed on the product labels. "I first got interested in this topic because people were telling me that the air fresheners in public restrooms and the scent from laundry products vented outdoors were making them sick," said Anne Steinemann, a University of Washington professor of civil and environmental engineering and of public affairs. "And I wanted to know, 'What's in these products that is causing these effects?'" Manufacturers of consumer products are not required to disclose the ingredients, so Steinemann analyzed the products to discover their contents. "I was surprised by both the number and the potential toxicity of the chemicals that were found," Steinemann said. Chemicals included acetone, the active ingredient in paint thinner and nail-polish remover; limonene, a molecule with a citrus scent; and acetaldehyde, chloromethane and 1,4-dioxane. Nearly 100 volatile organic compounds were emitted from the six products, and none were listed on any product label, she said. Plus, five of the six products emitted one or more carcinogenic "hazardous air pollutants," which are considered by the Environmental Protection Agency to have no safe exposure level, Steinemann said.

Steinemann chose not to disclose the brand names of the six products she tested. But in a larger study of 25 cleaners, personal care products, air fresheners and laundry products, now submitted for publication, Steinemann found that many other brands contained similar chemicals. Steinemann studied three common air fresheners (a solid deodorizer disk, a liquid spray and a plug-in oil) and three laundry products (a dryer sheet, fabric softener and a detergent), selecting a top seller in each category. She bought household items at a grocery store and asked companies for samples of industrial products. In the laboratory, each product was placed in an isolated space at room

temperature and the surrounding air was analyzed for volatile organic compounds, small molecules that evaporate from the product's surface into the air. Results showed 58 different volatile organic compounds above a concentration of 300 micrograms per cubic meter, many of which were present in more than one of the six products. For instance, a plug-in air freshener contained more than 20 different volatile organic compounds. Of these, seven are regulated as toxic or hazardous under federal laws. The product label lists no ingredients.

This study does not address links between exposure to chemicals and health effects. However, two national surveys published by Steinemann and a colleague in 2004 and 2005 found that about 20 percent of the population reported adverse health effects from air fresheners, and about 10 percent complained of adverse effects from laundry products vented to the outdoors. Among asthmatics such complaints were roughly twice as common. Manufacturers are not required to list the ingredients used in laundry products and air fresheners. Personal-care products and cleaners often contain similar fragrance chemicals, Steinemann said. And although cosmetics are required by the Food and Drug Administration to list ingredients, no law requires products of any kind to list chemicals used in fragrances.

"Fragrance chemicals are of particular interest because of the potential for involuntary exposure, or second-hand scents," Steinemann said. "Be careful if you buy products with fragrance, because you really don't know what's in them," she added. "I'd like to see better labeling. In the meantime, I'd recommend that instead of air fresheners people use ventilation, and with laundry products, choose fragrance-free versions." The European Union recently enacted legislation requiring products to list 26 fragrance chemicals when they are present above a certain concentration in cosmetic products and detergents. No similar laws exist in the United States. "I hope this study will raise public awareness, and reduce exposures to potentially hazardous chemicals," said Steinemann.

Cockroaches future-proofed against climate change

[The Journal of Experimental Biology, DOI: 10.1242/jeb.031310]

Hate cockroaches? Best pour yourself a stiff drink. The widely loathed insects can hold their breath to save water, a new study has found - and the trick could help them to thrive in the face of climate change. When cockroaches are resting, they periodically stop breathing for as long as 40 minutes, though why they do so has been unclear. To investigate the mystery, Natalie Schimpf and her colleagues at the University of Queensland in Brisbane, Australia,

Climate change? Not bothered

examined whether speckled cockroaches (*Nauphoeta cinerea*) change their breathing pattern in response to changes in carbon dioxide or oxygen concentration, or humidity.

They conclude that cockroaches close the spiracles through which they breathe primarily to save water. In dry environments the insects took shorter breaths than in moist conditions. "Cockroaches lose water across their respiratory surfaces when they breathe," says Schimpf, "so taking shorter breaths in dry conditions reduces the amount of water they will lose." The study deals a blow to the theory that cockroaches hold their breath to survive underground, where CO_2 levels can be poisonous. "They held their breath no longer in high-CO_2 than in low-CO_2 conditions," says Schimpf. Nor did the study support the idea that cockroaches hold their breath to avoid damage to their body tissue from chemical reactions with oxygen.

The same doesn't necessarily apply to other insects, warns John Terblanche at Stellenbosch University in South Africa. "Our research suggests that butterfly pupae hold their breath to prevent oxygen damage, rather than to conserve water," he says. The nifty breath-holding adaptation has allowed cockroaches to colonise drier habitats, says George McGavin of the University of Oxford, and may allow them to thrive in climate change. "Cockroaches have an awesome array of adaptations to life on dry land," says McGavin. "Living in the humid conditions of a rainforest, where they evolved, might be plain sailing, but cockroaches are adaptable and can cope in a wide range of environmental conditions." Will the sun ever set on the empire of the cockroach? Not any time soon, says McGavin. "Two hundred and fifty million years of physiological fine tuning has produced a creature that will be around for a long time to come," he says. "Cockroaches will do well in the face of climate change."

भारतीय विष अनुसंधान संस्थान (आई.आई.टी.आर.) में हिंदी सप्ताह का आयोजन

भारतीय विष विज्ञान अनुसंधान संस्थान (आई.आई.टी. आर.) लखनऊ में दिनांक 14.09.2009 को प्रातः 11:00 बजे हिंदी सप्ताह 14 से 20 सितंबर, 2009 के उद्घाटन समारोह का आयोजन किया गया। इस अवसर पर मुख्य अतिथि श्री समरेन्द्र कुमार, परियोजना संयोजक, आंचलिक विज्ञान नगरी, लखनऊ थे। श्री मुकुन्द सहाय, प्रशासनिक अधिकारी ने मुख्य अतिथि का औपचारिक परिचय दिया। संस्थान के निदेशक डॉ0 के0 सी0 गुप्ता ने मुख्य अतिथि को स्मृति चिह्न भेंट किया। मुख्य अतिथि ने अपने संदेश में कहा कि हिंदी आम आदमी की भाषा है और यह संपर्क भाषा भी है। हमें वैज्ञानिक अनुसंधानों को जनसामान्य हेतु हिंदी में प्रकाशित करने का प्रयास करना चाहिए ताकि ज्यादा से ज्यादा लोगों को इसका लाभ मिल सके। उन्होंने आमंत्रण हेतु निदेशक महोदय का आभार व्यक्त किया। संस्थान के निदेशक डॉ0 के0 सी0 गुप्ता ने मुख्य अतिथि द्वारा आमंत्रण स्वीकार करने के लिए आभार व्यक्त करते हुए कहा कि हमारे संस्थान में हिंदी की प्रगति अच्छी है और इसमें और प्रयास किये जा रहे हैं। संस्थान में वैज्ञानिकों को हिंदी में अपने अनुसंधान कार्यों को प्रस्तुत करने हेतु हिंदी संगोष्ठी का आयोजन भी किया जायेगा। हिंदी के विकास के लिए उन्होंने दिल से कार्य करने के लिए अपील की, उन्होंने हिंदी सप्ताह के दौरान आयोजित होने वाली प्रतियोगिताओं में सभी से बढ़-चढ़कर भाग लेने की अपील की। श्री प्रदीप कुमार, अनुभाग अधिकारी ने कार्यक्रम का संचालन किया और सभी के प्रति आभार व्यक्त किया।

दिनांक 18.09.2009 को पुरस्कार वितरण एवं समापन समारोह का आयोजन हुआ। इस अवसर पर संस्थान के निदेशक, डॉ0 के0 सी0 गुप्ता ने कहा कि जिस तरह से हिंदी सप्ताह मनाया गया और लोगों ने बढ़-चढ़कर भाग लिया, यह बहुत सराहनीय है। उन्होंने कहा कि केवल हिंदी सप्ताह मनाने से ही हिंदी को बढ़ावा नहीं मिलेगा बल्कि हिंदी में काम करने की रूचि पैदा होनी चाहिए। पूरे वर्ष हिंदी में कोई न कोई कार्यक्रम आयोजन के लिए संस्थान की राजभाषा कार्यान्वयन समिति अपनी महती भूमिका का निर्वाहन करे, जिससे हिंदी को और अधिक सशक्त बनाया जा सके। यदि प्रयास किया जाए तो हिंदी भाषा में कार्य किया जाना कोई मुश्किल कार्य नहीं है। हिंदी के प्रति जागरूक होने की आवश्यकता है। उन्होंने इस अवसर पर हिंदी सप्ताह के दौरान आयोजित वाद-विवाद, आशुभाषण, लेख, टिप्पणी व मसौदा लेखन, अहिंदी भाषी का हिंदी ज्ञान, हिंदी टंकण, अनुवाद, प्रश्नोत्तरी प्रतियोगिताओं और हिंदी में कार्य करने की प्रोत्साहन योजना के अन्तर्गत विजयी प्रतिभागियों को पुरस्कार और प्रमाण पत्र वितरित किया। श्री मुकुन्द सहाय, प्रशासनिक अधिकारी ने धन्यवाद प्रस्ताव दिया। कार्यक्रम का संचालन श्री प्रदीप कुमार, अनुभाग अधिकारी ने किया।

निदेशक महोदय, सभा को संबोधित करते हुए

आर्सेनिक विषाक्तता एवं स्वाइन-फ्लू

अबरार अहमद, पूजा सिंह एवं अशोक कुमार चौहान
पर्यावरण जैव प्रौद्योगिकी विभाग,
भारतीय विष विज्ञान अनुसंधान संस्थान

जिस प्रकार आजकल स्वाइन-फ्लू (H1N1) का प्रकोप है उसी प्रकार से आज से तीन वर्ष पूर्व बर्ड फ्लू (H5N5) का प्रकोप छाया हुआ था। विश्व स्वास्थ्य संगठन (World Health Organigation) यू.एस. के आंकड़ों के अनुसार सन् 2003 से 2006 तक बर्ड-फ्लू (H1N1) से 163 व्यक्ति संक्रमित हुए जिसमें से 91 व्यक्तियों की मृत्यु हो गयी। इसमें से अकेले वियतनाम में 93 व्यक्ति संक्रमित हुए और 42 लोगों की इससे मृत्यु हो गई। इण्डोनेशिया, थाइलैण्ड, चीन, तुर्की, कम्बोडिया और इराक आदि देशों में भी इसका संक्रमण फैला और इससे जनहानि हुई। भारत में बर्ड-फ्लू के कुल 126 व्यक्तियों में संक्रमण के मामले सामने आये। लेकिन इससे किसी की मृत्यु नहीं हुई।

बर्ड-फ्लू के विषाणु (वायरस) चिकन (मुर्गा या मुर्गी) में पाये जाते हैं। इसलिए लोगों ने चिकन खाना बंद कर दिया था। वास्तव में ये विषाणु पानी में तैरने वाले और उड़ने में असक्षम पक्षियों जैसे कि बतख, मुर्गा-मुर्गी और वाटर फाइल में प्राकृतिक रूप से वास करते हैं। जब ये विषाणु दूसरे पक्षी को संक्रमित करते हैं तो यह बीमारी फैलती है। उनके आवागमन से यह और भी दूर तक अपना प्रकोप दिखाती है।

अब यदि स्वाइन-फ्लू (H1N1) की चर्चा करें तो ज्ञात होगा कि वही विषाणु (बर्ड-फ्लू टाइप विषाणु) अपने कुछ जैनेटिक फूट प्रिंट (Genetic Foot Print) को परिवर्तित करके स्वाइनों में भी वास करते हैं तो इसे स्वाइन-फ्लू कहते हैं और ये विषाणु यहीं से फैलता है। इसे हार्सफ्लू, हॉग-फ्लू और पिग-फ्लू आदि नामों से भी जाना जाता है। पिछले एक दशक में 892 व्यक्ति स्वाइन संक्रमित पाये गये थे। इनमें से 253 व्यक्तियों की मृत्यु भी हो गई थी। वैश्विक स्तर पर 12,000 व्यक्तियों में भी इससे संक्रमण पाया गया और 4428 व्यक्तियों की इससे अब तक मृत्यु हो चुकी है। भारत में अब तक स्वाइन-फ्लू (H1N1) से संक्रमण के 239 मामले सामने आ चुके हैं जिसमें मरने वालों की संख्या 106 के लगभग है। इसमें से अकेले पुणे शहर में 31 लोगों की मृत्यु हुई। स्वाइन फ्लू के इतिहास पर नजर डाले तो H1N1 स्पेनिश फ्लू (Spenish Flu) प्रकोप से जो कि स्वाइन-फ्लू का ही एक रूप है, सन् 1918-1919 ई. में 500 लाख व्यक्तियों की मृत्यु हुई। इसके बाद सन् 1957-58 में H2N2 एशियन फ्लू (Asian Flu) से 7.5 लाख और 1968-69 में H3N2, हाँग-काँग (Hong Kong) फ्लू से 7.0 लाख लोगों की मृत्यु हुई। इसके बाद भी यह महामारियाँ बड़े स्तर पर हानि पहुँचाती रही हैं।

बर्ड-फ्लू एवं स्वाइन-फ्लू का विषाणु पहली बार सन् 1901 में इटली में एक बिल्ली के अन्दर पाया गया। बर्ड-फ्लू को वायवीय (Avian-Influya) एवं दूसरे को स्वाइन-फ्लू इन्फ्लूएन्जा भी कहते हैं। इन्फ्लूएन्जा सामान्यतः A, B एवं C तीन संबंधित विषाणुओं के संक्रमण से होता है, B एवं C प्रकार के विषाणु अधिकांशतः मानव में पाये जाते हैं और विभिन्न प्रकार की फ्लू बीमारियाँ पैदा करते हैं। उदाहरण के लिए आँख (Eye-Flu) नाक (Nose Flu) आदि। दोनों का आनुवंशिक पदार्थ (Genetic material) RNA होता है। बर्ड फ्लू विषाणु क्लास आर्थोमिक्सीविरिडी का सदस्य है। स्वाइन फ्लू विषाणु क्लास फ्लू लेविरिडी का सदस्य है।

A प्रकार के इनफ्लूंजा विषाणुओं को दो सब (उप) प्रकार में बाँटा गया है यह विभाजन उनके सतह पर पाये जाने प्रोटीन हीमोग्लूटिनिन एवं न्यूरामिनिडेज के आधार पर किया गया है। इस आधार पर 15, HA प्रकार के विषाणु एवं 9, NA प्रकार के विषाणु ज्ञात हैं। वैसे तो सभी तरह के HA एवं NA उप-प्रकार पक्षियों में पाये जाते हैं, लेकिन चार प्रकार के HA (H1, H2, H3 और H5) और NA (दो प्रकार का NA1 और NA2) मानव में परिसंचरण करते हैं।

विषाणु के सतह पर पाया जाने वाला प्रोटीन हीमोग्लूटिनिन होस्ट कोशिका के रेसेप्टर से जुड़ता (Attach) है जबकि न्यूरामिनिडेज प्रोटीन (NA) बड्डिंग को प्रमोट करता है जिससे कि नये विषाणु बन जाते हैं और संक्रमित व्यक्ति के भीतरी अंगों में फैल जाते हैं। इस प्रकार ये प्रोटीन विषाणु के फैलाव (Spread) में सहायता करता है। दोनों का आनुवंशिक पदार्थ बहुत ही अस्थायी होता है।

इनके अन्दर कोई भी रिपेयर (मरम्मत) तंत्र नहीं होता है। इसलिए एक बार उत्परिवर्तन होने के बाद उसमें कोई मरम्मत नहीं होती है। ये विषाणु Antigenic drift (एण्टीजेनिक ड्रिफ्ट) एवं Antigenic shift (एण्टीजेनिक शिफ्ट) के कारण अपना Antigenic गुणधर्म परिवर्तित करते रहते हैं, जिससे ये प्रतिरक्षी-कोशिकाओं के पहुँच या पहचान में नहीं आ पाते हैं और बीमारी फैलाने में सफल हो जाते हैं।

ट्रांसमिशन (संप्रेषण या संचरण) बर्ड-फ्लू के विषाणुओं का फैलाव (spread) पक्षियों में संक्रमण द्वारा होता है। जब यह संक्रमित पक्षी (मुर्गा या मुर्गी) मनुष्य के सम्पर्क में आता है तो मनुष्य को संक्रमित कर देता है। फिर यह मानव से मानव में फैलता है। लगभग इसी प्रकार से स्वाइन-फ्लू का संक्रमण होता है। इसमें मात्र एक अन्तर यह है कि इसके विषाणु स्वाइन (सुअर) में पाया जाता है मनुष्य शरीर में प्रवेश के बाद यह अपने सतह के हीमोग्लूटिनिन एवं न्यूरामिनिडेज प्रोटीन में बड़ी तेजी से उत्परिवर्तन (Mutation) करते हैं जिससे कि शरीर प्रतिरक्षा-तंत्र इसके विरूद्ध एण्टीबाडी नहीं बना पाते हैं।

लक्षण (Symptoms) दोनों में लक्षण परम्परागत इन्फ्लूएन्जा बीमारियों जैसे ही होते हैं। जैसेकि खाँसी आना, बुखार आना, गले में खरास, सिर में दर्द, मांसपेशियों में दर्द। गम्भीर मामलों में तेज-बुखार जो कि तंत्रिकाजनित (Neurologial) समस्यायें पैदा करता है। निमोनिया होना जो कि Sepsis लाता है। अत्यधिक उल्टी और दस्त के कारण निर्जलीकरण भी होता है। इससे शरीर में विद्युत-अपघट्य पदार्थों का असंतुलन हो जाता है और शरीर सुचार-संचालन बिगड़ जाता है। नये आयु के बच्चों और वृद्ध-वयस्कों में ये बहुत घातक होता है एवं मृत्यु का कारण भी बन सकता है।

बर्ड-फ्लू के मामले में इन्क्यूवेशन पीरियड औसतन 2-8 दिन और 8-17 दिन अधिकतम होता है। लेकिन स्वाइन-फ्लू में औसतन 5-15 दिन और 15-30 दिन अधिकतम Incubation Period होता है।

विषाणुवीय परीक्षण (Virological Diagnosis) दोनों ही मामलों में नेसोफैरिन्जियल (Nasopharyngeal swap) एवं रेस्पॉयरेटरी स्वैप (Respiratory swap) का Antigenic Assay करते हैं। RT-PCR के जरिये भी इन विषाणुओं का पता लगाया जा सकता है।

बर्ड-फ्लू को रोकने के उपाय

1. यदि किसी पोल्ट्री फार्म में बर्ड-फ्लू (H5N1) पाया जाता है तो उसके मुर्गे-मुर्गियों को नष्ट कर देना चाहिए।
2. माइग्रेटिंग-पक्षियों के आवागमन पर प्रतिबन्ध लगा देना चाहिए।
3. सुरक्षा के मापदण्ड जैसे मास्क, दस्ताना, चश्में, सुरक्षा वाले कपड़े पहनना इत्यादि के प्रयोगों को अपनाना चाहिए।
4. संक्रमित पक्षियों के पंखों, श्लेष्म, उत्सर्जी-पदार्थों मल-मूत्र से बचाव करना चाहिए।
5. डिटर्जेण्ट और साबुन से हाथ धोने से विषाणुओं की लिपिड-लेयर टूट जाती है और विषाणु समाप्त हो जाते हैं इसलिए अपना मुँह, नाक इत्यादि छूने से पूर्व एवं खाना खाने के पूर्व हाथ साबुन से भली-भाँति धोना चाहिए।
6. चिकन या अण्डे को 100 डिग्री सेंटीग्रेट से अधिक तापमान पर पका कर खाना चाहिए।
7. जिस क्षेत्र में यह मामले पाये जाते हैं उस क्षेत्र में सभी प्रकार के चिकन के व्यापार या आवागमन पर तात्क्षणिक प्रतिबन्ध लगा देना चाहिए।

स्वाइन-फ्लू के रोक-थाम के उपाय

1. जिस स्वाइन में यह पाया जाता है तो उसे दूसरे स्वस्थ स्वाइनों से दूर रखना चाहिए। उनके देखरेख में वे सारी सावधानियाँ लेना चाहिए जैसा कि बर्ड-फ्लू (H5N1) की बीमारी में लेना पड़ता है जैसे कि मास्क पहनना, दस्ताना पहनना, सुरक्षा वाले वस्तु धारण करना आदि।
2. अब यदि स्वाइन में संक्रमण है तो उसे मानव तक पहुँचने से रोकना चाहिए। संक्रमण वाले क्षेत्र में मानव-क्रिया-कलाप बिल्कुल कम कर देना चाहिए।
3. अब अगर यह संक्रमण मानव में पहुँच चुका है तो इससे मिलने वालों को मास्क पहनकर मिलना चाहिए और रोगी को अलग कमरे में रखना चाहिए।

उपचार :-

1. दोनों के ही इलाज के लिए जब बीमारी गम्भीर अवस्था में हो तो Tamiflu (Oseltamivir phosphate) 75mg दी

जाती है। यह दवा Neuraminidase प्रोटीन से जुड़कर विषाणु (Virus) बड्डिंग को रोकती है।

2. दूसरी असरदार दवा Relenza (Zenamivir phosphate) है।

3. रामान्टाडीन, अमन्टाडीन नामक दवायें Mild मामलों में दी जाती हैं।

4. अनामनडीन्स (Anamndeans) नामक दवा संक्रमित चिकन (पक्षी) एवं स्वाइनों को दी जाती है।

वैक्सीन :-

अभी हाल में ही विश्व को तीन बड़ी दवा निर्माता कम्पनियों ने स्वाइन-फ्लू लू के विरूद्ध वैक्सीन बनाने का दावा किया है। अमेरिका, जर्मनी, फ्रांस, मैक्सिको आदि देशों ने अपनी-अपनी स्टाक बुक करा ली है और आने वाले समय में इसका प्रयोग कर सकते हैं। भारत में इस वैक्सीन को ट्रायल के लिए विभिन्न प्रयोगशालाओं में परीक्षण के लिए भेज दिया गया है। परीक्षण के बाद ही इसका इस्तेमाल की आज्ञा मिल पायेगी क्योंकि यह वैक्सीन विदेशी-उत्पत्ति की है और ये हमारे यहाँ के फ्लू विषाणु से भिन्न प्रकार के हो सकते हैं।

स्वाइन-फ्लू लू और पर्यावरण

पर्यावरण और मानव-स्वास्थ्य के संबंध में एक कथन कहा जाता है कि यदि कोई वस्तु वातावरण के लिए हानिकारक है तो वह मानव स्वास्थ्य के लिए भी हानिकारक होगी। खाद्य और कृषि संस्थान के नवीनतम आँकड़ों के अनुसार मानव और पशुधन (Livestock) के संख्या बढ़ने से पशुधन के उत्पादन में परिवर्तन आये हैं जिससे वैश्विक स्तर (Global level) पर कृषि आधारित खाद्य पदार्थों में उत्थान हुआ है, जंगली पशुओं का व्यापार बढ़ा है और भी महत्वपूर्ण तरीके से देखें तो मानवों का आवागमन भी बहुत बढ़ा है जिससे कि मानव जातियों ने वैश्विक स्तर पर एक दूसरे के बीच और अन्य पशु-जातियों के बीच बीमारियों का आदान-प्रदान प्रारम्भ कर दिया।

आर्सेनिक विषाक्तता एवं स्वाइन-फ्लू :-

जोशोडया हैमिल्टन (जो कि मेरीन बॉयलोजिकल लेबोरेटरी, अमेरिका के वैज्ञानिक हैं) द्वारा हाल ही में चूहों पर किये अन्वेषणों से ज्ञात हुआ कि आर्सेनिक विषाक्तता और स्वाइन-फ्लू में गहरा संबंध है। उन्होंने चूहों के एक समूह को आर्सेनिक की 10 PPb और दूसरे चूहों के समूह को पाँच सप्ताह तक 100 PPb की आर्सेनिक की मात्रा पेयजल के साथ दी। और इसके बाद उन्हें स्वाइन-फ्लू के विषाणुओं से संक्रमित कराया और यह पाया गया कि 100 PPb की मात्रा वाले चूहों में पर्याप्त मात्रा में प्रतिरक्षी कोशिकाएं बनीं और फेफड़ों की तरफ पहुँचकर ऐसे रसायनों की उत्पत्ति की जिससे चूहों में स्वाइन विषाणुओं से संक्रमण के बाद प्रतिरक्षी क्रियायें बहुत ही देर से हुईं इस मामले में बहुत ही अधिक प्रतिरक्षी कोशिकाओं का इनफिल्टरेशन फेफड़ों को हुआ और बहुत अधिक इनफ्लूलेनटरी प्रतिक्रियायें हुई। जिससे फेफड़ों से खून निकला और उनका ह्रास हुआ। स्वाइन-फ्लू से संक्रमित मृत्यु दर उन मानवों में अधिक पायी गयी जिनमें सामान्य से ज्यादा (>10 PPb) आर्सेनिक की मात्रा थी।

अपने इस निष्कर्ष की पुष्टि के लिए मैक्सिको में पाये जाने वाले कुंओं के पानी का आर्सेनिक परीक्षण किया। उन्होंने पाया कि जहाँ पर मृत्यु की घटनायें प्रकाश में आयी थीं वहाँ के कुंओं में सामान्यतः आर्सेनिक की मात्रा 100 PPbसे अधिक थी।

अब आने वाले समय में वैज्ञानिक समाज के लिए यह तथ्य विचारणीय है कि आखिर आर्सेनिक से किस प्रतिरक्षी प्रक्रिया के क्षीण होने से स्वाइन फ्लू के संक्रमण के बाद मृत्यु हो जाती है।

Services offered by IITR

- Safety evaluation of chemicals and products : Agrochemicals, dyes, food additives, plastics and polymers, petrochemicals, cosmetics detergents, fibres and particulate material etc.
- IITR is accredited by National Accreditation Board for Testing and Calibration Laboratories (NABL) for chemical and biological testing
- Waste water analysis from chemical, paper and pulp, distillery, sugar, leather and food product industries
- Epidemiological surveys/studies on occupational diseases in industrial workers and suggesting remedial measures
- Ecotoxicological impact assessment
- Monitoring noise level in industrial environment
- Safety evaluation of drinking water
- Environmental Monitoring Studies

Any feedback on this publication is welcomed.
For information and feedback please write to :

Director
Indian Institute of Toxicology Research
P.O. Box 80, M.G. Marg, Lucknow-226 001
Telephone : 2628227, 2621856, 2611547
Fax : 91-522-2628227
E-mail : director@iitrindia.org

maheshwari press
maheshwaripress@gmail.com
Lucknow (0522) 4071816