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INSIDE

Indian Institute of Toxicology Research, Lucknow, India

WELCOME
DR KC GUPTA TAKES OVER AS DIRECTOR IITR

Dr KC Gupta, Acting Director, Institute of Genomics and Integrative Biology (IGIB), New Delhi assumed charge as Director, Indian Institute of Toxicology Research, Lucknow on March 27, 2009. Dr Gupta's areas of specialization are "Bioorganic chemistry, nucleic acid synthesis & design". His recent work relates to development of nano-particle aided transfection reagents for delivery of genes and their toxicity assessment and oligonucleotide based biochips as diagnostic tools for detection of genetic diseases.

He has over 80 research papers in national and international peer reviewed journals. He has national and international 15 patents to his credit and has also been instrumental in transferring two technologies in the area of nucleic acid chemistry. Dr Gupta is a Fellow of the National Academy of Sciences (Allahabad). He has guided over 12 students for their Ph.D. programme and an equal number are pursuing their doctoral programme under his guidance.

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ONE-DAY SYMPOSIUM ON "CURRENT ADVANCES IN BIOLOGICAL RESEARCH"

The institute established in 1965 as Industrial Toxicology Research Centre and renamed as Indian Institute of Toxicology Research (IITR) on February 01, 2008. To commomorate its first anniversary, a day long symposium was organized on "Current Advances in Biological Research" on February 02, 2009 at the institute.

The primary purpose of the one-day symposium was to highlight core biological research programmes being pursued in various institutions located in Lucknow. This platform provided an active interaction amongst scientists and students on the current activities and trends in the field of modern biology especially drug development, plant and herbal products, genetically modified crops, proteomics and genomics, toxicological test models and mechanism etc. The symposium was well directed towards bringing together the scientific thoughts from leading eminent scientists, who were willing to share their achievements, new methodologies and views in their respective fields of research. Two scientists of eminence from each of the Lucknow based CSIR institutes namely, Central Drug Research Institute (CDRI), National Botanical Research Institute (NBRI), Central Institute of Medicinal and Aromatic Plants (CIMAP) and Indian Institute of Toxicology Research (IITR) participated in it.

In his opening remarks Dr Ashwani Kumar, Acting Director, emphasized the need for effective collaborative networking among the four major Lucknow based CSIR labs. He also encouraged younger scientists to explore the possibilities of strengthening the research and to understand how new technologies can expedite our work, as IITR is a multifaceted research institute.

Prof. MM Godbole, Head, Endocrinology Department, Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow presided over the function and delivered the key-note lecture on: "Thyroid Hormone Deficiency and Brain Development: Molecular Clues".

IITR day celebration. Dr. Ashwani Kumar, Acting Director, IITR welcoming the guests Prof. Godbole and Dr. Manickam are sitting on the dais

He explained the role of thyroid associated problems and their implications during the brain development with elegant examples from experimental studies. Following this a technical session comprising 8 lectures of 45 minutes was delivered by scientists of the four CSIR laboratories.

The symposium was attended by a large number of guests from the city, eminent scientists, staff and research scholars of IITR and other institutes. The symposium provided a valuable overview of the modern biological research and development of new technologies that can benefit the society and environment.

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RESEARCH HIGHLIGHTS OF IITR

Bromelain inhibits COX-2 expression by blocking the activation of MAPK regulated NF-kappa B against skin tumor-initiation triggering mitochondrial death pathway

[Bhui K, Prasad S, George J, Shukla Y. Cancer Lett. 2009 Mar 30. (Epub ahead of print)]

Chemoprevention requires the pursuit for either single targeted or cocktail of multi-targeted agents. Bromelain, is a pharmacologically active compound, present in stems and fruits of pineapple (*Ananas cosmosus*), having anti-inflammatory, anti-invasive and anti-metastatic properties. Herein, the anti tumor-initiating effects of bromelain in 2-stage mouse skin tumorigenesis model is reported. Pre-treatment of mice with bromelain resulted in reduction in cumulative number of tumors (CNT) and average number of tumors per mouse. Preventive effect was also seen in terms of reduction in tumor volume up to 65%. Components of the cell signaling pathways, connecting proteins involved in cell death were targeted. Bromelain treatment resulted in upregulation of p53 and Bax and subsequent activation of caspase 3 and caspase 9 with concomitant decrease in Bcl-2. A marked inhibition in cyclooxygenase-2 (Cox-2) expression and inactivation of nuclear factor-kappa B (NF-kappaB) was recorded, as phosphorylation and consequent degradation of Ikappa Balpha was blocked by bromelain. Also, bromelain treatment curtailed extracellular signal regulated protein kinase (ERK1/2), p38 mitogen-activated protein kinase (MAPK) and Akt activity. The basis of anti tumor-initiating activity of bromelain was revealed by its time-dependent reduction in DNA nick formation and increase in percentage prevention. Thus, modulation of inappropriate cell signaling cascades driven by bromelain is a coherent approach in achieving chemoprevention.

Cytochrome P450 2E1 and head and neck cancer: Interaction with genetic and environmental risk factors

[Ruwali M, Khan AJ, Shah PP, Singh AP, Pant MC, Parmar D. Environ Mol Mutagen. 2009 Mar 30. (Epub ahead of print)]

The present case-control study investigates the association of polymorphisms in cytochrome P450 2E1 (CYP2E1), involved in the metabolism of tobacco carcinogens and alcohol, with Head and Neck Squamous Cell Carcinoma (HNSCC). In addition, the interaction of CYP2E1 (CYP2E1*5B and CYP2E1*6) with other genetic factors [null genotype of glutathione-S-transferase M1, GSTM1, X-Ray Repair Cross Complementing Group I, XRCC1 (Arg194Trp)], and environmental risk factors such as alcohol and tobacco in modifying HNSCC risk were investigated. Genotypes were determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay in male cases of HNSCC and an equal number of healthy male controls. Statistical analysis showed a significant increase in HNSCC risk in cases with variant genotypes of CYP2E1*5B (RsaI) (O.R. 3.44; 95% C.I. 1.45-8.14) and CYP2E1*6 (DraI) (O.R. 1.76; 95% C.I. 1.28-2.41). Haplotype analysis revealed that haplotype T-A was associated with a greater than 10-fold increase in risk for HNSCC. The data also revealed a several fold increase in HNSCC risk in cases carrying a combination of variant genotypes of CYP2E1 with the null genotype of GSTM1 or XRCC1 variant genotypes. Alcohol or tobacco use (both smoking and chewing) were found to interact with variant genotypes of CYP2E1 in significantly enhancing HNSCC risk. This increase in risk associated with an interaction of CYP2E1 genotypes with GSTM1 or XRCC1 or with tobacco and alcohol use demonstrates the importance of gene-gene and gene-environment interactions in the development of HNSCC.

The level of polyaromatic hydrocarbons in kajal and surma of major Indian brands

[Patel DK, Prasad S, Tripathi R, Behari JR. Int J Cosmetic Sci. 2009 Mar 18. (Epub ahead of print)]

Kajal and surma are eye cosmetics extensively used in the Indian subcontinent. Kajal is prepared by burning of vegetable oil and butter oil while surma by grinding of the stones. High performance liquid chromatography and gas chromatography-mass spectrometry were used for quantification and confirmation of 16 polyaromatic hydrocarbons (PAHs). Significant concentration of PAH was found in all the samples examined. The median concentration of PAH ranged from 0.14 (lowest, anthracene) to 31.18 $\mu g\ g^{(-1)}$ [dibenz(a,h)anthracene] in kajal sample and from not detectable concentration (naphthalene) to 197.47 $\mu g\ g^{(-1)}$ of benzo(a)pyrene in surma sample. Fifteen PAHs were detected in all the samples. Therefore the use of kajal and surma in eye should be restricted.

Therapeutic efficacy of Picroliv in chronic cadmium toxicity

[Yadav N, Khandelwal S. Food Chem Toxicol. 2009 Apr; 47(4):871-9.]

Cadmium (Cd), an industrial and environmental pollutant, is toxic to several tissues, most notably causing hepatotoxicity on acute administration and nephrotoxicity following chronic exposure. The therapeutic efficacy of Picroliv--a standardized fraction of *Picrorhiza kurroa*, was investigated in male rats treated with Cd as $CdCl_2$ (0.5 mg/kg, sc) 5 days/week for 24 weeks and Picroliv at two doses (6 and 12 mg/kg, p.o.) was given during the last 4 weeks.

The Cd induced levels of malondialdehyde and membrane fluidity and decreased levels of non-protein sulphydryls and Na+K+ATPase activity of hepatic tissue, along with liver function serum enzymes were restored to near normalcy on treatment with the higher dose of Picroliv. Enhanced excretion of urinary proteins, Cd, Ca and enzymes (lactate dehydrogenase and N-acetyl-beta-D-glucosaminidase) evident at 24 weeks of Cd exposure indicated severe renal damage. Picroliv appeared less effective in causing restoration of these urinary parameters as well as oxidative stress indices in the renal tissue. Picroliv not only reduced the accumulated levels of Cd, Zn and Ca and Cd-metallothionein in liver, but also enhanced the bile flow and biliary Cd. The morphological alterations in liver caused by Cd appeared less marked on Picroliv treatment. However, the renal morphology remained uninfluenced. Earlier data on 18 weeks of Cd and 4 weeks of Picroliv co-treatment showed significant amelioration of both hepatic and renal manifestations of Cd. The hepatic protection by Picroliv is clearly demonstrated in this study, while marginal lowering of urinary proteins and enzymes is a positive signal of renal protective efficacy of Picroliv, which could be augmented by adopting higher doses and extended regimen.

Regulation of cell growth through cell cycle arrest and apoptosis in HPV 16 positive human cervical cancer cells by tea polyphenols

[Singh M, Tyagi S, Bhui K, Prasad S, Shukla Y. Invest New Drugs. 2009 Mar 7. (Epub ahead of print)]

Cervical cancer is the second most common malignant neoplasm in women, in terms of both incidence and mortality rates worldwide. The polyphenolic constituents of tea (*Camellia sinensis*) have gained considerable attention because of its anti-cancer properties against a variety of cancers. Here the effects of green and black tea polyphenols (GTP and BTP), on cellular proliferation and cell death in the SiHa cells (human cervical cancer) expressing the human papilloma virus (HPV)-16 were studied. The result showed that both GTP and BTP inhibited proliferation of cells in dose and time-dependent manner. Cell cycle analysis showed anti-proliferative effect of GTP which is associated with an increase in the G2/M phase and apoptotic effect of BTP in 24 h treated SiHa cells. Further, on increase of incubation time to 48 h, GTP caused induction of apoptosis up to 20% of SiHa cells. The role GTP and BTP in apoptosis was further confirmed by reduction in mitochondrial membrane potential and increased levels of membrane phosphatidylserine. Thus, the data suggests that tea polyphenols exhibit anti-cancer potential against cervical cancer by inhibition of cell growth and induction of apoptosis.

Enhanced survival and function of neural stem cells-derived dopaminergic neurons under influence of olfactory ensheathing cells in parkinsonian rats

[Shukla S, Chaturvedi RK, Seth K, Roy NS, Agrawal AK. J Neurochem. 2009 Apr;109(2):436-51.]

Transplantation of neural stem cell (NSC)-derived dopamine (DA) neurons is associated with low survival of cells, which could be due to limited striatal innervations and uneven distribution of graft because of its dense neuronal core, limited host-graft interaction, poor axonal outgrowth, lack of continuous neurotrophic factors supply, and an absence of cell adhesion molecules mediated appropriate developmental cues. Olfactory ensheathing cells (OEC) express a variety of growth factors and cell adhesion molecules and promote axonal regrowth and functional recovery in spinal cord injury in animal models and patients. In the present study, the possibility to increase the survival, function, axonal outgrowth and striatal reinnervation of NSC by co-grafting with OEC in 6-OHDA lesioned parkinsonian rats was explored. In the presence of OEC, significantly enhanced survival of NSC-derived DA neurons and axonal fiber outgrowth was evident in the striatum of NSC+OEC co-grafted rats at 24 weeks post-grafting as compared with NSC alone grafted rats. The increased survival of NSC and their striatal reinnervation was further manifested in the form of significant and substantial restitution of motor function and neurochemical recovery in the co-grafted group. Significant enhanced expression of p75NTR (from OEC) and tyrosine hydroxylase (TH) (from NSC) confirmed the co-localization and survival of both types of cells at the transplantation site in co-grafted rats. Co-grafting results co-related well with our *in vitro* studies, which suggest that OEC not only significantly increase survival, neurite outgrowth and DA release of NSC-derived DA neuron but also protects against 6-OHDA neurotoxicity in co-culture conditions. These results collectively suggest that OEC increase the survival and function of transplanted NSC in 6-OHDA lesioned parkinsonian rats.

Plant derived antioxidants - Geraniol and camphene protect rat alveolar macrophages against t-BHP induced oxidative stress

[Tiwari M, Kakkar P. Toxicology in Vitro; 2009; 23: 295-301.]

Exploration of antioxidants of plant origin and scientific validation of their efficacies has unraveled bioactives from natural sources. In this study, two terpenoids camphene and geraniol were assessed for their cytoprotective and antioxidant potential using t-BHP stressed rat alveolar macrophages. Effect of these test substances along with a known plant derived antioxidant quercetin was seen on cell viability, some oxidative stress markers as well as on mitochondrial membrane potential. Both the test substances

geraniol and camphene increased the cell viability significantly as indicated by MTT assay and LDH release assay, during pre-treatment of test compound. Camphene and geraniol showed 29% ($P < 0.05$) and 45% ($P < 0.05$) increase in SOD activity, 28% and 120% ($P < 0.001$) increase in GSH content and restored the mitochondrial membrane potential during pre-treatment as compared to stressed cells. Camphene and geraniol were found to significantly decrease lipid peroxidation, inhibit NO release (83.84% and 64.61%) and ROS generation in the pre-treated cells as compared to stressed cells. The test compounds also showed significant protection against ROS during post-treatment of the test compounds. Results indicate the pharmacological potential of these phytochemicals in lung inflammatory diseases where oxidative stress is a critical control point.

Polymorphism in glutathione-S-transferases: a risk factor in alcoholic liver cirrhosis

[Khan AJ, Choudhuri G, Husain Q, Parmar D. Drug Alcohol Depend. 2009 May 1;101(3):183-90. (Epub 2009 Jan 21)]

In a case-control study, association of polymorphism in glutathione-S-transferases (GSTM1, GSTT1, GSTP1), involved in detoxification of reactive oxygen species (ROS), was studied with alcoholic liver cirrhosis. The study included 175 alcoholic cirrhotic patients (ACPs), 140 non-alcoholic cirrhotic patients (NACPs), visiting Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGI), Lucknow, India, 255 non-alcoholic controls and 140 alcoholic controls. The data showed an increase in risk to alcoholic cirrhosis in ACPs with GSTM1 (null) genotype when compared with non-alcoholic controls (OR: 1.7; 95% CI: 1.15-2.56) or alcoholic controls (OR: 1.7; 95% CI: 1.07-2.73). Significant increase in risk was also observed in ACPs with variant genotype of GSTP1 when compared with non-alcoholic controls (OR: 1.65; 95% CI: 1.12-2.43). A much higher risk to alcoholic liver cirrhosis was observed in patients carrying combination of null genotypes of GSTM1 and GSTT1 (OR: 2.8; 95% CI: 1.3-6.06) or variant genotype of GSTP1 and null genotype of GSTM1 (OR: 2.8; 95% CI: 1.58-4.90) or GSTT1 (OR: 2.16; 95% CI: 1.08-4.28). Likewise, greater risk for alcoholic cirrhosis was observed in patients carrying combination of GSTM1, GSTT1 (null) and variant genotype of GSTP1 (OR: 5.8; 95% CI: 2.17-15.80). The data further showed that interaction of GSTs with variant genotype of manganese superoxide dismutase (MnSOD), which detoxifies free radicals, or cytochrome P450 2E1, which generates free radicals, resulted in several fold increase in risk to alcoholic liver cirrhosis in ACPs when compared with non-alcoholic controls, thus demonstrating the role of gene-gene interactions in modulating the risk to alcoholic liver cirrhosis.

Patulin causes DNA damage leading to cell cycle arrest and apoptosis through modulation of Bax, p(53) and p(21/WAF1) proteins in skin of mice

[Saxena N, Ansari KM, Kumar R, Dhawan A, Dwivedi PD, Das M. Toxicol Appl Pharmacol. 2009 Jan 15;234(2):192-201.]

Patulin (PAT), a mycotoxin found in apples, grapes, oranges, pear and peaches, is a potent genotoxic compound. WHO has highlighted the need for the study of cutaneous toxicity of PAT as manual labour is employed during pre and post harvest stages, thereby causing direct exposure to skin. In the present study cutaneous toxicity of PAT was evaluated following topical application to Swiss Albino mice. Mice skin exposed to PAT for 4 h resulted in a dose (40-160 µg/animal) and time (up to 6 h) dependent enhancement of ornithine decarboxylase (ODC), a marker enzyme of cell proliferation. The ODC activity was found to be normal after 12 and 24 h treatment with patulin. Topical application of PAT (160 µg/100 µl acetone) for 24-72 h caused (a) DNA damage in skin cells showing significant increase (34-63%) in olive tail moment, a parameter of Comet assay (b) significant G 1 and S-phase arrest along with induction of apoptosis (2.8-10 folds) as shown by annexin V and PI staining assay through flow cytometer. Moreover, PAT leads to over expression of p(21/WAF1) (3.6-3.9 fold), pro-apoptotic protein Bax (1.3-2.6) and tumor suppressor wild type p(53) (2.8-3.9 fold) protein. It was also shown that PAT induced apoptosis was mediated through mitochondrial intrinsic pathway as revealed through the release of cytochrome C protein in cytosol leading to enhancement of caspase-3 activity in skin cells of mice. These results suggest that PAT has a potential to induce DNA damage leading to p(53) mediated cell cycle arrest along with intrinsic pathway mediated apoptosis that may also be correlated with enhanced polyamine production as evident by induction of ODC activity, which may have dermal toxicological implications.

DNA damaging potential of zinc oxide nanoparticles in human epidermal cells

[Sharma V, Shukla RK, Saxena N, Parmar D, Das M, Dhawan A. Toxicol Lett. 2009 Mar 28;185(3):211-8.]

At present more than 20 countries worldwide are manufacturing and marketing different varieties of nanotech-based consumer products of which cosmetics form the largest category. Due to the extremely small size of the nanoparticles (NPs) being used, there is a concern that they may interact directly with macromolecules such as DNA. The present study was aimed to assess the genotoxicity of zinc oxide (ZnO) NPs, one of the widely used ingredients of cosmetics, and other dermatological preparations in human epidermal cell line (A431). A reduction

in cell viability as a function of both NP concentration as well as exposure time was observed. ZnO NPs demonstrated a DNA damaging potential as evident from an increased Olive tail moment (OTM) of 2.13 +/- 0.12 (0.8 g/ml) compared to control 1.37 +/- 0.12 in the Comet assay after an exposure of 6 h. ZnO NPs were also found to induce oxidative stress in cells indicated by depletion of glutathione (59% and 51%); catalase (64% and 55%) and superoxide dismutase (72% and 75%) at 0.8 and 0.08 g/ml respectively. The data demonstrates that ZnO NPs even at low concentrations possess a genotoxic potential in human epidermal cells which may be mediated through lipid peroxidation and oxidative stress. Hence, caution should be taken in their use in dermatological preparations as well as while handling.

Molecular Characterization of Desmodium species- an important ingredient of 'Dashmoola' by RAPD analysis

[Irshad S, Singh J, Kakkar P, Mehrotra S. Fitoterapia.2009 Mar; 80(2):115-8.]

Identification of medicinal plants by their molecular signature is a fast growing tool. The identification of *Desmodium gangeticum (L.) DC.* (Shalparni, a constituent of Ayurvedic formulation "Dashmoolarishtha") was carried out using genomic approach. Authentic samples of *D. gangeticum(L.) DC., D. velutinum (Wild.) DC. and D. triflorum (L.) DC.* were analyzed and compared to commercial samples of various origin. Within twenty primers used, eleven gave 223 RAPD fragments. RAPD profiles of three species showed very low similarity index (0.21-0.39), whereas market samples showed high similarity of 0.82-0.89 with authenticated *D. gangeticum*.

ASSIGNMENTS ABROAD

Dr Ashwani Kumar, Acting Director visited Philadelphia, USA to attend 24th International Conference on Solid Waste Technology and Management (ICSW-2009) from March 15-18, 2009.

Dr Mukul Das Scientist attended Indo-EU Joint Expert Committee Review Panel Meeting for collaborative projects on functional foods at Brussels, Belgium from March 16-20, 2009.

Dr Deepak K. Agarwal visited Bangkok, Thailand to attend WHO-TDR harmonization Workshop on short course from February 9-14, 2009.

CURRENT TOPIC
BIOINFORMATICS IN DRUG DESIGNING AND TOXICOLOGY

Shailendra K. Gupta and Shivangi Sinha
ENVIS Centre, Indian Institute of Toxicology Research, Lucknow

Recent advancements in computer sciences along with equally significant developments in molecular biology and chemistry provide toxicology with powerful new Bioinformatics tools. This tool box of computational models increases the efficiency and the effectiveness by which the hazards and risks of environmental chemicals are determined. Computational toxicology mainly focuses on applying these tools across many scales, including vastly increasing the numbers of chemicals and the types of biological interactions that can be evaluated. In addition, knowledge of toxicity pathways gathered within the tool box will be directly applicable to the study of the biological responses across a range of dose levels, including those more likely to be representative of exposures to the human population [1]. This development of databases and the use of bioinformatics made science interesting and easier to carry out new researches using the existing biological data. The rapid exponential growth in high throughput techniques for genome sequencing, gene expression, protein profiling hailed the development of new algorithms and computational tools for acquiring, storing, analyzing and representing biological information.

Bioinformatics completely revolutionized the traditional process of drug designing. Virtual screening followed by *in vitro* experiment is recognized as a promising approach in today's drug discovery projects. Computer assisted drug designing (CADD) processes successfully contributed to develop numerous novel enzyme inhibitors, including inhibitors of Thymidylate synthase, HIV-1 protease, Dihydrofolate reductase, Acetylcholinesterase. Several softwares and databases developed for computer assisted drug discovery are illustrated in Table 1 and the process is summarized in Figure 1. The process starts with the target identification and its structural analysis. The ideal target is one that is closely linked to human disease and binds with small molecules (ligand) in order to carry out a function. The field of genomics and proteomics contributed greatly to identify novel potential targets retrieved by comparing

whole genome of host and pathogen over the last several years.

Analysis of 3D structure and identification of target sites: The process of CADD initiates with the detail structural analysis of target molecule and prediction of potential ligand binding site on its surface. The problem to detect putative binding sites can be approached in several ways. The common approach is to determine the cavities in the protein structure and assume the biggest cavity as the active site. More precise method searches for key amino acid residues involved in catalysis or cofactor are considered to form the binding sites. However, in the absence of experimental data, uncertainty remains in the accurate prediction of binding sites. Target sites for protein-protein interaction are difficult to identify as they are often flat, large and hydrophobic.

Drug designing methodology: Once the binding site is determined, thousands of compounds can be tested using high-throughput screening technologies, although the better way is to select lead compounds using computer driven assays. Molecular databases can be virtually screened to identify small molecules that are docked in the target sites, out of millions of molecules available. De novo generation approach is initiated by placing small fragments of molecules such as benzene ring, amino group etc. into the binding cavity. These small fragments are further linked among themselves to form best inhibitors.

Modification and evaluation of lead compounds: Methods for modification of lead compounds are meant to enhance activity, detect toxicity, increase pharmacokinetics and reduce side effects. These methods are normally based on structural data mining to detect and analyze biological assays of similar compounds. Several programs are designed for prediction of various ADME properties which has to be tested later during preclinical evaluation for determination of suitability of hits into therapeutic agents [2].

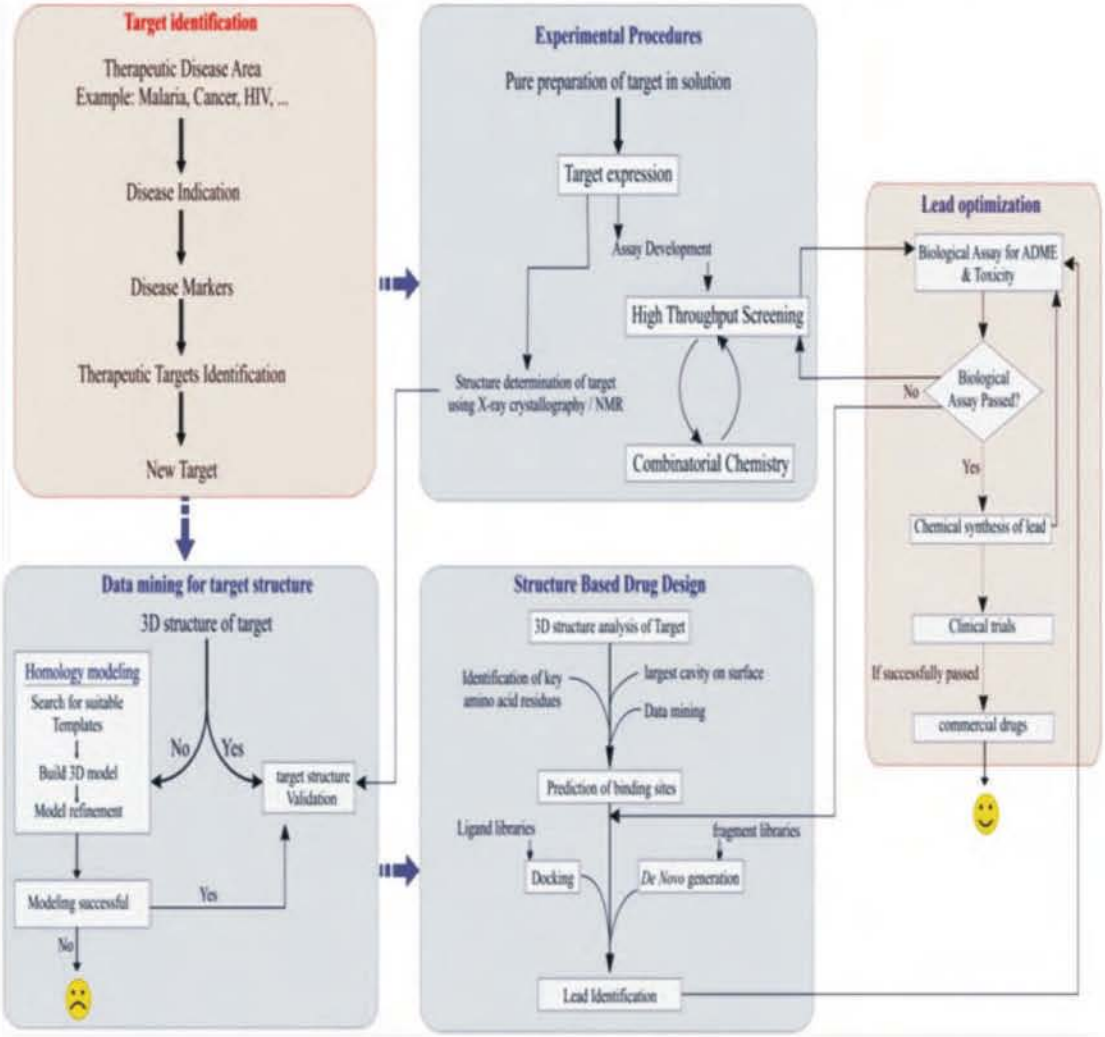

Figure 1: Schematic diagram of computer-assisted drug designing process

Table 1:
List of some prominent computer-assisted drug discovery programs

Program	Description	URL
Protein – Protein docking		
3D Dock Suite	FTDock, RPScore and MultiDock Perform rigid body docking of 2 biomolecules	http://www.bmm.icnet.uk/docking
ClusPro	Fully automated protein-protein docking. Perform rigid docking by DOT, ZDOCK, PIPER. Clustering of complexes	http://structure.bu.edu/Projects/PPDocking/cluspro.html
DOT	Rigid docking of macromolecules including Protein, DNA, RNA	http://www.sdsc.edu/CCMS/DOT/
FireDock	Refinement and re-scoring of rigid-body protein-protein docking solutions	http://bioinfo3d.cs.tau.ac.il/FireDock/
HEX	Protein docking and molecular superimposition program	http://www.loria.fr/~ritchied/hex/
HADDOCK	High Ambiguity Driven biomolecular DOCKing	http://www.nmr.chem.uu.nl/haddock/
ZDock	Fast Fourier Transform based protein docking program	http://zdock.bu.edu/
Protein – ligand docking		
Autodock	Rigid & flexible docking. Docking of the ligand to a set of grids describing the target protein	http://autodock.scripps.edu/
DOCK	Dock small molecules or fragments, allow flexible ligand docking, shape based algorithm	http://dock.compbio.ucsf.edu/
FlexX	Incremental construction approach of ligand and allow flexible protein-ligand docking	http://www.biosolveit.de/FlexX/
GLIDE	Complete suite for protein-ligand interaction based on systematic search techniques	http://www.schrodinger.com/
GOLD	Uses genetic algorithms for docking	http://www.ccdc.cam.ac.uk/products/life_sciences/gold/
LigPlot	For automatically plotting protein-ligand interaction	http://www.biochem.ucl.ac.uk/bsm/ligplot/ligplot.html
VEGA	Rank ligand on the basis of receptor-ligand interaction energy	http://users.unimi.it/~ddl/
Discovery Studio	Flexible docking, virtual high throughput screening, *de novo* ligand generation	http://www.accelrys.com

In recent years, number of docking systems developed to optimize the prediction of binding affinity using molecular mechanics force field methodologies. One most commonly used system is Autodock developed by G. M. Morris and co-workers. The system uses grid based techniques in which the interaction energy between the atoms of receptor & ligand are pre-calculated as the points of a grid. Hybrid genetic algorithm is used to adjust the position and confirmation of ligand in the binding cavity of protein. DOCK and Discovery Studio are based on negative image of binding site in which a sphere is generated around the cavity. During the process, virtual libraries are scanned to find the ligand that matches to the sphere. For all the ligands that fit in the sphere, full estimation of binding affinity is computed and ligands are arranged on the basis of different scoring functions. FlexX uses a fragmentation approach in which ligand is fragmented and incrementally reconstructed in iterative manner using the tree-search algorithm in the binding site of receptor. GOLD uses a template based on H-bond donors & acceptors of protein and uses genetic algorithm to sample over all possible combination of inter molecular hydrogen bond and ligand confirmations. One example of protein-ligand interaction is illustrated in Figure 2 using Autodock software, where the docking was performed between HIV-1 protease and nonpeptide cyclic ureas.

As structural genomics, proteomics, bioinformatics, computational power, knowledge of disease cycles and therapeutic targets continue to explode with new advances, further understanding in the structure-based drug designing are likely to follow.

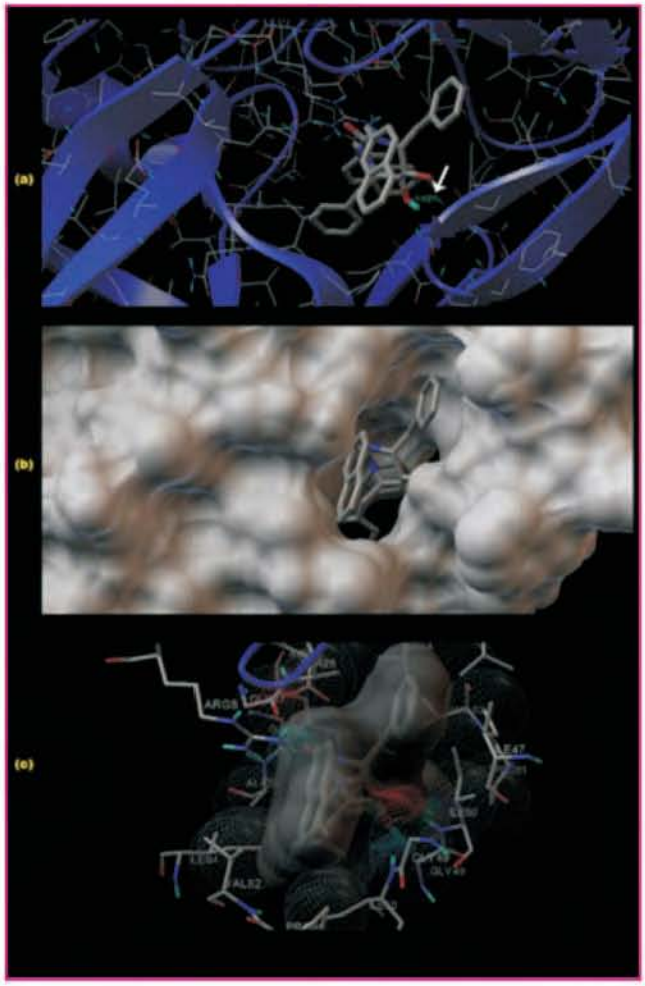

Figure 2:
Nonpeptide cyclic ureas, HIV-1 protease inhibitors, in the binding cavity of protein (PDB code 1hvr).
(a) Protein is displayed in its secondary structure. Arrow shows the hydrogen bonding between protein-ligand.
(b) Molecular surface of the protein is generated to properly illustrate the binding cavity.
(c) Residues near binding site are displayed as wireframe to analyze their interaction with ligand molecule.

Recent developments in toxico-cheminformatics: Supporting a new paradigm for predictive toxicology:

For the future of computational toxicology, integration and analysis of large toxicological data sets is of great significance. The current state of toxicity databases is something of a mess. The Distributed Structure Searchable Toxicity (DSSTox) public database provide a public interface for publishing downloadable, structure-searchable, standardized chemical structure files associated with toxicity data. The data are put into a model where they are easier to manipulate. Data are deposited in PubChem, structure search is possible in DSSTox and there are links out to other resources such as ChemSpider, PubChem, the EPA Aggregated Computational Toxicology Resource (ACToR), Lazar *in silico* tox etc.

In 2007, EPA launched the ToxCast program in order to develop a cost-effective approach for prioritizing the toxicity testing of large number of chemicals in a short period of time. Using data from state-of-the-art high throughput screening (HTS) bioassays developed in the pharmaceutical industry, ToxCast is building computational models to forecast the potential human toxicity of chemicals. The goal is to derive "signatures" from *in vitro* and *in silico* assays to predict *in vivo* endpoints. ToxRefDB has been used in profiling of liver effects for pesticides. [3]

Molecular Modeling Methods as a Virtual Screening Tool for the Assessment of Chemical Toxicity :

Molecular modeling methods provide an approach for estimating chemical activity when the relevant data is not available. When used in this way it becomes an important tool for screening chemicals for toxicity and hazard identification. Computational molecular methods may also be applied to model toxicity pathways when some of the relevant experimental data are unavailable. An element of a virtual screen for potential toxicity may be developed from the characterization of these toxicant-target interactions. One large and important subset of target-toxicant interactions is the interaction of chemicals with proteins. Many computational approaches for screening libraries of molecules for pharmaceutical application have been developed. These methods also may be applied to screen environmental chemicals for toxicity.

Bioinformatics and System Biology:

The quantity and range of omics data (genomics, proteomics, transcriptomics, metabolomics) along with recent technologies developed till date has made possible the research in bioinformatics to move beyond the study of individual biological components (genes, proteins etc). The requirement is now to develop cooperation between individual dataset to design more realistic models for system biology in order to understand the functioning of the biological systems. Computer models performing complete cell simulation are more in demand [4]. Various

computational environments were designed for studying simulation using System Biology Markup Language (SBML) like SimBiology Toolbox of MATLAB [5], SYCAMORE [6], and PROMOT [7].

Conclusion

Despite of some drawbacks, there is no doubt that bioinformatics is a field that has enormous capacity for revolutionizing biological research in the days to come. Along with providing more consistent and more precise computational tools for all the domain of biotechnology the biggest challenge for bioinformatics is to develop overall biology from a qualitative to a quantitative and predictive science. Many new upcoming areas where bioinformatics is going to play an important role are simulation of entire biological system, development of personalized drugs, identification of new strains for bioremediation and biofuel generation etc.

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RESEARCH DIGEST

Will Warmer Soil Be as Fertile?

[Environmental Health Perspectives Volume 117, Number 2, February 2009]

Growing concern about global climate change has focused increasing research attention on the carbon-regulating role played by soil. Collectively, the Earth's soils contain more than twice the amount of carbon found in the atmosphere. Scientists at the University of Toronto Scarborough (UTS) now report that global warming may significantly alter soil composition at the molecular level and that such changes could have a major impact on atmospheric levels of carbon dioxide (CO_2).

Decomposition of organic matter may change with rising soil temperatures

Organic matter, the decaying remains of plants and animals, enables soil to support plant life, providing plants and soil microbes with the energy and raw materials they need for growth. Soil microbes facilitate the decomposition of organic matter from litterfall (the leaves, twigs, and other plant materials that fall to the ground), and CO_2 is a natural by-product of this process. Rising atmospheric temperatures and/or CO_2 levels are likely to increase photosynthesis and plant productivity, according to the results of several studies over the past two decades; consequently, litterfall is expected to increase. Until now, however, the effects of warming on soil's molecular composition have been poorly studied. It is therefore unclear to what extent the carbon-containing components of soil matter will accumulate or degrade and thus how much carbon will be sequestered by the soil and how much released into the atmosphere as CO_2.

In a report published online 23 November 2008 ahead of print in Nature Geoscience, environmental chemist Myrna Simpson and her UTS colleagues wrote that rising temperatures would be expected to speed up the decomposition of labile (easily degraded) soil organic compounds such as the carbohydrates from leaf litter, whereas more biochemically resistant carbon-containing structures-such as the lignin from woody tissues and lipids from leaf cuticles-would be expected to remain stable over decades, possibly even centuries. Global warming may change present-day decomposition patterns by altering the soil microbial communities and activities, thus changing the overall flow of carbon into and out of the soil and affecting soil fertility as well.

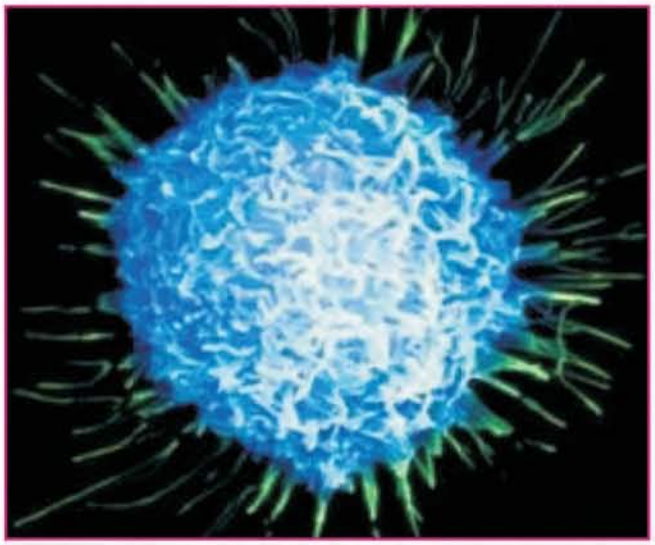
Dr Simpson and group found that the activity of some microorganisms increased in the warmer soil, resulting in a faster degradation of carbohydrates and other labile components. However, the study also showed that soil fungi numbers and activity also increased in the warmer soil, with a corresponding rise in abundance of lignin-derived compounds (reflecting decomposition by the fungi). "The implication of the increased degradation of lignins is that less carbon remains in the soil solid phase, and more CO_2 is being released from the soil into the atmosphere."

Conversely, certain other recalcitrant molecular structures-such as the alkyl structures found in the waxy coating or cuticles of leaves-remained resistant to decomposition and accumulated in the warmer soil. Thus, says Simpson, as global temperatures warm and more organic matter from litterfall is added to the soil, less of that matter will be in a form that's easily used by microbial decomposers in the soil, including many soil bacteria that are needed to sustain robust plant life. "In other words, the soil's molecular carbon composition is shifting [toward] a form that is not usable to microbes or plants, and more of the remaining carbon is being derived from leaf cuticles that persist in the soil."

Until now, soil-climate research has focused heavily on the total amount of carbon in the soil, but the more relevant issue may be changes in the carbon-based molecular structures contained in the soil's organic matter and how such changes will ultimately affect both microbial and plant life. "To understand the soil-climate interactions better, we will need more soil research to focus on the molecular level with an eye toward predicting both short- and long-range changes in the system."

Prostate cancer marker found in urine: A simple urine test for sarcosine could be used to detect cancer

[Nature 11 February 2009 | doi:10.1038/news.2009.94]



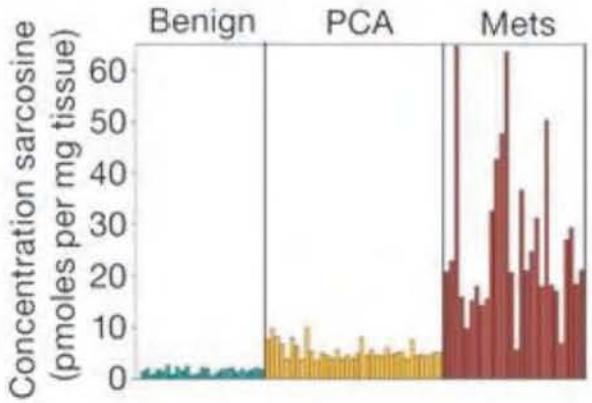
The findings could lead to less invasive prostate cancer tests.

The concentration of a small molecule found in urine could reveal how advanced a patient's prostate cancer is. The discovery could lead to simple, non-invasive tests for men who have the disease and might help avoid the need for biopsies.

Researchers identified the molecule, a derivative of the amino acid glycine called sarcosine, after close inspection of all the metabolites found in 262 samples from prostate cancer patients - all of whom had different stages of the disease - including benign cells to aggressive metastasized cancers able to spread to other organs. Metabolites are the substances involved in or produced by

the chemical reactions that take place in the body, and the concentrations of these chemicals can change as cancer progresses. Christopher Beecher at the University of Michigan in Ann Arbor, led the metabolomic analysis. Generally, researchers screen only for metabolites that they think might be affected during a disease. However, using mass spectrometry, Beecher's team looked at all the metabolites present in their samples. According to the Charity Cancer Research UK, more than 670,000 men diagnosed worldwide with prostate cancer each year. Survival rates vary due to different screening and treatment policies but in the United Kingdom around 30% of men die within 5 years of diagnosis. Now, prostate cancer is diagnosed and monitored by testing for high concentrations of prostate-specific antigen (PSA), often followed by a biopsy to determine whether the cancer is aggressive or slow-growing. If further testing and validation confirm the new results, sarcosine concentrations in urine could be used to predict the aggressiveness of a cancer. "We could actually detect this metabolite non-invasively in the urine of men with prostate cancer," says Arul Chinnaiyan, also at the University of Michigan, who led efforts to look at how sarcosine interacts with cancer cells.

Beecher's team found that at least six metabolites showed up in higher concentrations in the samples of metastatic cancer than in those of any other stage of the disease. Sarcosine was present in especially high concentrations. Chinnaiyan and his colleagues then identified the enzymes that make and degrade sarcosine and altered the genes that controlled the production of these enzymes in cells grown in culture. The research is published in Nature.



Sarcosine concentrations in benign cells (left); localised prostate cancer (centre) and metastatic prostate cancer (right).

"Any time they did anything that increased production [of sarcosine], the cells became highly mobile," says Beecher. The cells started to move so much they could get themselves into a sample of gelatine under their own force - a behaviour that cancerous cells also show. Simply adding sarcosine to benign prostate cells made them behave like cancerous cells. The implication is that sarcosine is not only an indicator of how aggressive a cancer has become, it also has a role in making cells move and become aggressively cancerous. The study could also provide new targets for treatments. "Not only have they got a marker that has a fundamental role in invasion and metastasis, the molecule itself is regulated by systems that have been directly implicated in prostate cancer," says Malcolm Mason, Cancer Research UK's prostate-cancer expert from Cardiff University, UK. "There's enough to say that [sarcosine] should be looked at as a possible drug target."

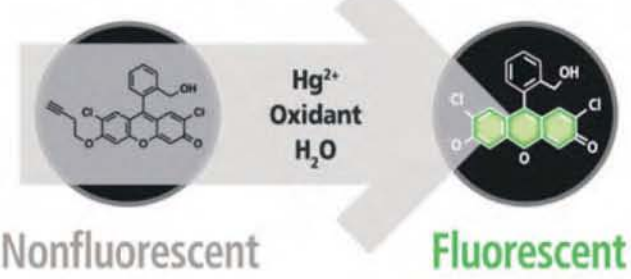
But Mason adds that the study needs to be validated further, and cautions that sarcosine is unlikely to be the basis of a widespread screening programme. Several large clinical trials for screening for the disease are under way, in Europe, he says, and until these results are in, it is hard to say what role sarcosine urine tests will have. They are most likely to be used to differentiate between men who need biopsies and those who don't. "This isn't a simple urine test that you can do at the chemist's," says Mason.

Beecher, however, has high hopes that sarcosine will find a place in prostate-cancer tests, and possibly even replace current tests. "This may actually be superior to PSA," he says. Other metabolites identified in the study could also be added to a test as "a panel of metabolites that we could monitor", says Chinnaiyan.

Fluorescent Probe Detects Mercury

[Environmental Health Perspectives Volume 117, Number 2, February 2009]

Mercury is toxic to the nervous system and may cause memory loss, cognitive and behavioral disturbances, headaches, insomnia, peripheral nerve damage, tremors, and motor dysfunction-all problems that have been observed to disappear upon removing the source of mercury exposure, according to the International Programme on Chemical Safety. Removing the source may now become easier with a new chemical probe that gives off a bright green glow when it reacts with mercury in fish and dental amalgams. Eventually the probe could be used to detect contaminated fish at home or mercury in wastewater at dental offices.



Developer Kazunori Koide, an associate professor in the University of Pittsburgh Department of Chemistry, based the probe on a simple principle taught in undergraduate organic chemistry classes: Mercury converts alkynes into ketones. Koide's team synthesized an alkyne-based compound from commercial dichlorofluorescein. When mercury converts the alkyne into a ketone, the reaction generates a green fluorescent signal whose intensity corresponds to the amount of mercury present. "It's a very straightforward approach," Koide says, "yet no one ever thought to do this."

The probe performed well in real-life tests described in the 10 December 2008 issue of the Journal of the American Chemical Society. A piece of salmon about the size of a dime was first treated with an oxidizing agent to release mercury bound up as methylmercury, then was soaked in the alkyne test solution. Within 30 minutes, mercury in the sample had converted the test solution to a ketone, which produced a strong fluorescent signal. Next, the researchers soaked a thin cloth with saliva and pressed it against an extracted tooth filled with silver amalgam (which may contain up to 50% mercury) obtained from a dental clinic. When the solution was applied to the cloth, its green fluorescent signal was substantially brighter than that of a control cloth also treated with saliva but not held against the tooth.

Because the sulfur-containing amino acid cysteine is known to bind tightly to mercury in fish, Koide wondered whether cysteine in sulfur-rich foods such as onions and eggs would react with mercury in dental amalgams. In another experiment, two extracted teeth containing amalgams were soaked for an hour in a cysteine solution. The filled teeth released a significant amount of mercury into the cysteine solution as compared with teeth that had been soaked in water at a temperature of 35ºC.

Other probes for mercury reported in the scientific literature are largely based on chemical reactions between mercury and sulfur groups. However, sulfur-based tests are prone to oxidation during storage, making them impractical for environmental testing. The standard method for detecting mercury in environmental samples-atomic absorption spectrometry-is expensive. "We're not trying to compete with atomic absorption spectrometry," says Koide. "We want to fill a gap by making a probe that the general public can use."

Koide's fluorescent probe "provides a clever chemical approach for detecting mercury in complex samples," says Chris Chang, an assistant professor of chemistry at the University of California, Berkeley. "The simplicity and sensitivity of this assay are promising for further application." Koide and colleagues hope to turn this chemical tool into a kit, and are modifying the method to make it consumer-friendly, such as finding safe chemicals to transform extremely toxic methylmercury in fish into less toxic mercury species before testing.

Some Food Additives Mimic Human Hormones

[Scientific American, March 27, 2009]

New research reveals that some common food additives behave like estrogen in the body

Fishy lobster? Some food additives that help to keep lobster and other shellfish looking fresh might be changing human hormones.

A discovery that two commonly used food additives are estrogenic has led scientists to suspect that many ingredients added to the food supply may be capable of altering hormones.

More than 3,000 preservatives, flavorings, colors and other ingredients are added to food in the United States, and none of them are required to undergo testing for estrogenic activity, according to the Food and Drug Administration. "We need to be mindful of these food additives because they could be adding to the total effect of other estrogen mimicking compounds we are coming into contact with," said Clair Hicks, a professor of food science at the University of Kentucky and spokesperson for the Institute of Food Technologists, a nonprofit scientific group.

"The benefits of using these additives in food need to be weighed against the risks they present," Hicks said. In a study published in December, Italian researchers

screened 1,500 food additives using computer-modeling software, a much faster and cheaper approach than testing lab rats.

The researchers first used modeling to identify 13 molecules that could hypothetically bind with an estrogen receptor, a group of molecules activated by the hormone. Like a clenched fist that fits into the palm of a hand, potentially estrogenic molecules will "fit" inside the receptor, indicating they could interact and alter hormones. Then, the researchers exposed cells to the 13 food additives, which confirmed that two have estrogen-mimicking properties. Known as "xenoestrogens," these substances have been linked to reproductive problems in animals and perhaps humans.

The first food additive, propyl gallate, is a preservative used to prevent fats and oils from spoiling that can be found in a range of foods including baked goods, shortening, dried meats, candy, fresh pork sausage, mayonnaise and dried milk. The second additive, 4-hexyl resorcinol, is used to prevent shrimp, lobsters, and other shellfish from discoloring.

"Some caution should be issued for the use of these two additives," said Pietro Cozzini, one of the researchers who conducted the study and a chemistry professor at the University of Parma in Italy. He added that further tests on rats are necessary to determine whether these additives could harm humans. Paul Foster, whose research focuses on the potential human health effects of endocrine disruptors, agreed. He said there is a big difference between adding estrogenic molecules to cells in a culture dish and actually seeing what happens when that dose is administered to an animal.

Double Trouble : Flu Intensifies Effects of Ozone

[Environmental Health Perspectives Volume 117, Number 2, February 2009]

Environmental health scientists have long speculated that the influenza virus could intensify the pulmonary effects of air pollution or vice versa. Like air pollution, influenza affects primarily the respiratory system, and ambient air pollutants may either lower resistance to viral infection or provide a vehicle that facilitates the spread of the virus, or both. There have been a number of laboratory-based animal studies on this potential relationship but no epidemiologic research. Researchers at the University of Hong Kong, in the first study of the influenza-air pollution interaction in humans, now report that respiratory hospitalizations and mortality significantly increased when ozone (O_3) levels rose during flu season [EHP 117:248-253; Wong et al.].

Influenza appeared to exacerbate the health effects of ozone pollution in Hong Kong.

The authors conducted a retrospective population-based study focusing on hospitalization and mortality rates for respiratory and cardiovascular disease. Medical data on patients diagnosed with respiratory or cardiovascular disease between 1996 and 2002 came from 14 Hong Kong hospitals. The authors determined "influenza intensity" during the same period as the percentage of respiratory specimens that tested positive for influenza each week. The Hong Kong Environmental Protection Department provided data on average daily concentrations of nitrogen dioxide (NO_2), sulfur dioxide (SO_2), particulate matter smaller than 10 µm (PM10), and O_3.

As O_3 levels increased during times of high influenza intensity, so did the number of hospitalizations and deaths from respiratory disease. The association was stronger in women than men, the researchers reported. There was no significant relationship between O_3 and cardiovascular disease hospitalizations or mortality, and the data reflected no significant modification by influenza on the health effects of the other pollutants studied. Hong Kong has two flu seasons, peaking in January-February and May-July. O_3 levels in Hong Kong typically peak in the sunniest months of September-December, when ultraviolet radiation reacts with nitrogen oxides and volatile organic compounds to form the noxious gas.

A surprising finding was a decrease in hospitalization for respiratory illness when peak PM10 concentrations coincided with flu outbreaks, whereas PM10 increases at other times were associated with increased hospitalizations. The researchers hypothesize that PM10 may diminish the flu effect by limiting the amount of ultraviolet light entering the atmosphere, which in turn would reduce the production of ozone. The authors found weak interactions between influenza and both NO_2 and SO_2, but

cautioned against drawing conclusions about individual pollutants that react in the atmosphere. NO_2, for example, can combine with oxygen to form O_3. The researchers propose that future studies focus on influenza's potential interactions with a combination of pollutants in the atmosphere.

India's drug problem : Chemists show how waste-water contamination affects ecosystem.

[Nature 457, 640-641, February 2009]

Water tested near Hyderabad contains some of the highest environmental drug levels

Waste flowing out of a treatment plant near Hyderabad in India pollutes the region's waters with some of the highest levels of pharmaceuticals ever detected in the environment. Researchers from Sweden report how this effluent has serious adverse effects on the development of tadpoles and zebrafish. The findings raise concerns for the health of wildlife and ecosystems in the region, as well as underscoring little-studied potential effects on human health. "The volume of drug production in that valley is overwhelming the system," says Stan Cox, a researcher at the Land Institute in Salina, Kansas. "Even though they have good [environmental] laws on the books, they're being swamped by the production."

For several years, the National Geophysical Research Institute in Hyderabad and the country's Central Pollution Control Board in Delhi have monitored heavy metal and other pollutants around the town of Patancheru, which is home to factories producing solvents and other chemicals. But although Patancheru is also home to numerous drug companies, the government has not monitored for drugs being released into the environment. In 2007, however, a team led by environmental scientist Joakim Larsson of the University of Gothenburg in Sweden published results from one waste-treatment facility, Patancheru Enviro Tech Ltd (PETL. Around 90 companies in the region that manufacture active pharmaceutical ingredients, or assemble final drug products, send their waste to PETL. With permission, Larsson's team sampled the waste exiting the plant; they found drugs including the antibiotic ciprofloxacin, at concentrations of up to 31,000 micrograms per litre, and the antihistamine cetirizine, at up to 1,400 micrograms per litre. The team estimated that the amount of ciprofloxacin entering the river from the plant could amount to up to 45 kilograms a day - the equivalent of 45,000 daily doses, says Larsson. "The government has not monitored for drugs being released into the environment."

In new work, he and co-workers exposed tadpoles and zebrafish embryos to diluted PETL effluent, equivalent to river water downstream of the plant. At the lowest concentration tested - equivalent to 1,500 cubic metres of effluent diluted in 750,000 cubic metres of river water, or a 0.2% concentration - the tadpoles experienced 40% reduced growth compared with controls. Concentrations of 8-16%, zebrafish embryos lost colour and movement within two days of fertilization, among other developmental effects.

Larsson's team has also found drugs in nearby lakes that do not receive effluent from the PETL plant - which suggests that drugs may also be entering the environment by means other than waste-treatment flow. Past reports, including a 2004 review commissioned by the Indian Supreme Court, noted that the PETL plant could not handle all of the waste arriving for treatment over the years. Local villagers speculated that drivers may have dumped their waste elsewhere. The problem is gaining media attention. In January, The Times of India reported that the office of prime minister Manmohan Singh asked the local pollution board to start collecting data on pharmaceuticals in Patancheru's waters. And an Associated Press report last month triggered a spate of local news stories highlighting the issue.

Sri M. Narayana Reddy, president of the Hyderabad-based Bulk Drug Manufacturers Association (India), questions the validity of the research. In the past decade, Reddy says, drug manufacturers have worked to clean up their effluent, but a legacy of pollution from three decades of chemical manufacturing remains in the region's groundwater and surface water. He also notes that no manufacturer would want to lose such large quantities of a valuable drug such as ciprofloxacin. "At 20 dollars a kilogram, that's not economical," he says. "We suspect the analysis."

But within the Swedish market, Larsson's team obtained a restricted list of which companies produce or buy active pharmaceutical ingredients from India. By matching the list to records from India, they discovered that, out of 242 Swedish products studied, the active ingredient was made in India in 123 cases. Publishing online on 29 January, Larsson and Jerker Fick of Umeå University conclude that 31% of Swedish products are produced at least in part in Patancheru. They propose that developed countries importing drugs should make sure that the supply chain is open, so that consumers know whether their medicines are made in an environmentally sustainable way.

The Swedish Medical Products Agency in Uppsala will lead discussions this year on how to address the country's de facto export of drug waste. The meetings will include input from the Stockholm-based Swedish Association of the Pharmaceutical Industry (LIF) as part of a special commission to review environmental impacts from manufacturing emissions nationally and internationally. "We cannot move forward on this alone," says Ethel Forsberg, director general of the Swedish Chemicals Agency, which is also party to the discussions.

The polluted waters in the area are used for agriculture and also possibly for household use. The local drug manufacturers "produce medicine of very good quality," she says, "but they really cause severe damage to these people living in India around a facility like this."

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एकीकृत नाशीजीव प्रबंधन

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(वैज्ञानिक हिन्दी व्याख्यान माला मार्च, 12, 2009)

एकीकृत नाशीजीव प्रबंधन की बात आजकल हम सभी करते हैं लेकिन इसका सही अर्थ क्या है इसे समझना आवश्यक है। एकीकृत नाशीजीव प्रबंधन का अभिप्राय केवल इस बात से नही है कि कीटनाशकों का प्रयोग खत्म कर दें बल्कि इसका अभिप्राय प्राकृतिक पर्यावरण को ध्यान में रखते हुए खेत तथा इसके आस-पास के वातावरण में उपस्थित नाशीजीवों को नियंत्रित करने के लिए केवल कीट नाशकों या अन्य विषैले रसायनों पर ही निर्भर न रहा जाए बल्कि ऐसी मिली जुली सुरक्षाविधि अपनायी जाए जो वातावरण की दृष्टि से सुरक्षित हो तथा इसमें प्रकृति में पाये जाने वाले लाभकारी परजीवी परभक्षी एवं फफूंदी तथा विषाणुओं को बढ़ावा दिया जाए और आवश्यकता पड़ने पर इनको भी प्रयोगशाला में पैदा कर खेतों में छोड़ा जाए।

ऐसा सोचा जा रहा है कि विश्व की जनसंख्या वर्ष 2050 तक 1100 करोड़ के आस पास हो जाएगी जो कि 1990 की तुलना में दो गुनी होगी। साथ ही साथ प्रति व्यक्ति भूमि उपलब्धता 0.3 है से घटकर 0.13 हो जाएगी। जनसंख्या की इस बढ़ते हुए दबाव को देखते हुए विगत कई वर्षों से उच्च उत्पादकता प्रजाति वाली एक ही तरह की फसल का उत्पादन ज्यादातर भूमि पर किया जाने लगा है। इसके लिए वनो-जंगलों का विनाश भी किया गया। एक तरफ तो उत्पादन में वृद्धि हुई लेकिन वहीं पर दूसरी तरफ फसल परिवेश को क्षति पहुँची इसके कारण नये कीड़े एवं बीमारियों का प्रकोप बढ़ता चला गया। बाद में ऐसा महसूस किया गया कि संसार में नाशीजीवों द्वारा एक तिहाई फसल की क्षति हो रही है। इस वजह से 50 लाख टन या इससे अधिक रसायनों का प्रयोग प्रतिवर्ष किया जाने लगा जिसका मूल्य 1200 अरब रुपयों के आस पास है। यह मूल्य मात्र कीटनाशी या उससे संबंधित रसायनों का ही है जबकि इससे होने वाली अन्य क्षतियों एवं खर्चों का विवरण नहीं है। अब मनुष्य प्रकृति के आमने सामने है और उसने अपनी सुख समृद्धि के लिए ही अपने आप को नये संकट में डाल दिया है। आजकल पूरे विश्व में 20 लाख लोग इन कीटनाशकों से प्रभावित हैं जिनमें से करीबन 20 हजार लोगों की आकस्मिक मृत्यु हो जाती है और कितने लोग कैंसर सहित अन्य घातक बीमारियों का शिकार हो जाते हैं इसकी तो गिनती ही नहीं है इसलिए अब

समय आ गया है कि हम पर्यावरण एवं प्राकृतिक संतुलन के केन्द्र बिन्दुओं को ध्यान में रखते हुए नाशीजीवों के प्रबन्धन के संबंध में ऐसी तकनीक अपनाएं जिससे कीटनाशक,फफूंदी नाशक, खरपतवार नाशक एवं उनसे सम्बन्धित रसायनों का उपयोग कम से कम हो।

एकीकृत नाशी जीव प्रबन्धन (आई.पी.एम.) की उत्पत्ति

फसल सुरक्षा को खेत-परिवेश के संदर्भ में पाँच चरणों में विभाजित किया गया है :-

विकास चरण, उपयोगी चरण, विपत्ति की शुरुआती चरण, घोर विपत्ति चरण और एकीकृत नियंत्रण चरण।

इस समय भारत में सभी फसलें प्रारम्भिक दो चरणों में हैं, लेकिन कपास विपत्ति की चरण में पहुँच रही है। आई पी एम की विकास की अवस्था चल रही हैं।

एकीकृत कीट नियंत्रण के बारे में 1959 में सोचा गया जिसमें रसायनों के साथ-साथ लाभकारी जीवों का भी उपयोग किया जाने लगा। इसमें बाद में सभी विधियों को मिलाकर 1970 में एकीकृत नाशीजीव प्रबंधन का सुझाव दिया गया। विश्व खाद्य संगठन के अनुसार एकीकृत नाशीजीव प्रबंधन ऐसी प्रक्रिया है जिसमें वातावरण एवं नाशीजीवों की जनसंख्या को ध्यान में रखते हुए मिला जुला कर इस प्रकार से नाशीजीवों का प्रबंधन किया जाए कि वे अपनी जनसंख्या को आर्थिक नुकसान स्तर तक न बढ़ा पाए। इस प्रक्रिया में कई विधियाँ अपनाई जाती है खासकर प्राकृतिक विधियाँ। इसके प्रमुख सिद्धांत है।

1. फसलों को बोने से लेकर काटने तक साप्ताहिक निगरानी कर मित्र व शत्रु कीट के बारे में जानकारी रखना।
2. कीटों को नष्ट करने के लिए उन्ही तरीकों को अपनाए जिनसे वातावरण प्रदूषित न हो।
3. समयानुसार आई पी एम के तरीको को अपनाकर नाशी जीवों को उनके आर्थिक क्षति स्तर के नीचे रखना।
4. रसायनिक कीटनाशकों, फफूंदी नाशकों इत्यादि को उचित मात्रा में उसी समय प्रयोग करें जब कीट रोगों की क्षति स्तर को पार कर रही हो।

एकीकृत नाशी जीव के मुख्य घटक :

1. खेत-परिवेश

परिस्थितिकी तंत्र में सामान्यतः ऐसी बहुत सी क्रियाएं होती हैं जिनके द्वारा पौधे और मिट्टी के घास-पात खाने वाले जीवों और प्राकृतिक शत्रुओं के तथा परिवेश के बीच संतुलन बना रहता है। इसमें सभी का अपना-अपना अलग महत्व है। पौधें में कीट एवं व्याधि आदि के बढ़ने के मुख्यतः दो कारण होते हैं : पौधे की जाति-प्रजाति और पौधे की बनावट, लेकिन इनमें प्राकृतिक शत्रुओं की संख्या इन पर पलने वाले कीटों की संख्या पर निर्भर रहती है। इस वजह से कीटनाशक रसायनों का प्रयोग करने के बाद कभी-कभी कीड़े प्रजनन क्षमता में वृद्धि कर अपनी जनसंख्या को आर्थिक नुकसान के स्तर तक बढ़ा लेते हैं। जबकि उनके प्राकृतिक शत्रु ऐसा नहीं कर पाते, क्योंकि उनके प्राकृतिक शत्रु सर्वप्रथम नष्ट होते हैं। कृषि परिवेश विश्लेषण में ज्यादातर कुछ नाशीजीव उनके प्राकृतिक शत्रु और आस-पास का वातावरण ही समाहित होता है। वास्तव में किसी एक कीड़े के प्रकोप होने पर उनकी जनसंख्या में अधिक वृद्धि होती है, जिसका मुख्य कारण वातावरण या मनुष्य स्वयं ही होता है। इसलिए खेत परिवेश को ध्यान में रखते हुए फसल की ऐसी प्रजातियां उगाई जानी चाहिए जो वहां के आस-पास के वातावरण के अनुरूप हो या जहाँ पर फसल लगाई जाये वहाँ पर इस तरह का वातावरण न हो जहाँ कीट या व्याधि उसे शीघ्र नष्ट कर दें।

2. आर्थिक प्रभाव सीमा का स्तर :

आर्थिक प्रभाव सीमा स्तर नाशी जीव को नियंत्रित करने सम्बंधित क्रियाओं जैसे कि कीटनाशकों का छिड़काव करना आदि से होने वाले प्रभाव के बारे में सोचकर निर्णय लेना चाहिये। इसमें यह ध्यान रखना है कि यदि कीटनाशकों का प्रयोग न करें तो उपज का नुकसान होगा, या छिड़काव करने की लागत ज्यादा होगी, या कीटनाशकों के छिड़काव से प्राकृतिक शत्रुओं की संख्या के स्तर या पौधे के स्वास्थ्य पर कोई प्रभाव पड़ेगा। प्रभाव सीमा का स्तर, निर्णय लेने की प्रक्रिया में मार्गदर्शन करता है। क्योंकि इसके द्वारा किसान यह निर्णय ले सकता है कि उसे भविष्य में क्या करना चाहिए कि कीड़े या बीमारी आर्थिक क्षति स्तर तक न पहुंचे। आर्थिक क्षति स्तर वह अवस्था है जिसमें नाशीजीव का नियंत्रण

बहुत जरूरी हो जाता है, नहीं तो किसान को भारी क्षति होती है। इसलिए इस क्षति स्तर तक नाशीजीव न पहुंचे उसके लिए सभी उपाय जैसे कि निरन्तर निगरानी, मित्र जीवों का संरक्षण, प्रबंधन की अन्य विधियाँ शामिल हैं।

आर्थिक क्षति स्तर, फसल एवम् उसकी प्रजाति फसल पैदा करने का स्थान किसानों के स्वयं परिवेश पर निर्भर करती है। जितना ज्यादा नियंत्रण पर खर्च होगा उतना ही कम लाभ होगा। यह भी देखा गया कि आर्थिक क्षति स्तर कभी-कभी पर्यावरण को ध्यान में रखकर नहीं सोचा जाता। इसलिए यदि हम पर्यावरण की क्षति स्तर को ध्यान में रखकर आर्थिक क्षति स्तर को सोचे तो कीड़े एवं बीमारियों के प्रबंधन में केवल उन्हीं कीटनाशकों और रसायनों का प्रयोग करें जो कि पर्यावरण को क्षति न पहुँचाते हों। इसलिए किसान केवल यह न सोचे की कीटों एवं बीमारियों आदि के नियंत्रण में उसे क्या फायदा या नुकसान हो रहा है। वास्तव में 90 प्रतिशत या उससे अधिक कीटनाशक रसायन उन कीड़ों को नष्ट नहीं कर पाते जिनके लिए उनका उपयोग होता है बल्कि वे विभिन्न तरीकों से वातावरण या खाद्य श्रृंखला में प्रवेश कर मानव को ही नुकसान पहुंचाते हैं।

एकीकृत नाशीजीव प्रबंधन की विधियाँ :

शस्य क्रियाएं (कर्षण क्रियाएं)

गर्मी की जुताई करके उसमें मौजूद कीटों की अवस्थाओं को नष्ट करना।

स्वास्थ्य एवं रोग रोधी किस्मों का प्रयोग करना।

समय से बुआई करें तथा फसल चक्र अपनाएं।

पौधे से पौधे और लाइन से लाइन की वांछित दूरी ही रखें।

आवश्यकता होने पर पौधें की जड़ों शोधित करके लगाएं।

संतुलित खाद का प्रयोग करें।

सिंचाई का समुचित प्रबन्ध करें।

कटाई जमीन स्तर से करें और कटाई के बाद फसल अवशेष को नष्ट कर दें।

यांत्रिक विधियाँ :

खेतों से अण्डों व सूड़ियों को एकत्र करके नष्ट करना।

कीट/रोग ग्रसित पौधे को नष्ट करना।

हिस्पा ग्रसित पौधें से पत्तियों का ऊपरी हिस्सा काट देना।

केशवर्म की सुडियों का रस्सी द्वारा पानी में गिरा कर नष्ट करना।

लाइव ट्रेप खेतों में लगाकर कीटों को नष्ट करना।

खरपतवारों को हाथ से व निराई, गुड़ाई करके नष्ट करना।

जैविक विधियाँ :

परजीवी एवं परभक्षी को आवश्यकता पड़ने पर बाहर से लाकर खेत में छोड़ना।

मित्र-जीवों को संरक्षण देना।

हानिकारक व मित्र कीटों का अनुपात बनाए रखना।

जैविक एवं पौधे जनित रसायनों का आवश्यकता पड़ने पर उपयोग।

संगरोध विधियाँ :

इसके अन्तर्गत कानून द्वारा किसी भी कीड़े या बीमारी अथवा खरपतवारों का नियंत्रित किया जाता है।

रासायनिक नियंत्रण :

कीटनाशक, फफूंदीनाशक, खरपतवारनाशक एवं अन्य रसायनों का प्रयोग अन्तिम उपाय के रूप में करें।

सुरक्षित रसायनों का उचित समय पर निर्धरित मात्रा में प्रयोग करें।

रसायनों का प्रयोग करते समय सावधनियाँ बरतें।

खरपतवार नाशकों का प्रयोग बताए गए निर्देशों के अनुसार करें।

एकीकृत नाशी जीव प्रबंधन से लाभ :

इस विधि में पारिस्थितिकि तंत्र और पर्यावरण की सुरक्षा होती है।

कीटनाशक एवं अन्य विषैले रसायनों से जल, वायु तथा भूमि की रक्षा होती है एवं मनुष्य में होने वाले दुष्प्रभाव को कम करती है।

कीड़ों एवम् बीमारियों में प्रतिरोध एवम् प्रजनन क्षमता को कम करती है, लाभदायक कीटों तथा मित्र जीवों की सुरक्षा करती है।

उत्पादन लागत को कम करके विष रहित खाद्य पदार्थ के उत्पादन के लिए।

निर्यात में बढ़ावा।

किसानों द्वारा ग्रामीण परिस्थिति में आसानी से अपनाई जाती है।

कीटनाशकों से बढ़ती दुर्घटनाओं एवं स्वास्थ्य समस्याओं को रोकने के लिए।

कृषकों में मित्र कीटों के प्रति जागरूकता बढ़ाने के लिये।

आई.पी.एम. तकनीक कृषकों को कृषि रक्षा के लिए सही निर्णय लेने की क्षमता प्रदान करती है।

वन्य जीवों की सुरक्षा करती है।

हिन्दी व्याख्यान माला का आयोजन

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Any feedback on this publication is welcomed.
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