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# TOXICOLOGY

## *Research Bulletin*

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Indian Institute of Toxicology Research, Lucknow  
(Formerly: Industrial Toxicology Research Centre, Lucknow)

## 66<sup>TH</sup> CSIR FOUNDATION DAY CELEBRATED AT IITR

CSIR Foundation Day Function was organised at Indian Institute of Toxicology Research (IITR) on September 26, 2008. Dr DK Saxena, Chairman of the Organizing Committee gave the genesis of the CSIR Foundation Day.

On this occasion officials of IITR who had completed 25 years of service and superannuated were honoured by Dr Ashwani Kumar, Acting Director, IITR Earlier on September 23, 2008 an essay competition was held for the children of CSIR employees. The topics of the essay contest were: 1. Water Conservation for Sustainable Development and 2. Alternate Energy Sources.

The prize winners for essay competitions were:

### Junior Group

Priya Awasthi – I Prize



Winners of painting competition at CSIR Foundation day function

Akshay Shukla – II Prize  
Divyanshu Sharma – III Prize  
Yashi Tripathi – Consolation

### Senior Group

Manas Misra – I Prize  
Ardhya Prakash – II Prize  
Shanya Das Rastogi – III Prize  
Manisha Dixit – Consolation



Km. Nivedita Maji

Dr Ashwani Kumar awarded prizes to the children. Km. Nivedita Maji D/o Shri BK Maji was honoured for securing over 90% marks in science subjects in the Class XII exam.

Dr RK Upreti, Convener of the programme proposed the vote of thanks.



Science exhibition (held at NBRI, Lucknow) on CSIR Foundation day function

### 1) Determination of antimicrobial resistance and virulence gene signatures in surface water isolates of *Escherichia coli*

[Ram S, Vajpayee P, Tripathi U, Singh RL, Seth PK and Shanker R. 2008. *Journal of Applied Microbiology*. 105: 1899-1908]

To determine the occurrence of *Escherichia coli* harbouring virulence markers of shiga- or enterotoxins and resistance to antimicrobials in surface waters. Surface water samples were collected at six locations of the river Gomti. *E. coli* isolates (n = 90) were characterized for their pathogenic potential using polymerase chain reaction to detect virulence genes as well as their sensitivity to antimicrobial agents using disc diffusion methods. In this study, 57.8% of *E. coli* isolates exhibited resistance to three or more antimicrobial agents. Sensitivity to cephalexin, gentamicin and norfloxacin was observed in 7.8%, 48.9% and 77.8% of isolates, respectively. Both stx1 and stx2 genes were present in 15.6% of isolates while remaining isolates had either stx1 (17.8%) or stx2 (6.7%). The stx1 gene (33.3%) was more prevalent than stx2 (22.2%). The results indicate that the LT1 and ST1 genes were positive in 21.2% of isolates. The presence of multi-drug resistance and virulence genes in *E. coli* isolated from surface water being used for domestic and recreational purposes may result in waterborne outbreaks. The data will be useful in monitoring surface waters for forecasting and management of waterborne outbreaks.

### 2) Multipronged evaluation of genotoxicity in Indian petrol-pump workers

[Pandey AK, Bajpayee M, Parmar D, Kumar R, Rastogi SK, Mathur N, Thorning P, de Matas M, Shao Q, Anderson D, Dhawan A. 2008. *Environ Mol Mutagen*. Sep 17. (Epub ahead of print)]

Petrol (gasoline) contains a number of toxicants. This study used human biomonitoring to evaluate the genotoxic effects of exposure to benzene in petrol fumes in 100 Indian petrol-pump workers (PPWs) and an equal number of controls. The study

was corroborated with *in silico* assessments of the Comet assay results from the human biomonitoring study. An *in vitro* study in human lymphocytes was also conducted to understand the genotoxicity of benzene and its metabolites. In a subset of the population studied, higher blood benzene levels were detected in the PPWs (n = 39; P < 0.01) than the controls (n = 18), and 100-250 ppb benzene was also detected in air samples from the petrol pumps. PPWs had higher levels of DNA damage than the controls (P < 0.01). In addition, the micronucleus assay was performed on lymphocytes from a subset of the subjects, and the micronucleus frequency for PPWs was significantly higher (n = 39; 14.79 +/- 3.92 per thousand) than the controls (n = 18; 7.54 +/- 3.00 per thousand). Human lymphocytes were treated *in vitro* with benzene and several of its metabolites and assayed for DNA damage with the Comet assay. Benzene and its metabolites produced significant (P < 0.05) levels of DNA damage at and above concentrations of 10 µM. The metabolite, p-benzoquinone, produced the greatest amount of DNA damage, followed by hydroquinone > benzene > catechol > 1,2,4,-benzenetriol > muconic acid. This study demonstrates that, using sensitive techniques, it is possible to detect human health risks at an early stage when intervention is possible.

### 3) Chemopreventive potential of resveratrol in mouse skin tumors through regulation of mitochondrial and PI3K/AKT signaling pathways

[Roy P, Kalra N, Prasad S, George J, Shukla Y. 2008. *Pharm Res*. Sep 13. (Epub ahead of print)]

This study was planned to investigate the chemopreventive potential of resveratrol, a phytoalexin found in seeds and skin of grapes, berries and peanuts in 7,12 dimethyl benz(a) anthracene (DMBA) induced mouse skin tumorigenesis. Topical treatment of resveratrol was given to the animals 1 h prior to DMBA for 28 weeks. At the end of the study period, the skin tumors were dissected out and western blotting was carried out to examine the regulation of proteins involved in anti-tumorigenesis in response to resveratrol. Chemopreventive properties of resveratrol were reflected

by delay in onset of tumorigenesis, reduced cumulative number of tumors, and reduction in tumor volume. Results of the western blotting showed that resveratrol treatment increased the DMBA suppressed p53 and Bax while decreased the expression of Bcl-2 and Survivin. Further, resveratrol supplementation resulted in release of cytochrome C, caspases activation, increase in apoptotic protease-activating factor-1 (Apaf-1) as mechanism of apoptosis induction. Resveratrol was also found to inhibit skin tumorigenesis through regulation of Phosphatidylinositol-3-kinase (PI3K) and AKT proteins which are implicated in cancer progression because it stimulates proliferation and suppresses apoptosis. Based on the results it can be concluded that resveratrol regulates apoptosis and cell survival in mouse skin tumors as mechanism of chemoprevention hence deserve to be a chemopreventive agent.

#### **4) Long-term functional restoration by neural progenitor cell transplantation in rat model of cognitive dysfunction: co-transplantation with olfactory ensheathing cells for neurotrophic factor support**

[Srivastava N, Seth K, Khanna VK, Ansari RW, Agrawal AK. 2008. *Int J Dev Neurosci*. Aug 12. (Epub ahead of print)]

Neural progenitor cell transplantation has emerged as a promising approach for cell replacement therapy in the brain of neurodegenerative diseases. These are multipotent stem cells with self-renewal capabilities and can give rise to cells of all the three lineages of nervous system and can be maintained and differentiated to desirable neuronal subtypes *in vitro* with known trophic factors. However, like fetal cells, neural progenitor cells after differentiating to specific neuronal type also require continuous neurotrophic factor support for their long-term survival following transplantation. Recent reports suggest that olfactory ensheathing cells are capable of providing continuous neurotrophic factor to the transplanted neural progenitor cells for their long-term survival. In the present investigation, an attempt has been made to validate functional restoration in kainic acid lesioned rat model of cognitive dysfunction following co-transplantation of neural progenitor cells with olfactory ensheathing

cells. Animals lesioned with kainic acid in CA3 subfield of hippocampal region were transplanted with neural progenitor cells, olfactory ensheathing cells or neural progenitor cells+olfactory ensheathing cells together. Twelve weeks post-transplantation functional restoration was assessed using neurobehavioral, neurochemical, and immunohistochemical approaches. Significant recovery in learning and memory (89%) was observed in co-transplanted group when compared to lesioned group. This was accompanied by significantly higher expression of choline acetyltransferase and restoration in cholinergic receptor binding in co-transplanted group (61%) over the animals transplanted either olfactory ensheathing cells or neural progenitor cells alone. Role of olfactory ensheathing cells in supplementing neurotrophic factors was further substantiated *in vitro* by pronounced differentiation of neural progenitor cells to choline acetyltransferase/acetylcholine esterase immunoreactive cells when co-cultured with olfactory ensheathing cells as compared to neural progenitor cells alone. The results strengthened the hypothesis that co-transplantation of olfactory ensheathing cells and neural progenitor cells may be a better approach for functional restoration in kainic acid induced rat model of cognitive dysfunction.

#### **5) Effects of soil amendments on the bioavailability of heavy metals from zinc mine tailings**

[Misra V, Tiwari A, Shukla B, Seth CS. 2008. *Environ Monit Assess*. Jul 16. (Epub ahead of print)]

A study was conducted to test the effects of soil amendments on the bioavailability of heavy metals in a zinc mine tailings containing soil to plants, using the Indian mustard plant (*Brassica juncea*) as a test organism. Zinc mine tailing containing soil was amended with humus soil (HS) and phosphatic clay (PC). The zinc mine tailing containing soil (ZMTS) was characterized for heavy metals. It was mixed with PC and HS, and four mixtures were prepared. The first mixture contained ZMTS, and served as a control. The second mixture contained ZMTS and PC in the ratio of 1:1 (w/w). The third mixture contained ZMTS and HS in the ratio of 1:1 (w/w). The fourth mixture containing ZMTS, PC and HS in the

ratio of (2:1:1) (w/w). A slight increase in the bioavailability of Pb, Cu, Zn and Mn was noticed with increase in the incubation time from 14 to 42 days. The bioavailability of Pb, Cu, Zn and Mn from ZMTS alone in Brassica plant was in the range of 94-99% up to 42 days. Addition of PC and HS to the ZMTS soil reduced the bioavailabilities of Pb by (15%), of Cu by (20%), of Zn by (20%) and of Mn by (25%) in the mustard plant. The data showed that PC in the presence of HS had a high affinity for the heavy metals in the order of Pb, Cu, Zn and Mn.

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

[Chandra R, Raj A, Yadav S, Patel DK. 2008. *Environ Monit Assess*. Jul 12. (Epub ahead of print)]

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 degrees 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 bacterial 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 liberation of significant amount of chloride due to bacterial dechlorination of chlorolignins and chlorophenols this showed 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.

## 8) Association of functionally important polymorphisms in cytochrome P4501B1 with lung cancer

[Shah PP, Singh AP, Singh M, Mathur N, Mishra BN, Pant MC, Parmar D. 2008. *Mutat Res*. Aug 25;643(1-2):4-10]

In the present study, genotype and haplotype frequencies of four polymorphisms of cytochrome P450 1B1 (CYP1B1) that cause amino acid changes (Arg-Gly at codon 48, Ala-Ser at codon 119, Leu-Val at 432 and Asn-Ser at codon 453) were studied in 200 patients suffering from lung cancer and equal number of controls. A significant difference was observed for the distribution of variant genotypes of CYP1B1Arg48Gly and Ala119Ser polymorphisms (CYP1B1\*2) in cases when compared to the controls. No significant difference was observed for the distribution of variant genotypes of CYP1B1Leu432Val (CYP1B1\*3) and CYP1B1Asn453Ser (CYP1B1\*4) polymorphism. When the four SNPs were analyzed using a haplotype approach, SNPs at codon 48 (Arg48Gly) and codon 119 (Ala119Ser) exhibited complete linkage disequilibrium (LD) in all the cases and controls. Significant differences in the distribution of the three haplotypes (G-T-C-A, G-T-G-A and G-T-C-G) were observed in the cases when compared to controls. Tobacco use in the form of smoking as well as chewing was found to significantly increase the risk of lung cancer in patients by interacting with CYP1B1Ala119Ser genotypes demonstrating the role of gene-environment interaction in lung cancer. Further, the risk of lung cancer increased several fold in the patients carrying the genotype combinations of CYP1B1Ala119Ser and CYP1B1Leu432Val with GSTM1, a phase II enzyme suggesting the importance of gene-gene interactions in enhancing the susceptibility to lung cancer.

## 9) Correlation of phthalate exposures with semen quality

[Pant N, Shukla M, Patel DK, Shukla Y, Mathur N, Gupta YK, Saxena DK. 2008. *Toxicol Appl Pharmacol*. Aug 15;231(1):112-6]

Phthalates are widely used man-made chemical released in the environment and human exposure is mainly through diet. As the phthalate plasticizers are not covalently bound to PVC, they can leach, migrate or evaporate into the environment and as a result have become ubiquitously contaminants. The present study investigates the correlation, if any, between the phthalate esters (DEP, DEHP, DBP, DMP, DOP) and sperm mitochondrial status, ROS, LPO, SCSA, and sperm quality. The study was conducted in the urban/rural population of Lucknow visiting Obstetrics and Gynecology Department, CSMMU, Lucknow. Semen analysis was performed according to the WHO guidelines while phthalate analysis by HPLC and LPO by spectrophotometer and the sperm mitochondrial status, ROS, SCSA using flow cytometry. The questionnaire data showed no significant difference in the demographic characteristics among the groups. In general, urban population was found to have statistically significant higher levels of phthalate esters than the rural. Further, infertile men showed statistically significant ( $p < 0.05$ ) higher levels of pollutants in the semen than fertile men. A negative correlation between semen phthalate level viz DEHP and sperm quality and positive association with depolarized mitochondria, elevation in ROS production and LPO, DNA fragmentation was established. The findings are suggestive that phthalates might be one among the contributing factors associated with the deterioration in semen quality and these adverse effects might be ROS, LPO and mitochondrial dysfunction mediated.

## **10) Alterations in redox potential of glutathione/ glutathione disulfide and cysteine/cysteine disulfide couples in plasma of dropsy patients with argemone oil poisoning**

[Babu CK, Ansari KM, Mehrotra S, Khanna R, Khanna SK, Das M. 2008. *Food Chem Toxicol.* Jul;46(7):2409-14]

Several incidences of adverse effects on human health have been reported in many countries, due to consumption of edible oil adulterated with argemone oil (AO). The clinical manifestation of the disease is commonly referred to as epidemic dropsy. In the present study, we determined the relationship between redox potentials (E(h)) of glutathione/ glutathione disulfide (GSH/GSSG), cysteine/

cysteine disulfide (Cys/CySS) couples and non-enzymatic antioxidants such as alpha-tocopherol and ascorbic acid status in plasma of dropsy patients (n=14) from an outbreak of argemone oil poisoning in Lucknow (March, 2005), India. Depleted GSH (55%) and concomitant enhancement (163%) of plasma GSSG content was observed in patients ( $P < 0.05$ ). Furthermore, lower content of Cys (42%) and CySS (25%) was noticed in patients ( $P < 0.05$ ) when compared to control subjects. Eh GSH and Eh Cys values were shifted by +46 mV and +12 mV towards more oxidizing environment in patients ( $P < 0.05$ ). In addition, alpha-tocopherol and ascorbic acid contents were found to be depleted significantly ( $P < 0.05$ ) in plasma of patients (59-58%). The alterations in redox potentials and antioxidants in plasma, which are synthesized in liver, may be responsible for histopathological changes in hepatic tissue of patients showing swelling of hepatocytes, fluid accumulation in spaces of Desci along with mild kupfer cell hyperplasia. Over all the present study shows that redox state of GSH/GSSG and Cys/CySS pools become oxidized which inturn causes depletion of alpha-tocopherol and ascorbic acid, thus providing a strategy to distinguish pro-oxidant and antioxidant events in patients.

## **11) Responsiveness of cerebral and hepatic cytochrome P450s in rat offspring prenatally exposed to lindane**

[Johri A, Yadav S, Dhawan A, Parmar D. 2008. *Toxicol Appl Pharmacol.* Aug 15;231(1):10-6]

Prenatal exposure to low doses of lindane has been shown to affect the ontogeny of xenobiotic metabolizing cytochrome P450s (CYPs), involved in the metabolism and neurobehavioral toxicity of lindane. Attempts were made in the present study to investigate the responsiveness of CYPs in offspring prenatally exposed to lindane (0.25 mg/kg b. wt.; 1/350th of LD(50); p. o. to mother) when challenged with 3-methylcholanthrene (MC) or phenobarbital (PB), inducers of CYP1A and 2B families or a sub-convulsant dose of lindane (30 mg/kg b. wt., p. o.) later in life. Prenatal exposure to lindane was found to produce an increase in the mRNA and protein expression of CYP1A1, 1A2, 2B1, 2B2 isoforms in brain and liver of the offspring at postnatal day 50. The increased expression of the CYPs in the

offspring suggests the sensitivity of the CYPs during postnatal development, possibly, to low levels of lindane, which may partition into mother's milk. A higher increase in expression of CYP1A and 2B isoenzymes and their catalytic activity was observed in animals pretreated prenatally with lindane and challenged with MC (30 mg/kg, i. p. x 5 days) or PB (80 mg/kg, i. p. x 5 days) when young at age (approx. 7 weeks) compared to animals exposed to MC or PB alone. Further, challenge of the control and prenatally exposed offspring with a single sub-convulsant dose of lindane resulted in an earlier onset and increased incidence of convulsions in the offspring prenatally exposed to lindane have demonstrated sensitivity of the CYPs in the prenatally exposed offspring. The data assume significance as the subtle changes in the expression profiles of hepatic and cerebral CYPs in rat offspring during postnatal development could modify the adult response to a later exposure to xenobiotics.

## **12) Gene expression profiles of mouse striatum in control and maneb + paraquat-induced Parkinson's disease phenotype: validation of differentially expressed energy metabolizing transcripts**

[Patel S, Singh K, Singh S, Singh MP. 2008. *Mol Biotechnol. Sep*;40(1):59-68]

The present study was undertaken to investigate the gene expression patterns of the striatum of control and maneb + paraquat-induced Parkinson's disease (PD) phenotype in mouse to identify the differentially expressed transcripts. The animals were treated with and without maneb (30 mg/kg, i.p.) + paraquat (10 mg/kg, i.p.), twice a week, for 3, 6, and 9 weeks. The RNA was isolated from control and treated mouse striatum and reverse transcribed, and equal quantities of labeled cDNA were mixed and hybridized with mouse 15 k arrays. Comparative transcription patterns showed the time of exposure dependent alteration in the expression of several transcripts associated with various pathways. RT-PCR reconfirmed the differential expression of some energy metabolizing transcripts. The study provides maneb + paraquat-induced differential expression of many transcripts using high-density microarray approach. Few transcripts, which were previously not reported to be associated with neuronal degeneration, were also identified. The results obtained thus suggest that maneb + paraquat induce neurotoxicity in the striatum in a time of exposure dependent manner via multiple pathways and defective energy metabolism could play a critical role



Dr. Anurag Tripathi joined as Scientist Gr. IV(1) on August 29, 2008. He did his M.Sc in Biotechnology (2004) and Ph.D. Biotechnology (2008) from Banaras Hindu University. His area of specialization during doctoral work was Immunology with emphasis on the effects of prolactin and growth hormone on macrophages. He has five publications to his credit in peer reviewed international journals.



Dr Abhay Raj joined IITR, Lucknow, in September 2008 as Scientist Gr. IV (I) in Environmental Microbiology Section. He completed his M.Sc. in 2002 in Environmental Science form University of Lucknow. After M.Sc. he joined as Research Scholar at IITR under the supervision of Dr Ram Chandra and he completed his Ph.D. in 2008 on "Isolation and characterization of lignin degrading bacteria for the treatment of

## APPOINTMENTS

effluent from pulp and paper mill". Dr Raj has presented his findings at national and international conferences. He has published nine research papers in peer reviewed journals.



Dr Nasreen Ghazi Ansari joined IITR Lucknow, in September 2008 as Scientist Gr. IV (1) in Metal Analysis Laboratory. She has completed her graduation and post graduation with specialization in organic chemistry from Rani

Durgawati University, Jabalpur. After M.Sc she joined Prof. Ashutosh Srivastava at Department of Chemistry, Rani Durgawati University, Jabalpur and completed her Ph.D on 'Structure Elucidation of Natural Product' in 2006. She has served as Residue chemist for more than six years at National Research Centre Weed Science, Jabalpur. Dr Nasreen has presented her findings at national and international conferences and has published three papers.

## SUPERANNUATIONS

Dr SK Bhargava, IV(5)

31/08/2008

Dr SC Srivastava, IV(3)

31/09/2008

Shri VK Singh, III(5)

31/08/2008

Dr (Mrs.) FN Jaffery IV(2)

31/09/2008

## CURRENT TOPIC

### BIODESULFURIZATION OF PETROLEUM FRACTIONS FOR A CLEANER ENVIRONMENT

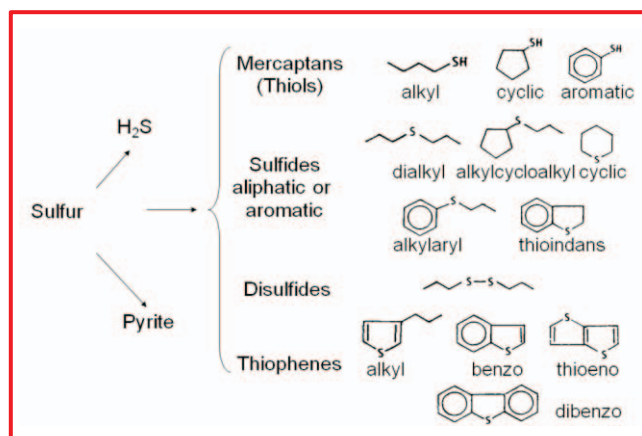
**Preeti Srivastava and Ashwani Kumar**

Environmental Biotechnology Section, Indian Institute of Toxicology Research, Lucknow.

#### Introduction

Fossil fuels including petroleum products have been the major source of energy for our various needs and are likely to remain so for many more years. More than 80 million barrels of oil is pumped out from the earth's surface, each day, and around 90% of it is processed for use as fuels. Sulfur constitutes up to 5% of crude oils, usually as organosulfur compounds (Fig. 1), which after combustion of fuel is released to the atmosphere in the form of gaseous sulfur oxides and dioxides etc (Monticello, 2000). These dissolve in atmospheric water vapour, lead to acid rain and cause various environmental problems including haziness, which substantially reduces average temperatures in affected areas (Charlson et al., 1992). SO<sub>2</sub> and related pollutants have also been linked to a number of human diseases e.g. bronchial irritation and asthma attacks in susceptible individuals (Mohebbali and Ball, 2008).

Sulfur compounds also cause severe corrosion problems in pipelines and pumping and refining equipment, premature failure of combustion engines, and poisoning of the catalytic converters that are used in automotive engines.

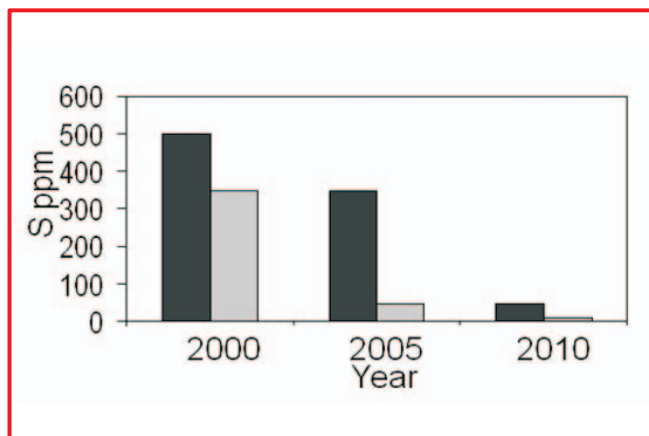


**Fig. 1 Some major types of sulfur containing compounds present in crude oil**



## Recommended levels of sulfur in petroleum products.

Reduction in sulfur emissions is highly desirable and many countries have, therefore, established stringent sulfur emission standards. In European Union (EU), the recommended level in diesel fuel in the year 2000 was 350ppm but the limit was revised to 50 ppm for the year 2005 and the recommended level for 2010 is 10ppm. Similarly in USA and Japan, the recommended level of sulfur is 10ppm for the year 2009 (Mohebbi and Ball, 2008). In India, the sulfur content according to Bharat Stage III norms is 350ppm in eleven major cities, and 500 ppm in the rest of the country. The recommended level for the year 2010, Bharat Stage IV, is 50ppm in the eleven major cities and 350ppm in rest of the country (Auto fuel policy report, 2002).



**Fig. 2** Changing limits of sulfur content in diesel for major cities in India (black) and EU (grey)

## Hydrodesulfurization

Conventionally, sulfur is removed from petroleum fractions by a process known as Hydrodesulfurization (HDS). It is also referred sometimes as hydrotreatment or hydroprocessing. Briefly, it includes the flushing of petroleum with hydrogen at high temperature of 300-350°C and at high pressure, in the presence of sulfided Nickel or Molybdenum as catalyst, to remove sulfur as hydrogen sulfide (Fig. 3a). It is capable of removing most of the inorganic as well as some organic sulfur compounds. Some organosulfur compounds such as dibenzothiophene, benzothiophenes and their derivatives, however, are not removed completely by this process. For their removal, a modified HDS

method, referred as deep HDS, is needed, which requires a higher temperature i.e. 400-450°C and presence of some specialized catalysts. While dibenzothiophenes and benzothiophenes are removed by this process, their alkylated derivatives such as 4,6- dimethyl dibenzothiophene, 4- methyl dibenzothiophene etc. are not removed. The reason for their recalcitrance is possibly because their sulfur moiety is sterically hindered and remains inaccessible to the catalyst. The recalcitrance depends upon the degree of alkylation and the positioning of alkyl groups in the organosulfur compounds. Their reactivity, for example, decreases in the order: Dibenzothiophene > 4 methyl dibenzothiophene > 4,6 dimethyl dibenzothiophene and so on.

Presently there are two major disadvantages of Hydrodesulfurization. First, it is not able to achieve the new sulfur limits set by regulatory agencies and second, the process is very costly as it works at extremely high conditions of temperature and pressure and requires specialized catalysts. Scientists throughout the world are working to find alternative solutions to reduce sulfur. Some of which are (i) extraction using ionic liquids (ii) Adsorption (iii) Photochemical oxidation and (iv) Biodesulfurization.

## Biodesulfurization (BDS)

Biodesulfurization, where in microorganisms are used to break the target compounds to release sulfur from petroleum fractions, offers the advantage of removing the sulfur selectively without affecting the calorific value of fuel. It is energy efficient, and is therefore environmentally and economically favorable.

Several microorganisms have been isolated for this purpose by using Dibenzothiophene (DBT) as the model compound. These convert DBT into 2-hydroxybiphenyl in four steps, with concomitant release of sulfur, by a so called 4S pathway. DBT-sulfoxide and DBT-sulfone are the intermediated products of this reaction. The end product 2-hydroxybiphenyl remains in the fuel and therefore the calorific value is not lost. Recently, in some of the microorganisms an extended 4S pathway, called 4SM pathway, has been reported (Xu et al., 2007), where 2-hydroxybiphenyl is further converted into

methoxybiphenyl as shown below in Fig. 3b. Genes *dszA*, *dszB*, *dszC* and *dszD*, encode the enzymes for desulfurization reaction. While *dszA*, *dszB* and *dszC* are found in an operon in the order *dszABC*, the *dszD* gene, which encodes for the Flavin reductase, is found unlinked in all the organisms reported so far.

Various genetic engineering measures have been tried to improve the desulfurization activity. Thus, since *dszABC* operon is repressed in the presence of inorganic sulfate, its native promoter was replaced with a non-repressible promoter and was found to enhance the desulfurization activity (Noda et al., 2002). Also, It was found that the desulfinase, *DszB*, catalyses the rate limiting step of the pathway, and increasing its levels, either by removing the overlap between *dszA* and *dszB*, or by rearranging the genes in the operon such that *dszB* becomes the first gene in the operon, resulted in

increased desulfurization activity (Li et al., 2007; Li et al., 2008). Further site directed mutagenesis was also attempted on *dszB* to increase the catalytic activity and thermostability of the enzyme (Ohshiro et al., 2007). Similar experiment was also done for *DszC*, which catalyzes the first step of the pathway, to improve its substrate range (Arsendorf et al., 2002).

Microorganisms are also capable of removing other organosulfur compounds; such as 4,6 dimethyldibenzothiophene and other alkylated dibenzothiophenes and benzothiophenes (Gunam et al., 2006), which are otherwise recalcitrant to hydrodesulfurization. Since at high temperatures, viscosity of petroleum is reduced and reaction turnover rates are higher, thermophilic microorganisms for desulfurization have also been isolated and characterized (Torkamani et al., 2008).

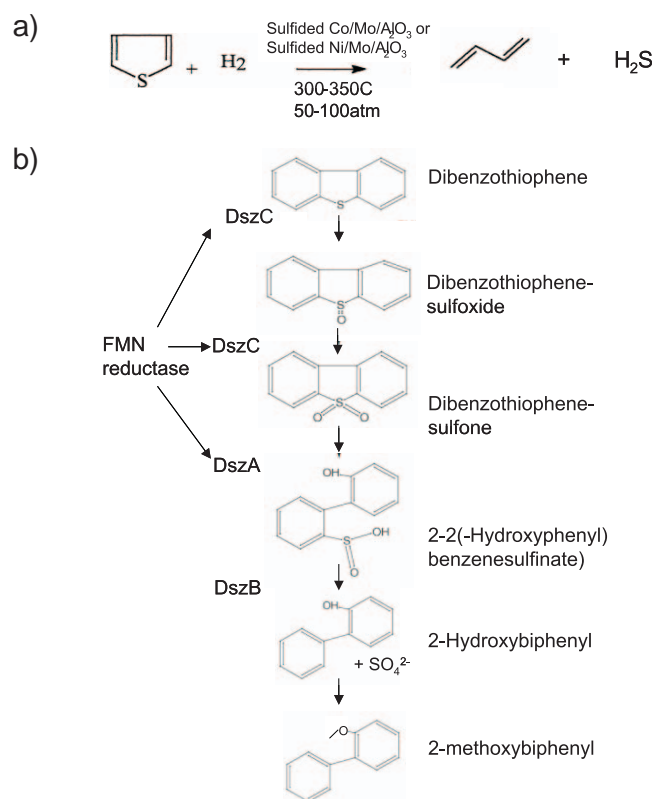


Fig. 3 a) HDS reaction for Thiophene; b) BDS pathway for Dibenzothiophene

## Limitations of biodesulfurization technology

For commercialization, the desired desulfurization activity is 1.2-3mM/ g Dry Cell Weight/ h. Extensive research work on selective sulfur removal for more than sixteen years could not yield any strain that can meet these requirements. The maximum activity achieved so far is only 320uM/ g DCW/ h (Kilbane J II, 2006). The first attempt to commercialize biodesulfurization technology was initiated by Energy Biosystem Corporation, USA (later renamed as Enchira Biotechnology Corporation). They were prepared with the complete bioreactor setup, but new recommended lower limits of sulfur forced them to quit. Some 'out of the box approaches' are, therefore, required for effective biodesulfurization.

## Future prospects in biodesulfurization research

Although many microorganisms are capable of removing sulfur from organosulfur compounds that are otherwise recalcitrant to hydrodesulfurization, the desulfurization rates need to be increased to meet the commercial demands. With the development of new genetic tools viz. metagenomics, integrative genetics, and artificial evolution, the capabilities of microorganisms can be enhanced to reduce the sulfur to negligible amounts.

Separately, there are a lot of heavy crude reserves, which are underutilized because of the high sulfur content and viscosity problems. In North America alone, over 3 trillion barrels of known petroleum reserves are largely untapped or underutilized because of these problems. With increasing consumption rates, the time when available petroleum will predominantly or exclusively be heavy high sulfur petroleum is not far off. Biodesulfurization, can also play role in reducing the viscosity of crude oil, which is largely due to some superstructures that are linked due to aliphatic sulfide linkages. Selective biodesulfurization at the time of extraction of crude oil before sending the crude to the refineries can, therefore, be very useful. Biodesulfurization, if done just before doing the dewatering and desalting steps in crude oil extraction, will not require any modification in the refinery set up.

Thus, biodesulfurization, in combination with other technologies, can be effectively used for both the removal of sulfur and the decrease in viscosity of heavy oils and residuum. It will not only mitigate environmental problems but will also contribute towards better yield of value added products from petroleum.

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## RESEARCH DIGEST

### Food companies reject clones over consumer fears

[New Scientist magazine, 2673 17 September 2008, page 6]

NESTLÉ and Kraft don't wish to sell the products from cloned animals because consumers won't buy them, even when these products have been declared as safe by US Food and Drug Administration (USFDA) and the European Food Safety Authority (EFSA).

US pressure groups "The Center for Food Safety and Friends of the Earth" say that 20 food companies they asked would not sell cloned food - even though both the US Food and Drug Administration and the European Food Safety Authority ruled this year that food from cloned animals is safe to eat. Most firms are worried that consumers won't buy the products, rather than whether there is any evidence against the regulatory bodies' decision, says Lisa Bunin of the CFS. "Consumers are currently not receptive to ingredients from cloned animals." Their high price means clones will probably be used to improve the stock, rather than for milk or meat. Only eight small companies said they would not use products from the clones' offspring. The big guns may join them if market rejection spreads. The European Parliament called for a ban on any cloned animal products.

### Bisphenol A, a plastic bottle chemical linked to heart disease

[Journal of the American Medical Association (vol 300, p 1303)]

Exposure to a compound, bisphenol A (BPA) commonly found in plastic food containers is linked to heart disease and type 2 diabetes, according to the first large epidemiological study in humans. The findings come just as the controversy surrounding this chemical, is heating up, with two US government bodies issuing conflicting advice about its safety. BPA is used in the coatings that line food tins, in the hard clear plastics that make baby bottles and in dental sealants. More than 2 million tonnes are produced worldwide every year, and over 93% of Americans have evidence of exposure in their urine. But most studies investigating potential dangers have been done in animals, which metabolise the chemical more slowly than humans, raising questions about how reliable such findings are.

In the a study Tamara Galloway and colleagues at the University of Exeter, UK, analysed data from 1455 human adults as part of the National Health and Nutrition Examination Survey (NHANES) conducted in 2003 and 2004. The researchers looked at whether there was an association between urinary BPA concentrations and certain diseases.

They found that, after adjusting for age and sex, concentrations of BPA were higher in people with cardiovascular disease and type 2 diabetes. People with the most BPA exposure were three times as likely to have cardiovascular disease, and 2.4 times as likely to have diabetes, as those with the

lowest levels. Nira Ben-Jonathan, at the University of Cincinnati, who has studied BPA for over 12 years, was impressed with the paper and said that reported suppression of adiponectin, a hormone that regulates insulin sensitivity, would predispose a person to diabetes. Csaba Leranth, at Yale University in New Haven, Connecticut, whose group did the first study of BPA in primates, says a human study was much needed and "I find the data very convincing,".

This research will help inform the policy of the US Food and Drug Administration, which held a public hearing into the chemical. Last month, the FDA issued a draft report saying current exposures of the chemical posed no danger. Just a few weeks later, however, the National Toxicology Program, a government-funded body charged with providing unbiased, scientifically sound evaluations of available evidence, issued its final report on the chemical. This concluded that current human exposure levels were cause for concern. "Changes are happening at doses most humans are exposed to," says John Bucher, NTP Associate Director. Anila Jacob, at the Environmental Working Group, a non-profit organisation based in Washington, DC, says the discrepancy between the two bodies is due to how they chose to weigh the evidence.

## **Chemical breakthrough turns sawdust into biofuel**

[NewScientist.com news service, 18 July 2008]

A wide range of plant material could be turned into biofuels; thanks to a breakthrough that converts plant molecules called lignin into liquid hydrocarbons. The reaction reliably and efficiently turns the lignin in waste products such as sawdust into the chemical precursors of ethanol and biodiesel. In recent years, the twin threats of global warming and oil shortages have led to growth in the production of biofuels for the transportation sector.

**Food crisis-** The biofuels industry has relied instead on starchy food crops such as corn and sugar cane to provide the feedstock for their reactions. But that puts the industry into direct competition with hungry humans, and food prices have risen as a result. A second generation of biofuels could relieve the pressure on crop production by breaking down larger

plant molecules – hundreds of millions of dollars are currently being poured into research to lower the cost of producing ethanol from cellulose. But cellulose makes up only about a third of all plant matter. Lignin, an essential component of wood, is another important component and converting this to liquid transport fuel would increase yields. However, lignin is a complex molecule and, with current methods, breaks down in an unpredictable way into a wide range of products, only some of which can be used in biofuels.

**Balancing act-** Now Yuan Kou at Peking University in Beijing, China, and his team have come up with a lignin breakdown reaction that more reliably produces the alkanes and alcohols needed for biofuels. Lignin contains carbon-oxygen-carbon bonds that link together smaller hydrocarbon chains. Breaking down those C-O-C bonds is key to unlocking the smaller hydrocarbons, which can then be further treated to produce alkanes and alcohol. But there are also C-O-C bonds within the smaller hydrocarbons which are essential for alcohol production and must be kept intact. Breaking down the C-O-C bonds between chains, while leaving those within chains undamaged, is a difficult balancing act.

**Breaking bonds-** Kou's team used their previous experience with selectively breaking C-O-C bonds to identify hot, pressurised water – known as near-critical water – as the best solvent for the reaction. Water becomes near-critical when heated to around 250 to 300 °C and held at high pressures of around 7000 kilopascals. Under those conditions, and in the presence of a suitable catalyst and hydrogen gas, it reliably breaks down lignin into smaller hydrocarbon units called monomers and dimers. The researchers experimented with different catalysts and organic additives to optimise the reaction. They found that the combination of a platinum-carbon catalyst and organic additives such as dioxane delivered high yields of both monomers and dimers. Under ideal conditions, it is theoretically possible to produce monomers and dimers in yields of 44 to 56 weight % and 28-29 wt% respectively. Weight % is the fraction of the solution's weight that is composed of either monomers or dimers. Removing the hydrocarbons from the water solvent after the reaction is easy – simply by cooling the water again, the oily

hydrocarbons automatically separate from the water. It is then relatively simple to convert those monomers and dimers into useful products, says Ning Yan at the Ecole Polytechnique Fédérale de Lausanne, Switzerland, and a member of Kou's team. That results in three components: alkanes with eight or nine carbon atoms suitable for gasoline, alkanes with 12 to 18 carbons for use in diesel, and methanol.

**Efficient process**-“For the first time, we have produced alkanes, the main component of gasoline and diesel, from lignin, and biomethanol” says Yan. “A large percentage of the starting material is converted into useful products,” he adds. “But this work is still in its infancy so other aspects related to economic issue will be evaluated in the near future.” John Ralph at the University of Wisconsin in Madison thinks the work is exciting. He points out that there have been previous attempts to convert lignin into liquid fuels. “That said, the yields of monomers [in the new reaction] are striking,” he says.

## Acidifying oceans are brewing up an underwater din

[NewScientist.com news service September 2008]

The big blue ocean is getting noisier. Sound can now travel farther than it did a century ago, thanks to carbon emissions that have made the oceans more acidic. Researchers have known for some time that acidity can influence how far sound travels in seawater. In the 1970s, acoustic measurements showed that the reach of low-frequency sounds varies between oceans. A whale's call, for example, travels farther in the north Pacific than in the north Atlantic, due to differences in pH. Exactly how the process works is unclear, especially at frequencies below 1 kilohertz, which include whale calls, crashing waves and whirring ship engines. “At these frequencies the exact molecular mechanism is still a bit fuzzy,” says Peter Brewer of the Monterey Bay Aquarium Research Institute in California. Some suggest that “ion pairs” of carbonate, bicarbonate, boric acid and borate are naturally “tuned” to absorb sound wave energy of 1 kHz and below. The acidity of the water affects the balance between these chemicals.



Oceans are becoming more acidic because of rising levels of CO<sub>2</sub> in the atmosphere, which dissolves in seawater to form carbonic acid. Brewer and his colleagues wanted to find out if these changes could be enough to affect sound transport. They analysed a database of ocean acidity during the 20th century, which showed that, on average, ocean pH levels dropped by 0.12. Using previous experimental data and field observations of how pH affects sound, they calculated how much this drop would affect the absorption of sound waves at 0.44 kHz – the note “A” used to tune an orchestra. They found that by the early 1990s, sound was being absorbed 15% less than in the late 19th century. Some studies predict ocean pH could drop by an average of 0.3 before the end of this century (*Nature*, vol 425 p 365). The team calculates that this would cause a 40% decrease in the absorption of sounds below 1 kHz. “The ocean will have higher levels of ambient noise, marine mammals will communicate at greater range, and military or industrial sounds will travel further,” Brewer says. “They have done well to raise this issue,” says Tim Leighton of the University of Southampton, UK. He points out the changes so far are relatively small, so the effect may be significant only in deep, quiet waters. Whether or not the differences will affect animal communication or military operations will require further study, Brewer says. However, he points to a study by Mark McDonald of Whale Acoustics, Colorado and John Hildebrand at Scripps Institution of Oceanography, California, suggesting that blue whales are calling at lower frequencies than they used to, possibly because it is easier to communicate this way.

भारतीय विषविज्ञान अनुसंधान संस्थान (आई.आई.टी.आर.) लखनऊ में दिनांक 15.09.2008 को प्रातः 11:00 बजे हिन्दी सप्ताह 14 से 21 सितम्बर, 2008 के उद्घाटन समारोह का आयोजन किया गया। इस अवसर पर मुख्य अतिथि श्री शशांक, निदेशक, दूरदर्शन, लखनऊ थे। उन्होंने कहा कि संस्थान के कार्यकारी निदेशक के हिन्दी ज्ञान से मैं प्रभावित हूँ। संस्थान में हिन्दी कार्य के लिये उन्होंने बधाई दी। अपने सम्बोधन में उन्होंने कहा कि हिन्दी में ऐसी क्षमता है कि वह सभी भाषाओं को अपने में समाहित कर लेती है। हिन्दी हमारे लिए नदी की तरह है। आक्सफोर्ड अंग्रेजी शब्दकोश में हिन्दी के बहुत से शब्द लिए गए हैं। यह हिन्दी की समृद्धि को दर्शाता है। हिन्दी भाषा का महत्व सम्पर्क भाषा के रूप में स्वीकार किया गया है। इससे पूर्व कार्यक्रम में मुख्य अतिथि को स्मृति चिन्ह भेंट करते हुए संस्थान के कार्यकारी निदेशक डॉ० अश्वनी कुमार ने मुख्य अतिथि द्वारा आमंत्रण स्वीकार करने के लिए आभार व्यक्त किया। उन्होंने कहा कि हमारे संस्थान में हिन्दी की प्रगति केवल समारोहों तक ही सीमित नहीं है बल्कि इसमें निरन्तर प्रगति हुई है। उन्होंने यह भी कहा कि हमारा कार्य राष्ट्रीय एवं अंतरराष्ट्रीय दोनों स्तरों पर है। फिर भी हिन्दी में कार्य करने का पूरा प्रयास किया जाता है। उन्होंने हिन्दी सप्ताह के दौरान आयोजित होने वाली प्रतियोगिताओं में सभी से बढ़-चढ़ कर भाग लेने की अपील की। श्री तारिक कुतबुद्दीन, वहिष्ठ प्रशासन नियंत्रक ने मुख्य अतिथि का औपचारिक परिचय देकर समारोह का संचालन किया।



मुख्य अतिथि श्री शशांक श्रोताओं को सम्बोधित करते हुये।

दिनांक 19.09.2008 को अपराह्न 3:00 बजे हिन्दी सप्ताह के पुरस्कार वितरण एवं समापन समारोह का आयोजन हुआ। संस्थान के कार्यकारी निदेशक, डॉ० अश्वनी कुमार ने कार्यक्रम की अध्यक्षता करते हुए कहा कि देश में सभी समान भाव से हिन्दी सप्ताह मना रहे हैं। हमें हिन्दी का सम्मान करना चाहिए तभी हिन्दी को बढ़ावा मिलेगा। हिन्दी भाषा हमें एक दूसरे से जोड़ने का काम करती है। उन्होंने सभी प्रतियोगिताओं के आयोजकों को धन्यवाद दिया। उन्होंने इस अवसर पर सप्ताह में आयोजित वाद-विवाद, आशुभाषण, लेख, टिप्पण व मसौदा लेखन, अहिन्दी भाषी का हिन्दी ज्ञान, हिन्दी टंकण, अनुवाद एवं विवज प्रतियोगिताओं और हिन्दी में कार्य करने की प्रोत्साहन योजना के अन्तर्गत विजयी प्रतिभागियों को पुरस्कार और प्रमाण पत्र प्रदान किया। आयोजक श्री तारिक कुतबुद्दीन, वहिष्ठ प्रशासन नियंत्रक ने कार्यक्रम के आयोजन हेतु सभी संबंधित के सहयोग हेतु हृदय से आभार प्रकट किया।

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Any feedback on this publication is welcomed.  
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