

Centre for Science and Environment

**Conference on Food Safety and Environmental
Toxins**

Biomarker and Body Burden

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Concept of Body burden

- **Chemical 'Body Burden'** refers to the accumulation of synthetic chemicals found in pesticides, cosmetics, industrial solvents, heavy metals, etc in our bodies. At any given time, hundreds of chemicals can be found in blood, urine, breast milk and even umbilical cord blood.
- **Testing of 9,282 people had revealed the presence in adipose tissues of various compartments in their bodies of 116 chemicals, including 34 pesticides [The U.S. Centers for Disease Control and Prevention (CDC)].**
- **Many chemicals are metabolized but others linger in human bodies for a lifetime risking individuals for certain diseases such as cancer and Parkinson's disease**

Chemical remobilization of chemicals stored in adipose tissues

- Many lipophilic chemicals pass into organism's cells through the fatty layer and once inside the organism, these chemicals are stored in fatty tissues and begin to accumulate.
- However, when fat reserves are called upon to provide energy for an organism, remobilization occurs within the organism.
- If appreciable amounts of toxins are stored in fat and fat reserves are quickly used, significant toxic effects may be seen from the remobilization of the chemical.

The target lipid-water partition coefficient K_{LW} (L/kg lipid) is defined as the ratio of chemical concentration in target lipid C_L (mol/g lipid mmol/kg lipid) to the aqueous concentration C_W (mmol/L)

$$K_{LW} = C_L / C_W$$

Octanol-water partition coefficient (K_{OW}) = C_O / C_W

Represents bioaccumulation potential of a chemical.

Influence of chlorine substituents on the chemical-physical properties of hydrocarbons

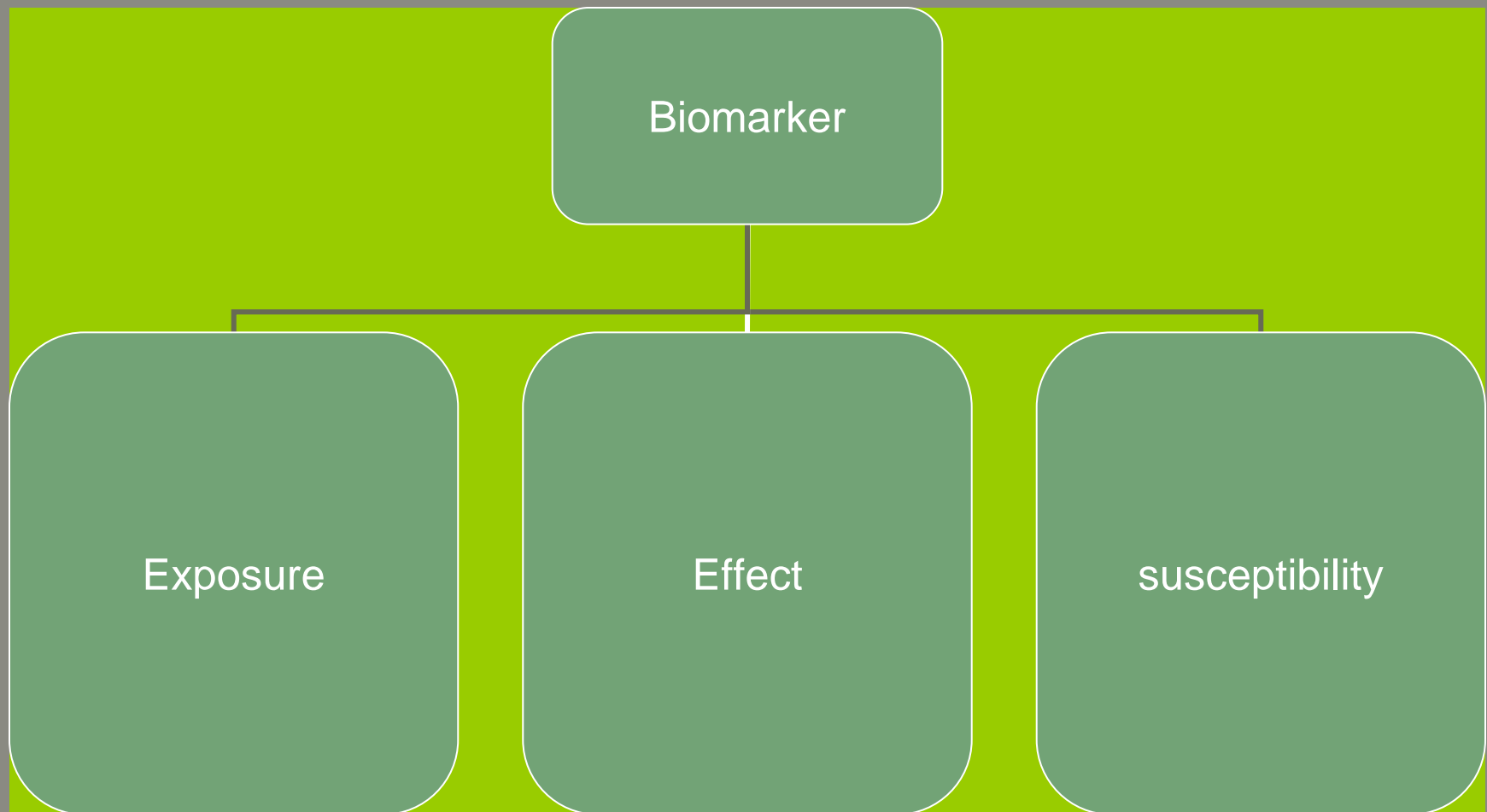
Compound	Number of Chlorine Atoms	S (mg/L)	log K _{OW}
Benzene	0	1,780	2.13
Hexachlorobenzene	6	0.006	6.18
Phenol	0	82,000	1.45
Pentachlorophenol	5	14	3.7
Biphenyl	0	5.9-7.5	3.89
PCB 209	10	0.000004	8.23
Dibenzo- <i>p</i> -dioxin	0	0.842	4.3
2,3,7,8-Cl ₄ DD	4	0.000008	7
Cl ₈ DD	8	0.0000004	8.2

S = Water solubility, K_{OW} = Octanol/water partition coefficient

Biomarkers

Classification of Biomarkers

(National Research Council, 1987)



Biomarker of exposure $\xrightarrow{\text{Transient}}$ **Biomarker of effect**

Biomarker of exposure $\xrightarrow{\text{Persists}}$ **Biomarker of disease**

NEERI's Contribution to Biomarker Research

- **DNA damage markers(CA,MN, comet)**
- **SNP in detoxifying enzymes coding genes(CYP,GST NOQ1)**
- **Neuro-regulation markers(Dopa.PL)**
- **Stress protein markers (Hsp,CRP)**
- **EDC exposure marker(Vitellogenin)**
- **Apoptotic biomarker**
- **Metal binding proteins**
- **Metabolite markers**
- **Protein biomarkers**

Toxic Challenge-Manganese

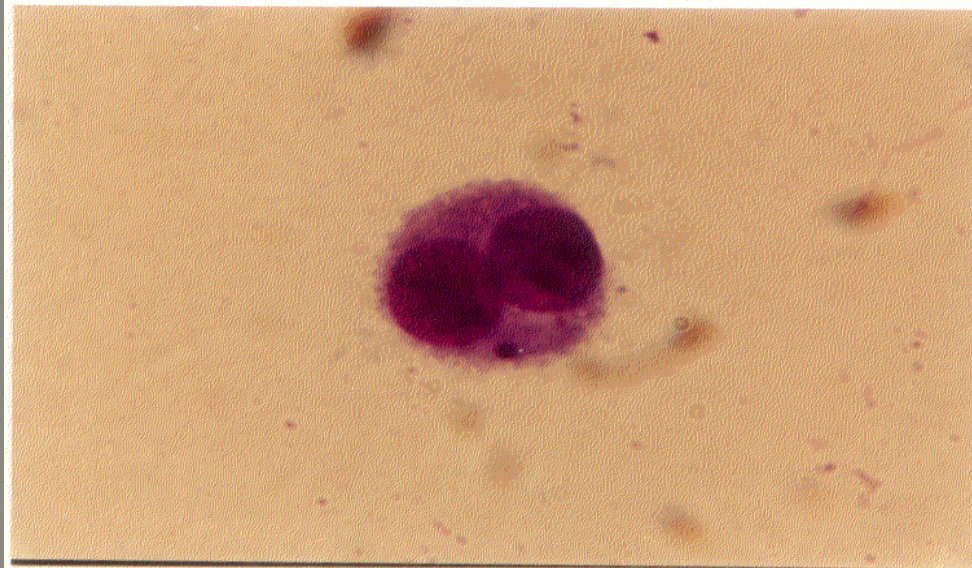
Neuro-regulation Markers (Dopa.PRL)

- It is hypothesized that dopamine (DA) level regulates the release of prolactin (PRL) from anterior pituitary.
- In Mn toxicity, auto-oxidation of free DA reverses the inhibition on PRL via tuberofundibular pathway increasing its blood level.
- Several workers have reported elevated levels of PRL on occupational exposure to Mn.
- Higher Mn concentration was detected not only in blood but also in urine along with elevated serum PRL in occupationally exposed workers.

DNA Damage Markers (CA, MN, Comet)

In vitro study with cultured lymphocytes revealed that:

- The percentage of chromosomal aberration and micronucleus formation increased with increase in MnCl_2 dosage.
- Comet assay also revealed a positive DNA damage
- A dosage of 360 $\mu\text{g/L}$ showed maximal genotoxicity in the cultured lymphocytes.
- This was associated with generation of oxidative stress in lymphocyte cultures.





Control



Exposed (Type C)

Type of Comets

Type A

Type C

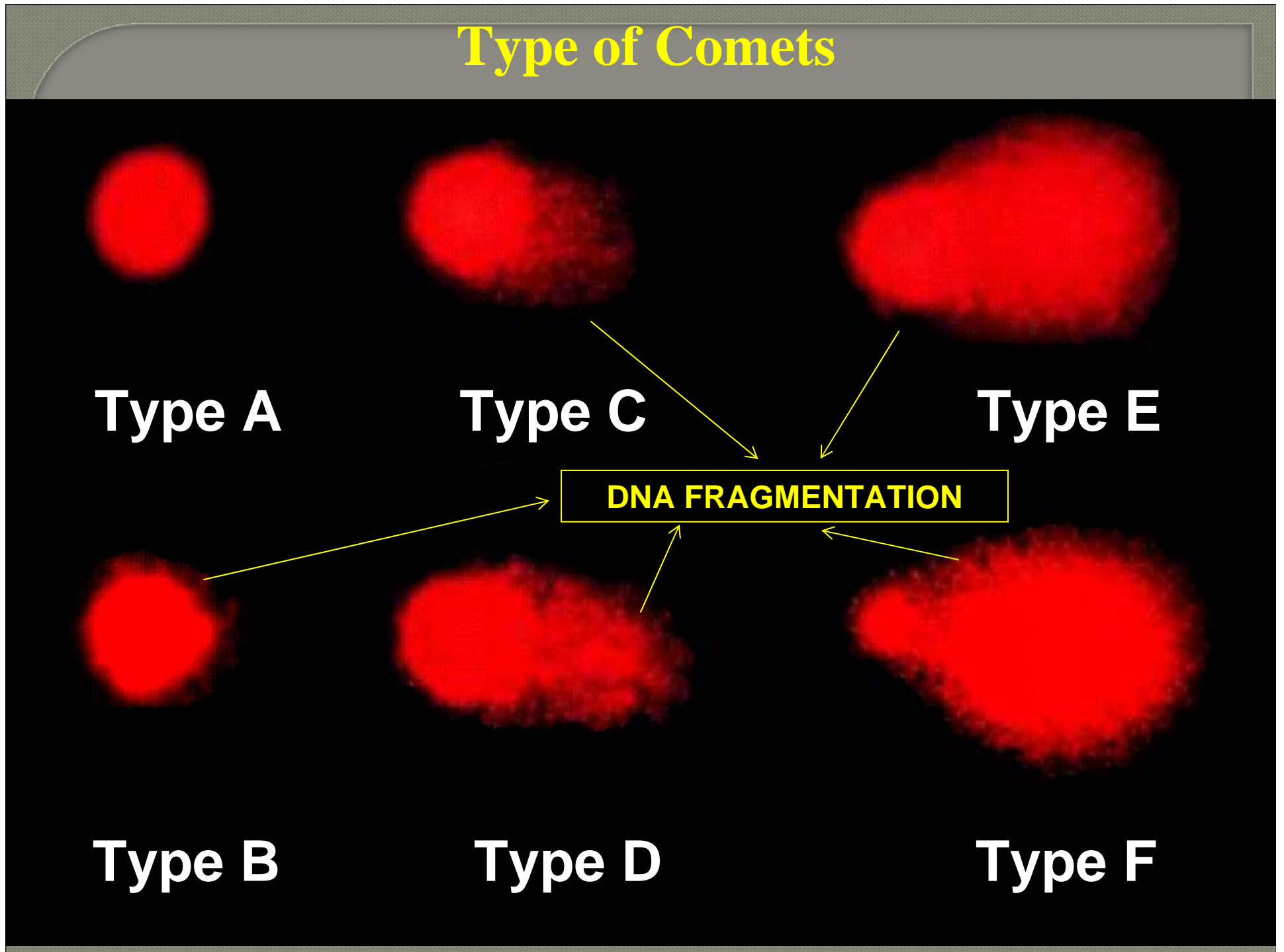
Type E

DNA FRAGMENTATION

Type B

Type D

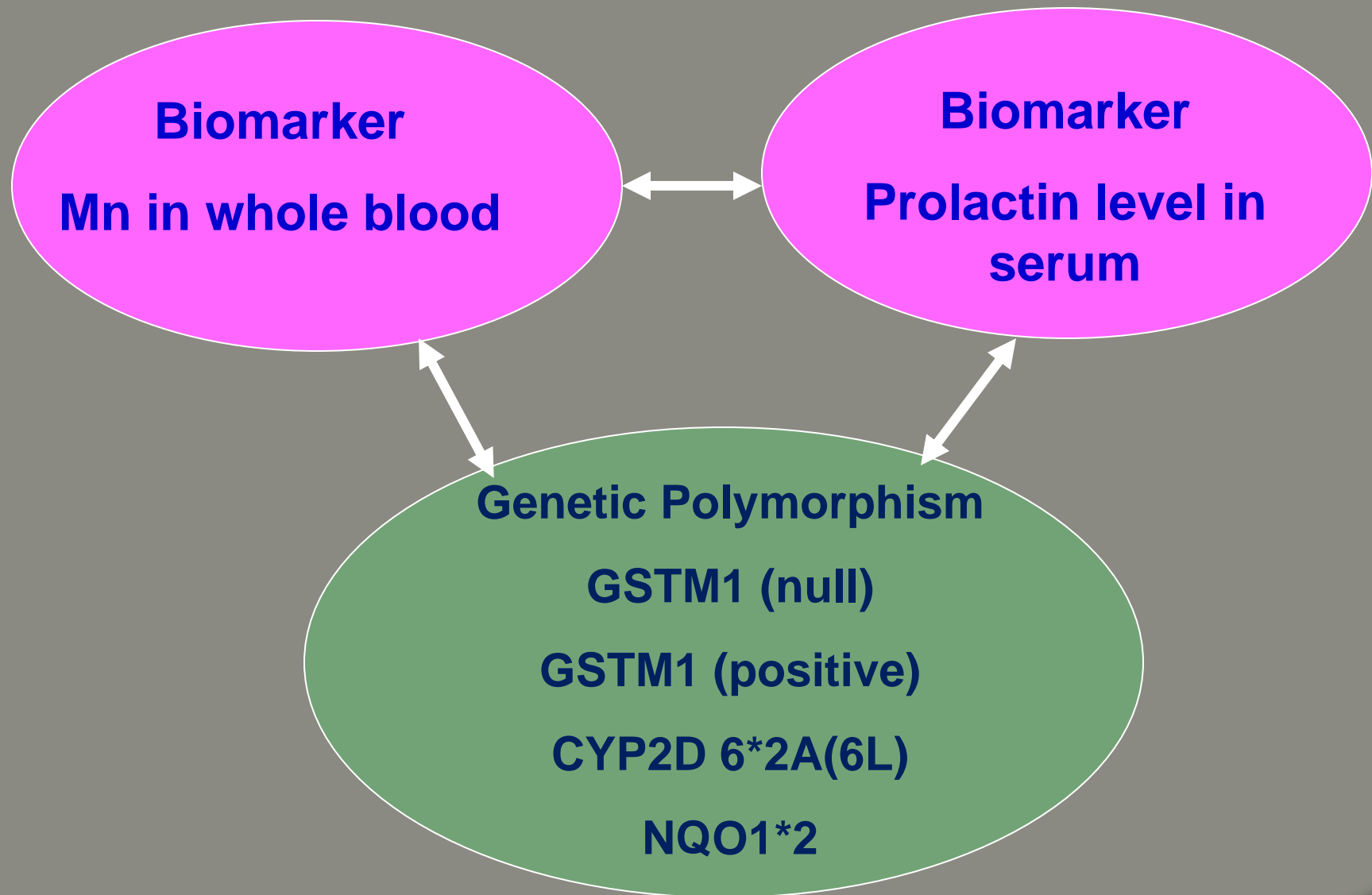
Type F



**SNP in Detoxifying Enzymes Coding
Genes**
(CYP, GST, NOQ1)

Correlation Study :

Genetic Polymorphism and Mn Biomarkers

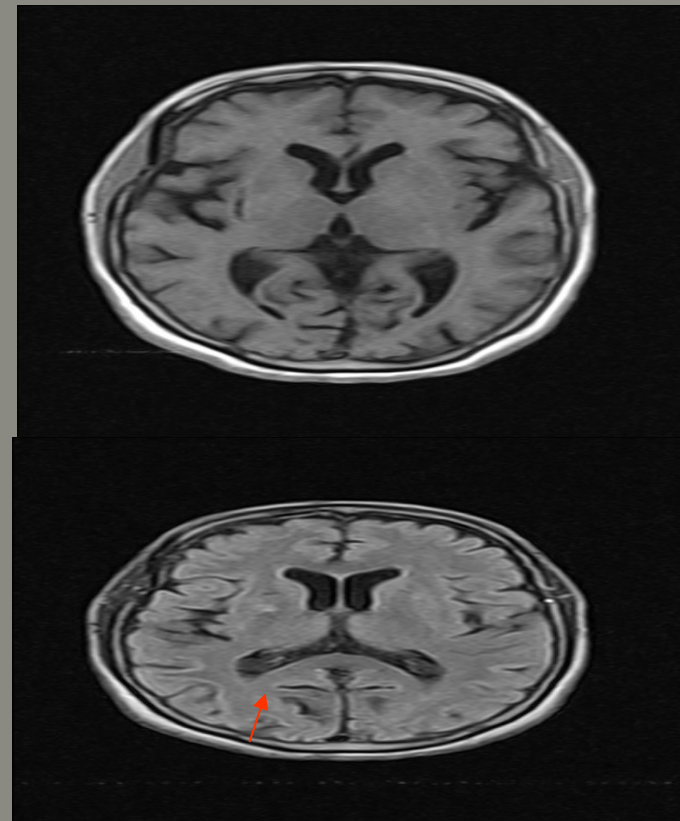
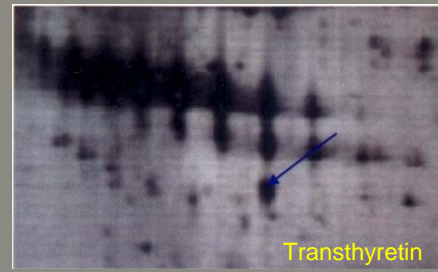
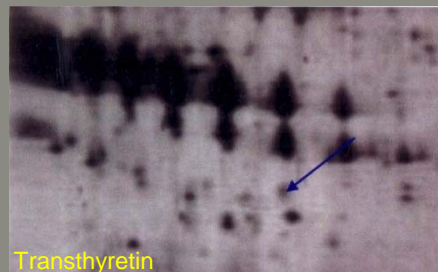
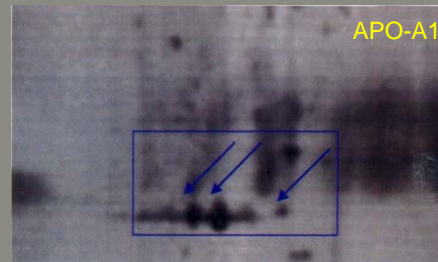
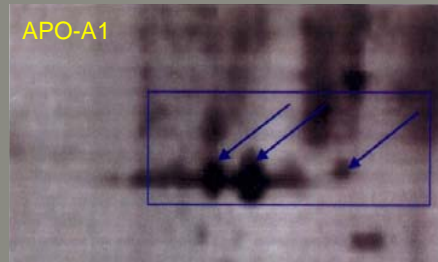
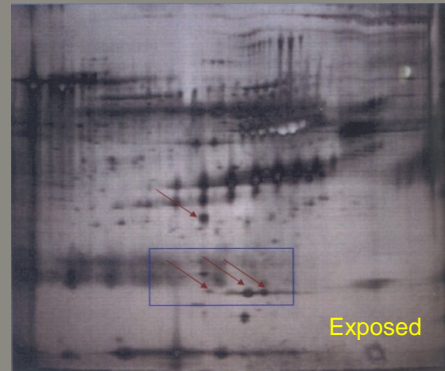
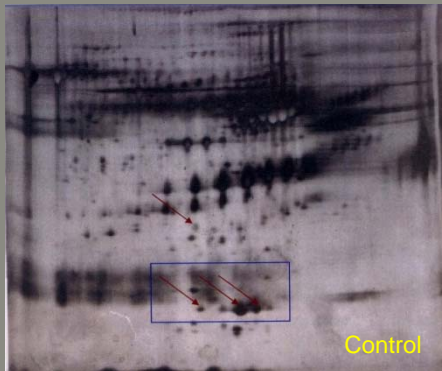


- **CYP2D6 C→T 2850 variant acts as a protective factor in manganese miners.**
- **GSTM1 polymorphism does not have influence on manganese and prolactin level.**
- **Association between Mn level and NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1) polymorphism could not be established.**

Proteomic Profile of Blood Samples

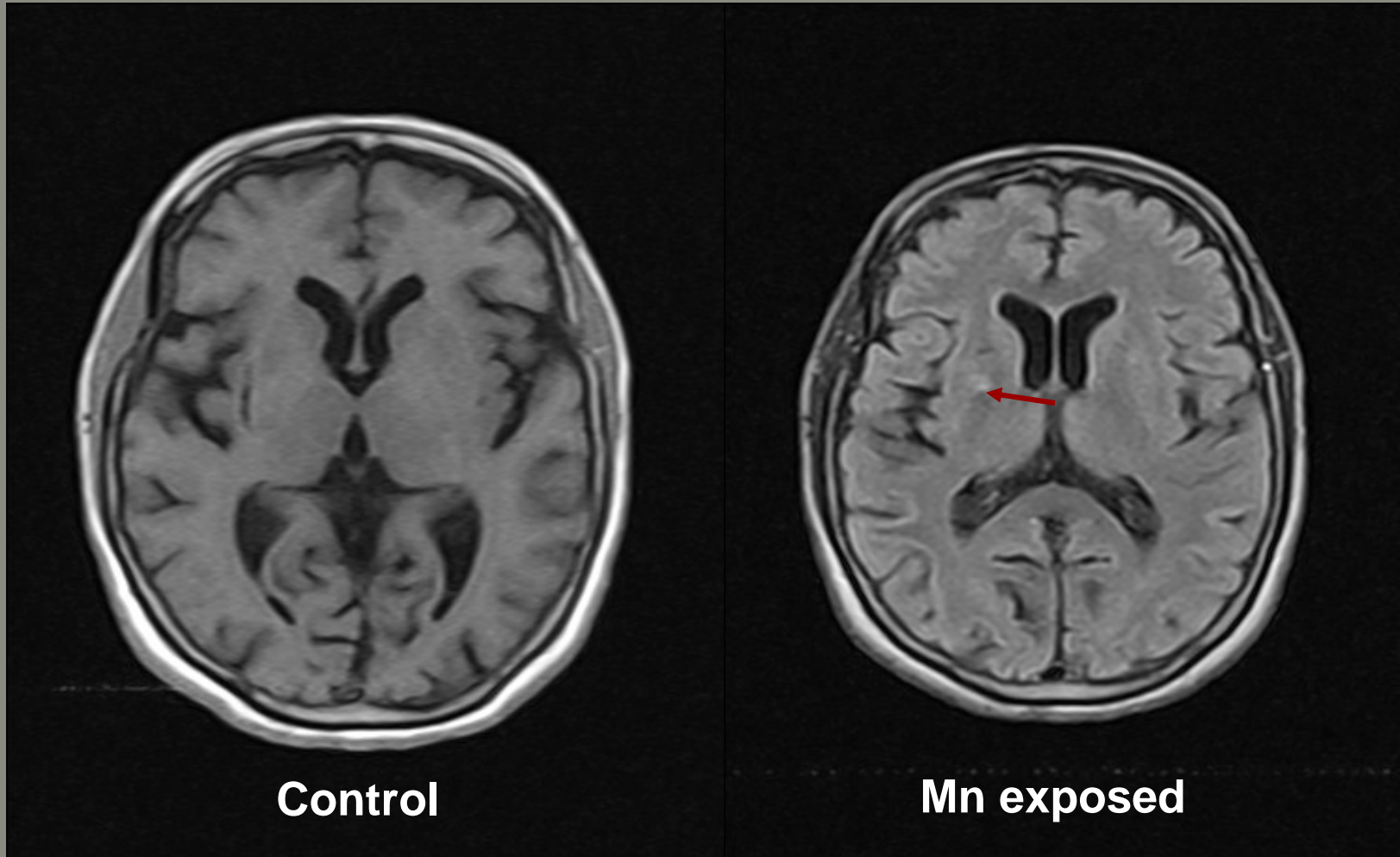
- Control
- Manganese exposed population
 - ❖ Mn level >20 ug/L

Proteomic Profile of Manganese Exposed Population and Control



MRI Image showing Mn deposition in brain of exposed individual

MRI Scan of Control and Exposed Individuals





**Manganese dioxide (10ppm) induced HSP
induction in human lymphocyte culture**

- **Transthyretine (TTR) levels were increased whereas Apo-AI levels were decreased in the manganism group.**
- **Mn is reported to cause oxidative stress by formation of free radicals.**
- **Hence, the increase in the level of TTR might be due to oxidative stress caused by Mn and, probably, the increase in TTR level increase might be a protective mechanism.**
- **In the present study, decreased level of Apo-AI was observed in manganism patients. Apo AI levels were also found to reduce during liver toxicity, in vascular dementia and Alzheimer's diseases.**

Manganese – Biomarker

➤ Exposure biomarker

- Mn level in blood- exposed GM 30.8 ug/l \pm GSD 8.0 ; control GM 2.0 ug/l \pm GSD 3.0

➤ Effect biomarker

- Prolactin level in serum- exposed GM 16.3 ug/l \pm GSD 1.6 ; control GM 3.3 ng/ml \pm GSD 1.7 .
- Up- regulation of TTR and down regulation of Apo –A1.

➤ Susceptible biomarker

- CYP2D6L/L (V) may have a lower risk and long latency of manganism than the CYP2D6W/W and CYP2DW/L genotype

PAHs Exposed Biomarker

PAH - Biomarker

Exposure biomarker

1-Hydroxypyrene-

exposed - (GM 22.18 $\mu\text{g/g}$ creatinine \pm 5.6) control (GM 6.5 $\mu\text{g/gm}$ creatinine \pm 5.17)

Effect biomarker

Up-regulation of fibrinogen gamma-A chain precursor and apolipoprotein A1 to mitigate oxidative stress and provide atheroprotective effects respectively

Susceptible biomarker

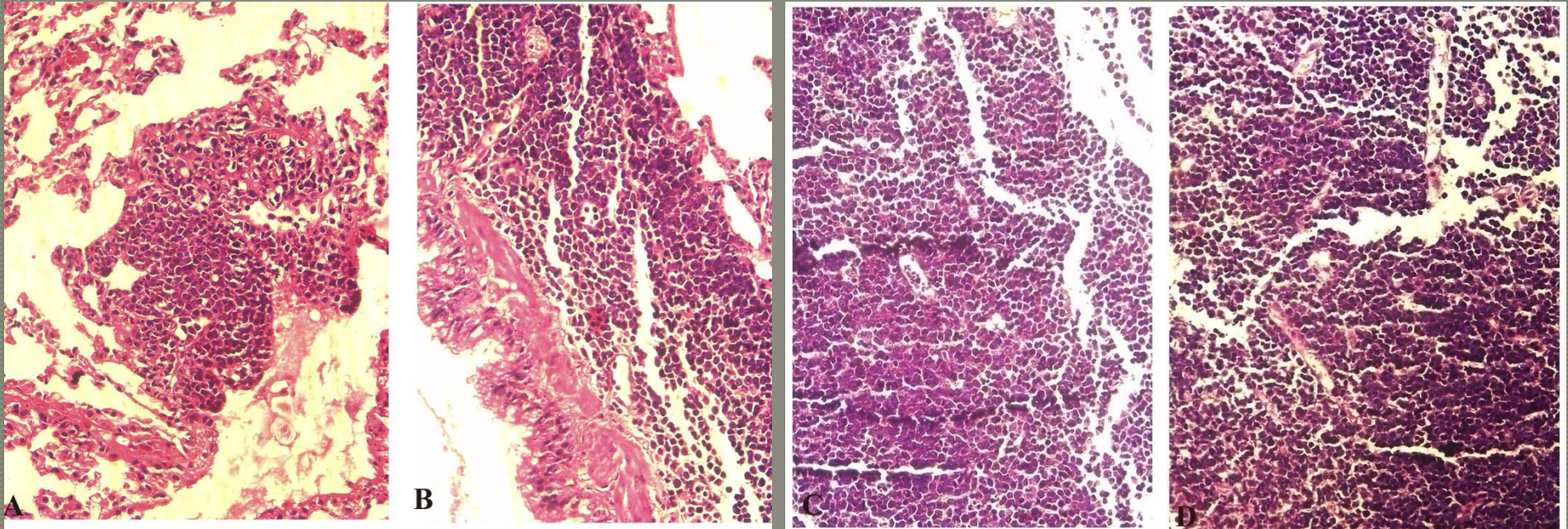
GSTM1 null genotype seems to be the susceptible biomarker of all genotype tested. GSTM1 null genotype showed higher level of 1-OHP in urine of exposed population

Bitumen Exposure Biomarkers

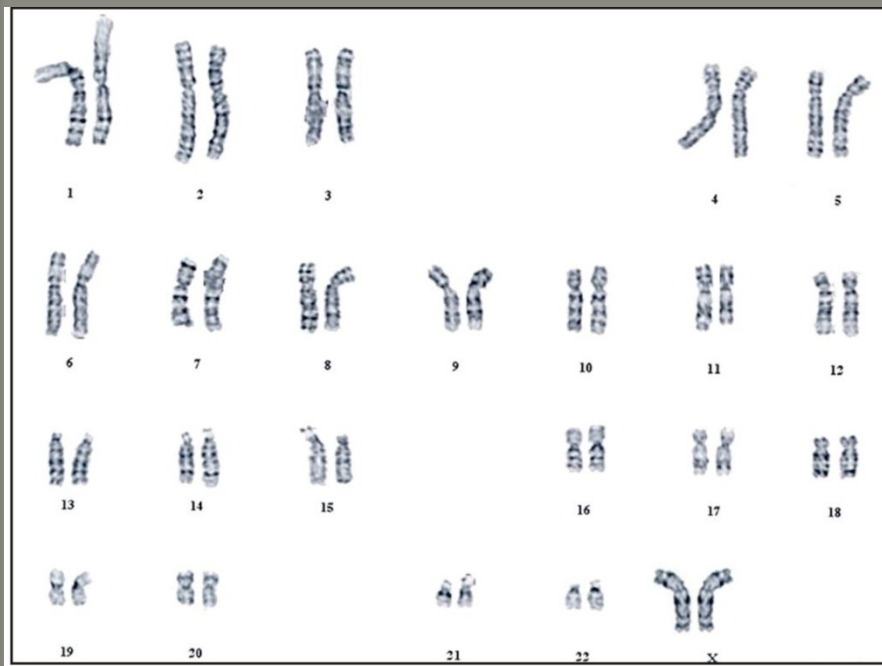
- Bitumen is a complex mixture containing predominantly naphthanic, aliphatic compounds, cyclic alkanes, polycyclic aromatic hydrocarbons (PAH), polycyclic aromatic compounds (PCACs) and metals (eg iron, nickel, vanadium).
- PCACs are a class of chemicals that include PAHs and heterocyclic derivatives in which one or more of the carbon atoms in PAH ring system has been replaced by a hetero atom of nitrogen (N-PCAC), oxygen (O-PCAC) or sulphur (S-PCAC). The proportions of these chemicals vary greatly, depending on the sources of crude oil.
- GC/MS analysis of bitumen showed the presence of polycyclic aromatic hydrocarbons (PAHs) and polycyclic aromatic compounds (PCACs) as also reported by Brandt et al., (2000) which include 1,6 dimethyl naphthalene, dibenzothiophene, dibenzofuran, C₂ anthracene, benzo(a) pyrene, phenyl methyl naphthalene, pyrene, methyl fluoranthene, benzo(a)anthracene, benzo(ghi)perylene, methyl PAH 228, benzo (b) fluoranthene, 2, methyl naphthalene, 1,2,3,4, dibenzo(a,e)pyrene, methyl PAH and dibenz(a,h)anthracene some of which are carcinogenic.

- Adverse health effects of exposure to bitumen include respiratory, skin immunological, carcinogenic (lung, bladder, skin, brain, stomach, liver cancers and leukemia) and genotoxic effects.
- The genotoxic potential of bitumen extract was assayed using multiple end points. such as chromosomal aberrations, and micronucleus assay.
- The carcinogenic potential of bitumen mixture was investigated using transformation studies.
- These assays were carried out *in vitro* using human osteosarcoma (HOS TE 85) cells, an immortalized cell line.
- The studies demonstrated the genotoxicity of bitumen in terms of increased frequency of chromosomal aberrations and micronucleus in HOS cells exposed to bitumen extracts when compared to untreated HOS cells.

- The original human osteosarcoma cell line HOS TE 85 has a diploid karyotype (Erenpreisa et al., 2000). HOS T1 and HOS T2 cells, challenged with bitumen extract, exhibited a change in karyotype with cells showing increased aneuploid metaphases with trisomy of chromosome 8.
- Huret, (2007), in his database of trisomy 8, has shown that various diseases are characterized by trisomy of chromosome 8 as sole anomaly or accompanied with other chromosome anomalies.



Trypsin-induced, giemsa-banded karyotype of (A) HOS TE 85 cells and (B) bitumen transformants HOS T1 and HOS T2 cells. Note the gain of chromosome 8 in (trisomy of 8) and HOS T1 & HOS T2



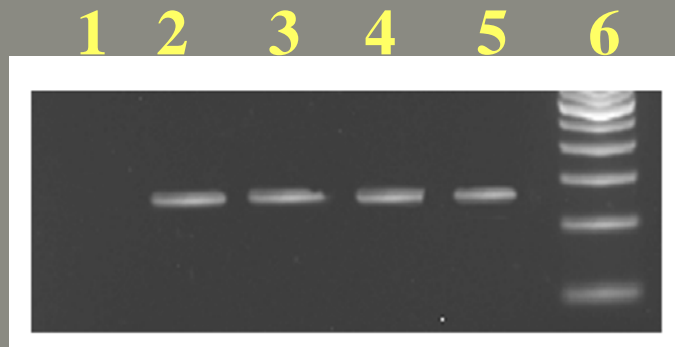
Trypsin-induced, giemsa-banded karyotype of (A) HOSTE 85 cells and (B) bitumen transformants HOS T1 and HOS T2 cells. Note the gain of chromosome 8 in (trisomy of 8) and HOST1 & HOST2

- Even though bitumen extract could induce morphological changes as well as anchorage independency, which are characteristic of the chemical induced neoplastic transformation, the clones of bitumen transformants HOS T1 and HOS T2 were incapable of forming tumours in nude and SCID (Severe Combined Immunodeficiency) mice.
- This could probably be due to insufficient tumorigenic potential of HOS T1 and HOS T2 cells or transplanted cells being rejected by NK cells of host.
- Such a rejection has also been reported by Bratslavska et al., (2000), wherein human T-cell lymphotropic virus type (HTLV-1) transformed HOS TE 85 cell culture i.e. RaHOS cells were unable to form tumour in nude mice.

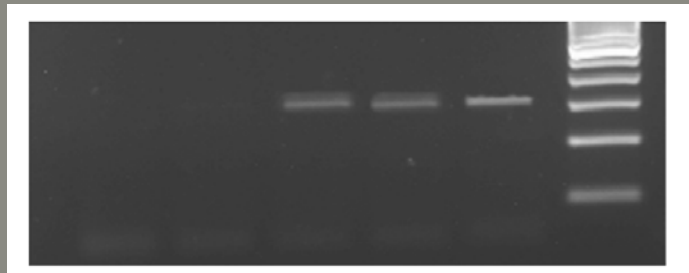
- However micrometastasis appeared in the lungs of SCID mice, injected with HOS T2 cells, which indicates that bitumen exposed HOS cells were transformed and acquired tumorigenic potential which was weak as compared to KHOS cells which exhibit high tumorigenic potential in inducing primary tumours.
- The protein expression profile of bitumen transformed cells (HOS T1 and HOS T2 clone) showed down regulation of keratin 2A and galectin 1 and up regulation of reticulocalbin precursor (RCN1) and Peptidyl-Prolyl Isomerase (Pin1) in HOS T1 and T2 cells.

- The two proteins RCN1 And PIN1, which are reported to be over expressed in many cancer cells, is observed to be upregulated in the bitumen treated HOS cells. It was therefore suggested that the identified upregulated proteins may be responsible for the transformation of HOS cells as could be evidenced by the anchorage independence assay and other cellular characteristics of transformation
- The down regulation of Galactin 1 was observed in HOS T1 and HOST2 which is usually over expressed in aggressive cancers and is known for its participation in cancer cell adhesion. (Demydenko 2002). Hence in the study, it is proposed that the down regulation of protein Galactin 1 may responsible for the non induction of tumor by the transformed clones HOST1 and HOS T2 in the nude as well as SCID mice which may be attributed to their non adhesion to target organ(s).

MDR Expression-Bitumen Exposed



RP II Expression (267 bp)
(House Keeping)



MDR1 Expression (308 bp)

- Lane 1: Negative Control
2: HOS (parent cell)
3: HOS T1
4: HOS T2
5: MCF 7 cDNA(Positive Control)
6: 100 bp Ladder

EDC Exposure Marker
(Vitellogenin)

Biomarker emergence following exposure to endocrine disruptive chemicals in the environment

- The bioaccumulation of DEP in testis, liver, brain, gills and more importantly in muscle tissues of fish increased significantly ($P < 0.01$) with increase of dose from 1 to 5 ppm and an exposure period of a week to two was sufficient to bring about changes in quantifiable parameters studied.
- This is the first report describing metabolic changes and vitellogenin induction following exposure of *C. carpio* to DEP dose that is **as low as 1/500th fraction of LC50**.

Endocrine disruption and metabolic changes following exposure of Cyprinus carpio to diethyl phthalate. Pesticide Biochemistry and Physiology 88 (2007)36–42

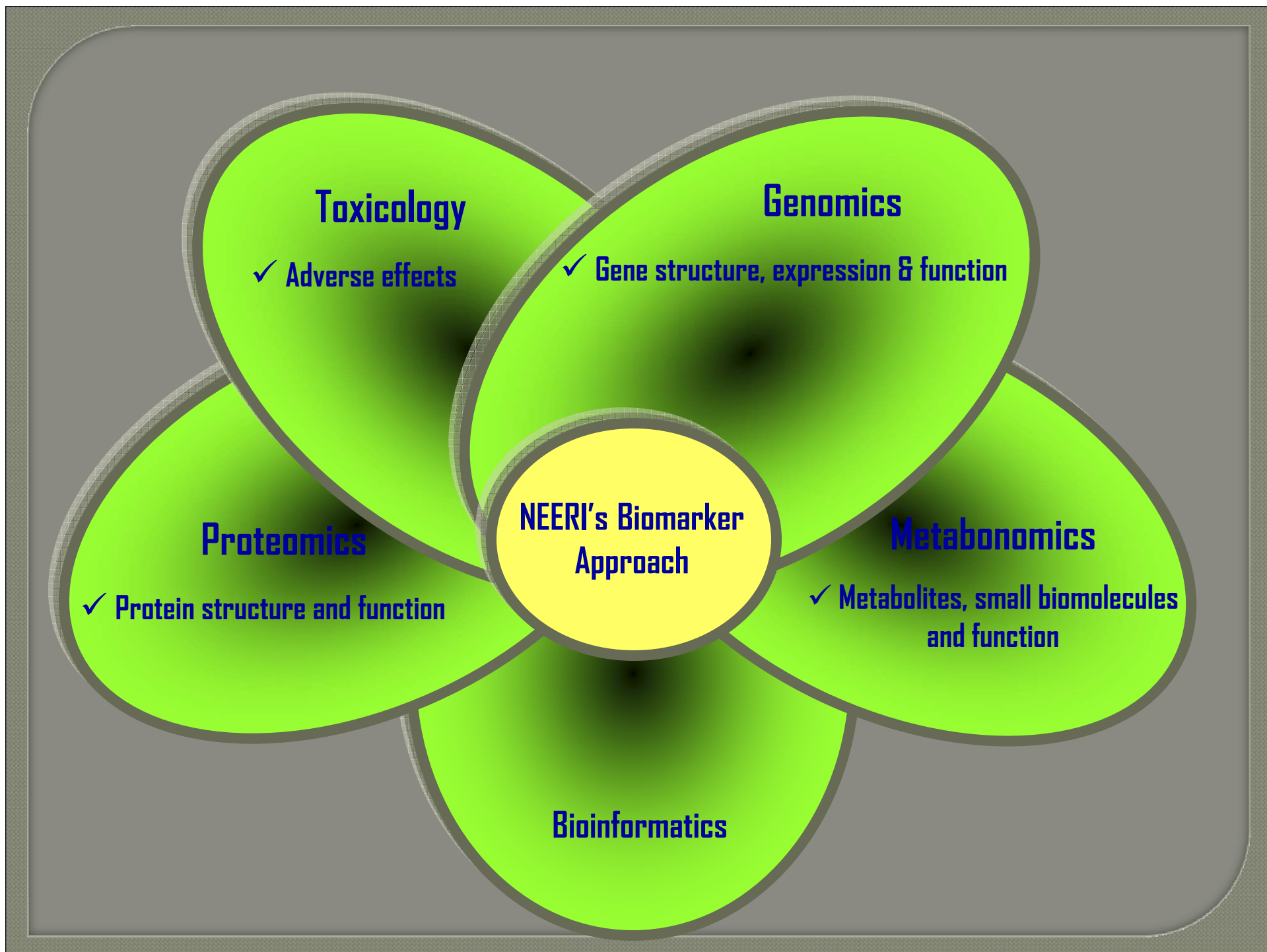
- Similar results were found with another EDC 4-*tert*-butylphenol (4-TBP).
- Fishes were treated with 1/10th (0.69mg/L), 1/5th (1.38mg/L), and 1/3rd (2.3mg/L) dose 96th h of LC50. Whereas there was significant ($P < 0.01$) decrease in alkaline phosphatase and aspartate aminotransferase activity; alanine aminotranferase, acid phosphatase [3.1.3.2] activity and vitellogenin production in muscle were increased compared to control.

- With all the dose levels tested, testicular-somatic index (testis size) was reduced ($P < 0.01$) and histo-architectural changes in testicular and liver tissue were found even in group given 1/3rd dose of LC50.

One-tenth dose of LC50 of 4-tert-butylphenol causes endocrine disruption and metabolic changes in Cyprinus carpio Pesticide Biochemistry and Physiology 86 (2006) 172–179

- Methyl paraben and endosulfun was also found to be an EDC

ROADMAP



- **Impact of genetic diversity on the response to exposure to toxic agents.**
- **Molecular mechanisms of action of toxic environmental pollutants**
- **Identification of cancer biomarkers**
- **Identification of EDCs and sex change chemicals in target organisms**
- **Redox imbalance**
- **Molecular Mechanisms of Heavy Metal Toxicity**

Personnel Involved in Task Force Project

- **Dr. T. Chakrabarti**
- **Dr. K. Krishnamurthi**
- **Dr. S. Saravana Devi**
- **Dr. T.K.Ghosh**
- **Mr. Pravin Naoghare**
- **Dr. S.Pramanik**
- **Dr. Abhijit Barse**
- **Mrs. Alka Dhondge**
- **Ms. Raka A. Biswas**
- **Ms. Meenal Agrawal**
- **Mr. N. Vinayagamoorthy**
- **Mr. Arup R. Biswas**



Thanks

➤ The bitumen transformants HOS T1 and HOS T2 showed high plating efficiency (i.e. ability to grow) in soft agar. On the other hand, the parental HOS TE 85 cells have negligible plating efficiency in soft agar.

- This anchorage independence assay is considered the most stringent assay for detecting transformation of cultured cells (Xie et al., 2007).
- The ability to form colonies in soft agar is a characteristic of many cancer cell lines

Investigation further showed that the bitumen transformants HOS T1 and HOS T2 acquired altered growth pattern and exhibited typical morphological changes such as :

- ability to grow in multilayer as aggregates on top of each other, forming dense bodies called foci at high cell densities (i.e. loss of contact inhibition) exhibiting uniform polygonal shape, were smaller in size and had less processes with clearly defined borders.

- increased number of cells having nucleus with irregular shape, indented nuclear envelop, increased frequency of hyperchromatic nuclei and coarsely granular chromatin.
- Morphometric analysis showed reduction of cell size of HOS T and HOS T2 due to retraction of cytoplasm.
- increased number of nucleolus as compared to parental HOS cells. Increased number of nucleoli has been observed in cancer cells.
- Increased number of cells with irregular shaped nucleoli and increased number of micro nucleoli.
- The N:C ratio was increased in bitumen challanged HOS T1 and HOS T2 as compared to parental HOS cells as could be seen in the present investigation. The tumorigenic KHOS cells also had higher N:C ratio.

