

Toxicology International

Vol. 12 no.1, pp29-45 Abstracts of articles presented at 23rd Annual Meeting

**Silver Jubilee Year Celebrations of Society of Toxicology (STOX) India
NATIONAL SYMPOSIUM ON GOOD LABORATORY PRACTICE AND
REGULATORY ISSUES &
23rd ANNUAL CONFERENCE OF STOX
28-30th October, 2004**

**Sree Chitra Tirunal Institute for Medical Sciences and Technology,
Thiruvananthapuram, Kerala, India**

ABSTRACTS

Session: Pharmaceuticals and Drugs

KEYNOTE ADDRESS

Drug Discovery: Toxicological Aspects

Lakdawala AD and Majeed M

SAMI Labs Limited, Peeniya Industrial Area, Bangalore 58

The global drug discovery scenario has undergone significant paradigm shift to integrate various stages of discovery process in order to minimize the attrition rate at initial stage of lead optimization in order to optimize cost and time factors. During last twenty years, twenty drugs have been withdrawn from the market for safety reasons, majority because of hepatotoxicity, followed by blood disorders or drug interactions. Two important attributes responsible for the failure of 50-70 % molecules during drug discovery and development process are ADME and Toxicity. This burdens 800 million dollars cost of discovery. Pre-clinical toxicological evaluation encompasses many facets like Acute, Sub-acute and Chronic toxicity of various duration involving segments like mutagenicity, reproductive, carcinogenicity tests. These tests are expensive and time consuming involving large animals with limited degree of reliability. The technological innovations of the recent past have facilitated the process of discovery in cost and time efficient manner to minimize the attrition rate by providing reliable data on toxicological front. The two key stages of drug development viz.: (i) predictive toxicology, the elucidation of toxic mechanisms and identification of toxicity biomarkers, and (ii) pre-clinical safety studies where tissue slides are evaluated for toxic drug effects or abnormalities have been addressed by new and rapidly advancing composite technical modality that automates the analysis of digital tissue images obtained from microscope slides called Automated Pathology (AP). AP can not only help the pathologist process more data more quickly and accurately, but also empowers the pathologist to correlate events occurring within tissues with their causative genomic and proteomic events, as well as outcomes at the clinical level. Presently, drug metabolism and toxicity in the human subject is outcome of clinical trials. Acute human toxicity can be predicted fairly well but chronic toxicity only reasonably on average, and nothing can be done for idiosyncratic toxicity. The experiments based on in silico models. Evolution of informatics tools has facilitated the drug discovery and development process significantly. Computational methods to predict drug safety liabilities are available. However, each program has perceived strengths and weaknesses. It is therefore important to incorporate multiple programs to evaluate the datasets as a sentinel filter for liability assessment. The cutting edge technologies in molecular toxicology encompassing in vitro, geno, reproductive and carcinogenic toxicity and role of Toxicogenomics and Transgenic animals to speed drug discovery and development process will be discussed.

LEAD PAPERS

Iatrogenic diseases: a course in toxicology in pharmacy curriculum

Ravindra R Raju

Arnold and Marie Schwartz College of Pharmacy and Health Sciences, Long Island University, Brooklyn, NY 11201, USA

In the recent decades, the curriculum in pharmacy colleges in the US has undergone major changes that include switching from a five year Bachelor's degree program to a six year entry level Doctor of Pharmacy (Pharm D) degree program. Emphasis on the clinical aspects has been added. A pharmacist also engages himself/herself in patient counseling and works as a team member with other health professionals. This course in toxicology is taught at this college during the spring semester of the fifth year as a three credit course. Didactic portion consists of three lecture hours per week for about fourteen weeks. Each lecture hour is a power point presentation describing the deleterious effects of the drugs and diagnostic agents/procedures. It is supplemented with actual case scenarios published in the medical journals. After discussing all the systems of human body, significant time is spent on poisoning and overdoses of prescription and over the counter medications. This course helps the students to learn how various pharmacological agents produce adverse effects on human beings by biochemical, cellular and molecular mechanisms and prepares them as future pharmacists in counseling the patients.

Evolution of drug discovery and development

Sheshagiri Rao

Discovery Research, Dr. Reddy's Laboratory, Hyderabad

Drug discovery and development is a creative, complex and a highly interdisciplinary process. On an average, it is estimated that it takes about 10-12 years for a new drug to reach the market at a cost of \$800 million. In recent times the process of drug discovery and development has undergone a sea of change. Serendipity has played a key role in the discovery of medicinal properties and therapeutic use of plant and animal origin for the treatment of diseases and paved way for therapy from ancient times. Even a few decades ago, the discovery of new drugs was by trial and error method. The late nineteenth and first half of the twentieth century witnessed drug discovery by random pharmacological evaluations of both natural and synthetic compounds for medicinal properties. The later half of the twentieth century witnessed a phenomenal development of rational approaches to drug discovery. This resulted in a gradual shift from the chemistry based approach to a *biology knowledge based* drug discovery. These approaches were initially based on the biochemical aspects of disease and more recently on molecular biological and genetic bases of disease. Progress in receptor biology has ushered in a new era in drug discovery (discovery of H₂ receptor antagonist, cimetidine). The development in enzymology and the understanding of role of peptides in physiological and pathological states lead to the discovery of non-peptide angiotensin converting enzyme (ACE) inhibitors. Cloning of genes has led to the development of methodologies for specific receptor directed and enzyme directed drug discoveries. The development of structure activity relationship (SAR) and quantitative structure activity relationship (QSAR) techniques has resulted in the discovery of many potent and relatively safe drugs in several therapeutic areas. However, the concomitant developments of biological sciences and computer technologies have helped in the evolution of highly sophisticated techniques that could significantly shorten the drug discovery timelines. The human genome elucidation identified 2000 kinases which could be potential targets for drug discovery. The combinatorial chemistry and high throughput screening (HTS), novel *in vivo* rapid pharmacokinetic screening promise to shorten the time required to generate lead compounds. The genomic and proteomic approaches to drug discovery may lead to the development of drugs that are tailor-made to suit individual requirement. Ultimately, the effectiveness with which these technologies will be deployed for cutting edge drug discovery research will be determined by the ingenuity of the human mind.

RESEARCH PAPERS

Effects of repeated (28 days) exposure to metoprolol succinate in rats

Deore MD, Dadarkar SS, Bakre DG and Garne MM

Deptt. of Pharmacology and & Toxicology, Bombay Veterinary College, Mumbai 400 012

The effect of metoprolol succinate, a selective β_1 antagonist, was studied in rats after oral exposure to it for 28 days. The study was conducted on 50 Wistar rats divided into five equal groups. Groups A, B, C, and D received oral doses (mg/kg) of metoprolol succinate @ 1000, 750, 500 and 300 respectively in two divided doses daily for 28 days. Whereas group E was an untreated control. The effects were evaluated on the basis of alterations in the haematological parameters (Hb, PCV, RBC and platelet count WBC, differential leucocyte count), biochemical parameters (serum bilirubin, sodium, potassium, sugar, liver function tests and kidney function tests) and cardiometrics. The clinical symptoms, effects on body weights, mortality, post mortem findings and histopathological changes were also studied. The results revealed that metoprolol succinate caused mild CNS depression and adverse effects on body weights at 1000 mg/kg. The mortality was observed at the doses above 500 mg/kg in all the groups. The post mortem lesions and histopathological findings suggested that apart from

lesions on liver, kidney, intestine and heart was also affected as revealed by cardiometrics. Rounding of heart and increase in heart weight indicated cardio toxicity of metoprolol succinate. A dose of 300 mg/kg was found to be dose causing oral sub acute toxicity of metoprolol succinate in rats.

Maturation arrest of neutrophils: possible reason for decline in peripheral leucocytes count in selenosis

Rampal S, Rakesh Kumar, Sood NK and Sandhu HS

Department of Vety. Pharmacology & Toxicology, Deptt. of Vety. Pathology, PAU, Ludhiana.

The sub-chronic toxicity of selenium was experimentally induced in crossbred cow calves by repeated oral administration of sodium selenite for 98 consecutive days. Typical signs of selenosis were observed with higher dose. Total leucocyte count showed a progressive decline during the treatment period and was 9613 ± 62.5 , 8289.5 ± 7.6 and $7896 \pm 27.9 \times 10^6$ mm³ in the control, low and high dose group respectively on 98th day of treatment. The decline in TLC was due to decrease in the count of neutrophils. The neutrophil count was reduced to 1/3rd in the animals with selenosis. The evaluation of bone marrow smears revealed that selenium induces myelosuppression. There was a significant increase in the promyelocyte and myelocyte cell count whereas metamyelocyte count was non significantly increased. However, there was significant decline in count of band cells and mature neutrophils indicating that there was an amaturation arrest at the level of metamyelocyte.

Session : Plant drugs/natural medicines, cosmetics

LEAD PAPERS

Cattle diseases associated with plant toxicities in Western Ghats of Karnataka

Narayana K and Shridhar NB

Deptt. Pharm & Toxicology, Veterinary College, Bangalore

There are more than a dozen clinical conditions occurring in cattle and buffaloes of western ghat regions of Karnataka. The etiology for such clinical entities is unknown. In the process of clinical investigation, a number of naturally occurring plants have been implicated. We have fed the suspected plants to cattle and buffaloes and done a phytochemical analysis. The plant as such and its extracts were given to cattle, rat and rabbit. The confirmed plant toxicities include *Ficus tsjahela*, *Ficus virens*, *Dichapetalum geloindes*, *Mimosa pudica*, *Mimosa invisa*, *Gnidia lauca* (*Lasiosiphon eriocephalus*), *Chromolaena odorata*, *Hevea brasiliensis*, *Chukrasia tabularis* and *Calycopteris floribunda*.

Herbal health care for livestock can avoid antibiotic drug residues in foods of animal origin

Punniamurthy N

Veterinary University Training and Research Centre, Directorate of Centre for Animal Health Studies, Thanjavur

Modern veterinary practice has numerous constraints, including the lack of supply and prohibitive cost of essential veterinary drugs and shortage of manpower, especially in the interior rural areas; the problem is more acute, if the livestock involved are less productive indigenous animals. All foods of animal/poultry origin (milk, eggs and meat) are viewed by many with apprehension that they may have some deleterious substance(s) which are harmful to health. Globally, an estimated 50% of all antimicrobials serve veterinary purposes. Bacteria that inevitably develop antibiotic resistance in animals comprise food borne pathogens, opportunistic pathogens and commensal bacteria. Concern over antibiotic residues in foods of animal origin is two fold (1) potential threat to direct toxicity in humans (2) whether the low levels of antibiotic exposure would result in alteration of micro flora, cause disease and the possible development of resistant strains compromising antibiotic therapy in clinical situations. The international recognition of the 'field to table' approach to food safety emphasizes the need for prudent use of antibiotics in animal/agriculture production. Based on the experience from human medicine, there are good scientific reasons to restrict the use of antibiotics and preserve the long-term efficacy of existing antibiotics and limit the risk of transfer of antibiotic resistance. Use of indigenous/traditional veterinary medicine is a cost effective treatment alternative for livestock farmers who maintain only a few heads of livestock, especially in primary health care for sustainable livestock production. Ethno veterinary medicine (EVM) includes the indigenous knowledge pertaining to the health care of animals, which is preserved mostly through oral traditions for generations. The desperate and resource-poor livestock farmers have kept alive and continue to use this vibrant and dynamic traditional medicine system (TMS). The efforts by individuals and institutions in the recent past to revive the traditional systems of veterinary health care on scientific lines have been sporadic and ephemeral. There is growing interest in traditional medicinal plants, used in EVM. Yet, most of this knowledge apparently exists only in folk traditions. A few fresh herbal traditional remedies validated for mastitis/metritis

would go a long way in safeguarding the interests of the rural livestock holders and the society at large with safe animal products free from antimicrobial residues.

RESEARCH PAPERS

Toxicology study of *Semecarpus anacardium* on histology of liver and some of its enzymes in albino rat

Choudhari CV and Deshmuku PB

Science College, Nanded 431 605, Maharashtra.

Bhallataka (*Semecarpus anacardium* Linn.) is well known highly potent medical plant. In Ayurveda it is considered as Rasayana. Toxicity study of (*Semecarpus anacardium*) was conducted in albino rats (Wistar strain) of either sex at acute and sub chronic levels. SAE from ripen nut was orally administered to albino rats along with feed. After treatment of sub lethal doses @250 mg, 500mg & 750 mg/ kg b wt for 7 days and two sub-sub lethal doses @83.33mg & 166.6 mg/kg b wt for 21 days, results were compared with control group animals. Acute and sub-chronic study revealed adverse effects on GOT, GPT, LDH & SDH activities of liver. SAE was found to have adverse effects on activity levels of GOT, GPT, LDH and SDH of liver, revealing liver disorders. Histopathological study was also made.

Session: Cellular, molecular and neuro-toxicology, and in vitro alternative methods

LEAD PAPERS

Acetaldehyde disrupts intestinal epithelial tight junctions and adherens junctions: implication in the pathogenesis of alcoholic liver disease

Rao RK, Seth A and Sheth P

Department of Physiology, University of Tennessee Health Science Center, Memphis, TN, USA

Ethanol is metabolized by alcohol dehydrogenase and aldehyde dehydrogenase, generating acetaldehyde and acetate. Acetaldehyde is the most toxic metabolite of ethanol. In the gastrointestinal tract, ethanol is metabolized by mucosal and bacterial alcohol dehydrogenase into acetaldehyde. Due to the low activity of aldehyde dehydrogenase in bacteria alcohol consumption results in accumulation of acetaldehyde in the colonic and ileal lumen and tissue. Over thirty percent of alcoholics develop alcoholic liver disease. Although the precise mechanism involved in the pathogenesis of alcoholic liver disease is not clear, a significant body of evidence indicates that endotoxemia and endotoxin mediated hepatocellular damage play a crucial role in the pathogenesis of alcoholic liver disease. Alcoholics with the symptoms of alcoholic liver damage also exhibit increased intestinal permeability to macromolecules. In the present study, we conducted series of experiments to determine the mechanism of alcohol-induced intestinal permeability to endotoxins. Our studies demonstrate that acetaldehyde, but not ethanol, disrupts intestinal epithelial tight junctions and adherens junctions, and increases paracellular permeability to endotoxins by a tyrosine phosphorylation-dependent mechanism. Acetaldehyde inhibits the protein tyrosine phosphatase, PTP1B, by the formation of acetaldehyde-PTP1B adduct, and increases tyrosine phosphorylation of E-cadherin and b-catenin, leading to the disruption of E-cadherin/b-catenin/PTP1B and occludin/ZO-1 complexes. Acetaldehyde-induced disruption of tight junctions and adherens junctions is associated with the loss of interaction of these proteins with the actin cytoskeleton, and their migration from the intercellular junctions into the intracellular compartments. Epidermal growth factor (EGF), a gastrointestinal mucosal protective factor, prevents acetaldehyde-induced disruption of tight junctions and adherens junctions by a MAP-kinase-dependent mechanism. L-Glutamine, a conditionally essential amino acid for the gastrointestinal mucosa, prevents acetaldehyde-induced disruption of tight junctions and adherens junctions by trans activation of EGF-receptor. Studies addressing the mechanisms of such epithelial disruption and the protective factors that prevent ethanol and acetaldehyde-mediated disruption of epithelial tight junctions are critically important in the investigations toward the search of preventive and therapeutic strategies for alcoholic liver disease.

Blood material interactions at the interface

Chandra P Sharma

Division of Biosurface Technology, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Poojappura, Thiruvananthapuram 695 012

Blood compatibility of materials and devices is dependent upon the surface energy parameters interacting with various blood components such as proteins and cells etc. at the interface. Such concepts will be reviewed with respect to the role of surface modifications in enhancing the blood compatibility. Current status will be discussed in relation to the monolayer deposition of phospho/glyco lipids with cholesterol mimicking the cell membrane and their interaction with blood proteins and cells and how such modifications enhance the blood compatibility. The AFM results will be presented indicating the relevance of varied surface modifications with reference to the cardiovascular devices. However, there is still a challenge to make much more efforts to develop surfaces, mimicking the biological interface.

Protection of alfa-ketoglutarate against seizures induced by neurotoxic substances: a possible mechanism for its protection

Hiro-aki Yamamoto

Institute of Community Medicine, University of Tsukuba, Tsukuba, Ibaraki, 305-8575 Japan

The effects of a-ketoglutarate, a substrate of enzyme on the Krebs cycle on seizures induced by neuro toxic substances including cyanide and kainic acid were examined in mice. An intraperitoneal (ip) injection of kainic acid (45 mg/kg) or subcutaneous injection of potassium cyanide (9 mg/kg) produced broad-spectrum limbic and severe sustained seizures in all of the treated mice. The seizures were abolished when a-ketoglutarate (1 g/kg) was injected intraperitoneally in the animal 1min before kainic acid or potassium cyanide administration. In addition, the administration of kainic acid or cyanide caused damage to mtDNA in brain frontal and middle cortex of mice. These effects were completely abolished by the ip pre-injection of a-ketoglutarate (1g/kg). *In vitro* exposure of kainic acid (0.25, 0.5 or 1.0 mM) or cyanide (1.0, 2.0 mM) to brain homogenate inflicted damage to mtDNA in a concentration dependent manner. The damage of mtDNA induced by 1.0mM kainic acid or 1.0mM cyanide was attenuated by the co-treatment with a-ketoglutarate (2.5 or 5.0 mM). Further-more, *in vivo* and *in vitro* exposure of kainic acid or cyanide elicited an increase in lipid peroxidation. However, the increased lipid peroxidation was completely inhibited by co treatment of a-ketoglutarate. These results suggest that a-ketoglutarate plays a role in the inhibition of seizures and subsequent mtDNA damage induced by reactive oxygen species produced by the neurotoxic agent, kainic acid and cyanide. These seizures and mtDNA damage were also completely abolished an ip injection of the other substrate (1 g/kg) of enzymes on the Krebs cycle including, oxaloacetate, succinate, malate, fumarate and citrate. However, preinjection of maleate (1 g/kg) or malonate (1 g/kg), which is non component on the Krebs cycle did not prevent against kainic acid-induced seizures. These results suggest that the Krebs cycle may play a key role for protection against seizures induced by the neurotoxic agent, kainic acid and cyanide, a potent producer of reactive oxygen species including ·OH.

RESEARCH PAPERS

Micro-Comet assay in CHO cells: an *in vitro* method for genotoxicity assessment of chemicals

Alok Dhawan, Mahima Bajpayee, Alok K Pandey, Devendra P and Seth PK

Industrial Toxicology Research Centre, Lucknow

A global effort on reduction, refinement and replacement of animals has led to a paradigm shift from animal to *in vitro* models for the toxicity evaluation of chemicals. There is also a concern for early detection of the genotoxic potential of new chemical entities constantly being added to the environment, causing adverse effects even at low- level, long-term exposure. A micro plate method was developed using Chinese Hamster Ovary (CHO) cells cultured and treated in a 96-well micro-titer plate. Single cell gel electrophoresis/ Comet assay was performed and the method was standardized and validated using a known mutagen, ethyl methanesulfonate. A concentration dependent increase in the DNA damage was observed between 1×10^{-4} M to 2×10^{-3} M. This method was used for the assessment of the genotoxicity of endosulfan, its isomers (endosulfan alpha, endosulfan beta) and metabolites (endosulfan sulfate, endosulfan diol, endo-sulfan ether, endosulfan hydroxyether and endosulfan lactone). A significant dose dependant (1×10^{-6} M⁻¹ x 10^{-5} M) increase in DNA damage for all the compounds was observed. This was evident by an increase in the Olive Tail Moment (arbitrary units), Tail DNA (%) and Tail length (mm) ($p < 0.05$) as compared to the control. Endosulfan lactone was found to be the most genotoxic followed by hydroxyether > sulfate > diol > alfa > ether > beta > endosulfan. The study shows that the organochlorine pesticide endosulfan, its isomers and metabolites are genotoxic and endosulfan exerts its genotoxicity through its metabolites. The data demonstrates that micro-Comet assay is a rapid and sensitive *in vitro* method with a better throughput for genotoxicity assessment of chemicals.

Histopathological effects of pendimethalin in a 90 day study in Wistar rats

Geeta Nirody, Kamala K, Vijay Kumar, Deepika Rani S, Lokesh PV, Lourdappa Shetty and Suresh CS
Toxicology Department, Rallis Research Centre, Bangalore

The toxicological effect of pendimethalin(N-(1-ethyl propyl)-2,6 dinitro-3,4-xylidine) a herbicide, was studied in Wistar rats in a 90 day (sub-chronic) study. The rats were given pendimethalin in feed @ 200ppm, 1000ppm and 4000 ppm in three treated groups with a control group and a control and high dose recovery group. Each group consisted of 10 male and 10 female rats. The terminal fasting body weight was decreased in high dose males and females and high dose recovery females. A significant increase in the absolute and relative weight of liver was observed in high dose males and females. The relative weight of kidney was significantly higher in high dose males and females. There were no treatment related gross changes. Microscopically, higher incidences of hepatocellular hypertrophy in liver in males and females, decreased eosinophilic granules in secretory ducts in salivary glands, increased hemosiderosis in spleen in females and hyaline droplet nephropathy in kidneys in males were observed in treated animals. In liver (both sexes) and in kidneys (males) the microscopic changes were associated with the increase in the organ weights. All these microscopic changes were found to be reversible as the incidences and severity of the lesions were low in the high dose recovery animals.

***In vitro* acetylcholinestrace activity in various pesticide exposed and diseased human blood**

Rahman MF, Mahaboob M and Grover P

Biochemical Toxicology, Indian Institute of Chemical Technology, Hyderabad

In toxicological studies the biochemical and enzymatic variations are powerful tools especially *in vitro* studies where less number of animals will be used and also in short period of time results can be achieved. Therefore, in the present investigation an attempt was made to study the *in vitro* effect of RPR-II, RPR-V, monochrotophos (MCP) and Acorus calamus on target biochemical enzyme acetylcholinestrace in human blood. These compounds inhibited this enzyme by *in vitro* assay in concentration dependent order and also based on their IC50 inhibition. With regard to blood AchE MCP was found more potential Than RPR -II, RPR-V and A. calamus. Further these compounds decreased Vmax and Km values indicating uncompetitive inhibition. The biochemical changes induced by a test compound have significance in its toxicological studies, alterations in biochemical parameters before clinical signs and symptoms indicate either the safety of the toxicant or its detrimental effect and these parameters can be used in predictive toxicology.

Session: Reproduction, teratology, mutagenicity and carcino-genicity

LEAD PAPERS

Assessment of sperm: a key factor in reproductive toxicology

Roy Chowdhury A

Regional Occupational Health Centre (E), Indian Council of Medical Research, Block DP, Sector V, Salt Lake, Kolkata

Assessment of sperm is one of the most commonly employed methods for studying male reproductive toxicology. Exposure to chemicals from different environmental sources causes harmful effect on sperm. Sperm analysis can be a very effective instrument for detecting testicular dysfunction, especially in the presence of azoospermia, or oligospermia. Work exposure of men has been associated with changes in sexual behaviour, libido and potency, infertility and increased spontaneous abortions in their wives. Oxidative damage to sperm head DNA and nuclear protein were observed after exposure to cadmium, nickel and cobalt. These defective sperm cells transmit wrong genetic information from generation to generation which produce various health impairments in subsequent generations. The methodology of semen examination still needs much research to establish the best methods for use in industrial situations to evaluate the male reproductive risk. The analysis of sperm count, concentration and morphology has been used as a best marker for the assessment of reproductive risk. Flow cytometric analysis of sperm head nuclear DNA of lead exposed workers revealed significant fragmentation of sperm head nuclear DNA. This result supported by higher percentage of abnormal sperm head morphology after chronic lead exposure. Moreover, there are several chemicals, which demonstrated the sperm head DNA damage and followed by heritable defects in the offspring. Therefore, assessment of sperm cell injury by the exposure of industrial chemicals may be a better indicator to warn the reproductive hazards by which the extension of damage of heritable materials in germ cell may be extrapolated.

A role of programmed cell death (apoptosis) in mediating birth defects: a report on three teratogens

Neeraj Sinha

Division of Toxicology, Central Drug Research Institute, Lucknow

Congenital malformation or birth defects are a major public health concern. The causes of birth defects are varied, but the etiology of most malformations is unknown. In earlier times human beings have wondered at the process that normally culminates in the birth of a healthy baby and has endeavored to understand the origin of birth defects. Infections, malnutrition, ionizing radiation and various drugs and chemicals etc have been reported to be as the causes for the birth defects but how do chemical insult at the cellular and molecular level translate to a birth defects is a point of attraction now a days. To illustrate the pathogenesis and mechanism of birth defects emphasis has been laid down on cell cycle perturbations and cell death by various workers. As the programmed cell death (PCD) i.e. apoptosis has been reported to have a critical role in normal morphogenesis and functional connectivity between the CNS and distal structures, the present work was emphasized to see if PCD has any role in bringing birth defects and thus can be considered as an end point for exploring teratogenic potential of a compound or not. For this teratogenic potential of these known teratogens i.e. Cyclophosphamide, Carbamezapine and Mitomycin C has been tested by culturing 11 gestational day old rat whole embryos and then performing a battery of tests which included quantitation of apoptotic cells, DNA 3' end labeling, and DNA laddering as the hall mark of apoptosis. The out come will be discussed in details during the session.

Concept of chemoprevention by dietary constituents in medium term carcinogenicity bioassay

Yogeshwer Shukla

Environmental Carcinogenesis Division, Industrial Toxicology Research Centre, Lucknow

Environmental compounds are likely involved in the development of many human cancers. Their elimination would be expected to help with the prevention of cancers. However, this is not a practical proposition; therefore, it is important to discover naturally occurring or synthetic compounds that might suppress or prevent the process of carcinogenesis. Several long term and short term tests have been used routinely for the evaluation of carcinogenic/ anti-carcinogenic potential of these agents. But the long term processes are time consuming, requires large number of animals and short term processes may not provide sufficient evidence for the carcinogenic/anticarcinogenic potential of the these agents. Therefore, a medium term bioassay was developed for the rapid and reliable detection of carcinogenic /anticarcinogenic agents. To achieve this induction of altered hepatic foci (AHF) was quantitated using certain positive and negative biomarkers of cellular process. These biomarkers include quantitative induction of placental isozyme of glutathione-S-transferase (GST-P) and g-glutamyl transpe-ptidase (GGT) foci and decrease in adenosine-tri phosphatase (ATPase), alkaline phosphatase (AlkPase) and Glucose-6- phosphatase (G6Pase) foci in rat liver. Quantification of these biological markers of cellular proliferation is the reliable marker for AHF and has been verified for the detection of carcinogenic /anticarcinogenic agents in literature reports. In the present study, the anticarcinogenic potential of various dietary constituents was evaluated using medium term liver bioassay. All of these agents decreased the diethyl nitrosamine induced GST-P and GGT positive foci whereas, potentiate the ATPase, AlkPase and G6Pase deficient foci. Thus these dietary agents possess chemo preventive potential against DEN induced hepatocarcinogenesis.

Assessment of two-generation reproduction toxicity study in rat

Rajendran S

Jai Research Foundation, Vapi, Gujrat

The assessment of the effect of test substance on reproduction of animal is an essential part of experimental toxicology. The experimental toxicology study data (produced following GLP principal) is the major source of information for the regulators, for evaluation of the substances, which enable them to make critical decisions so as to approve, restrict or disapprove a substance. Therefore, the integrity and reliability of data have to be of prime importance for safety evaluation of the product. Reproduction and fertility effects study is designed (as per OPPTS 870.3800 and OECD-416 guidelines) to provide general information concerning the effects of a test substance on the integrity and performance of male and female reproductive system, including gonadal function, the estrous cycle, mating behaviour, conception, gestation, lactation and weaning and on the growth and development of the offspring (neonatal morbidity, mortality and target organs). The principle of the study is to administer the test substance to parental (P) animals prior to and during their mating, during the resultant pregnancies and through the weaning to their First Filial (F1) offspring. The substance is then administered to selected F1 offspring during their growth into adulthood, mating and production of an F2 generation, until F2 generation is weaned. The critical observation, advances and the data interpretation

Studies of the teratological assessment of 'Sobatum' in rats

Mohan PV and Devi KS

Toxicology Group, BMT Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram

Sobatum, the active fraction of the plant *Solanum trilobatum* was obtained from the petroleum ether:ethyl acetate (75:25) extractable portion. Sobatum was proved as an anticancer agent by *in vivo* methods. The aim of the study is to evaluate, whether sobatum has any potential to induce skeletal and soft tissue anomalies in rat fetuses. In this assay there are four groups, group I as vehicle control, group II and III received 100mg and 200mg sobatum/kg body weight and group IV as positive control (caffeine). The control, positive control and sobatum were administered to pregnant rats by oral gavages from 6th day to 15th day of gestation. On 21st day, laprotomy was carried out and the position, number of live and dead fetuses including resorbed fetuses in the uterus was recorded, and all the fetuses were examined by routine teratological testing parameters. The results indicated that, there was a significantly less body weight gain and feed intake in the positive control group. There is no remarkable incidence of external anomalies noted in the control and treated groups, however significantly high incidence of skeletal abnormalities observed in the positive control group. Hence it can be concluded that the sobatum at different dose level did not have the potency to induce skeletal or soft tissue malformations in rats.

Session: GLP and Regulatory Toxicology

KEYNOTE ADDRESS

Good Laboratory Practice and Regulatory issues

Devi Saran Tewari

National GLP Compliance Monitoring Authority, Deptt.of Science and Technology, New Delhi

Government and industry are concerned about the quality of non-clinical health and environmental safety studies upon which hazard assessment is based. As a consequence, Organization for Economic Co-operation and Development (OECD) has formulated and published the GLP principles. Currently, it is mandatory that in all OECD countries, non clinical safety data for the purpose of registration of industrial chemicals. Pharmaceuticals, veterinary medicines, cosmetics, pesticides etc, should be generated in GLP complying test facilities/laboratories. The purpose of these principles of Good Laboratory Practice is to promote the development of quality test data. If the individual country can confidently rely on test data developed in other countries, duplicative testing can be avoided, thereby saving time and resources. In India, Department of Science and Technology has established the National Good Laboratory Practice (GLP) Compliance Monitoring Authority. This national programme takes into consideration the requirements of regulatory authorities, these facilities and global perspective. The national GLP programme has obtained the approval of the Government of India to adopt the OECD Principles of GLP and compliance monitoring as well as the OECD norms for managing its operational system. It includes participation in OECD activities on GLP issues so as to contribute technically and also in the management of the GLP programme as its stakeholder. In addition to this, an apex body has been constituted, which has the concerned Secretaries to the Government of India as its members. This apex body is to ensure the operation of the programme in accordance with the OECD norms. The programme also has a technical committee to help the secretariat of national GLP programme in evaluating the competence of test facilities on the basis of the inspections organized by GLP- certification body. Inspectors who are experts with their qualifications, experience etc meeting the norms of the national GLP programme, evaluate the technical competence of the applicant test facility/laboratory in all respects for its compliance to OECD principles of GLP and OECD test guidelines. These inspectors are the technical experts trained by national GLP compliance monitoring authority on GLP system. After obtaining the required approval of the Government, national GLP compliance monitoring authority was constituted on August 5, 2002. Thereafter, it started inviting applications from test facilities. The inbuilt feature of the programme is that the regulatory authorities would be accepting the safety studies conducted in OECD member countries as well as GLP certified Indian test facilities. The programme is now fully operational and has started giving GLP certification. To create awareness about the GLP programme to the nation, a number of workshops on "Good Laboratory Practice" were organized in different parts of the country. And to create awareness amongst the Government officials of different Ministries/Industry, a one-day workshop on 'GLP and regulatory issues' was organized by involving foreign speakers from seven OECD member countries. The first training course for GLP inspectors was also organized by National GLP Compliance Monitoring Authority and few of the test facilities were found to have satisfied the norms of the OECD's principles of Good Laboratory Practice and would be given GLP Certification.

GLP implementation in India: current status and future strategies

Yogendra K. Gupta and Deepak K. Agarwal

Industrial Toxicology Research Centre, Lucknow 226 001

Good Laboratory Practice (GLP) is a quality system concerned with the organizational process and the conditions under which non-clinical health and environmental studies are planned, performed, monitored, recorded, archived and reported. The objectives of implementing the principles of GLP are to promote the development of test data of comparable quality that forms the basis for mutual acceptance among countries, to avoid duplicative testing and technical barriers to trade, and to improve the safety to human health and environment. The principles of GLP are implemented as regulation in 40 member countries of OECD including USA and Japan and several non-member countries (including India) are signatory to OECD-GLP compliance programme through MAD agreement. Recognizing the merit and impact of GLP compliance on the global trade and testing business, several Indian test facilities have been voluntarily complying with GLP principles and some have been certified by Germany and The Netherlands. In 1994, CSIR spearheaded the implementation of GLP as a national policy that culminated in the establishment of a National GLP Compliance Monitoring Authority in 2002. In the past two years, this body has organized national conferences and training programmes for mass awareness on GLP principles, created a cadre of GLP inspectors that conducts laboratory inspections and study audits for GLP certification of Indian test facilities, and participated in OECD test guideline coordination programme. Regulatory submissions for Drug Controller general of India and Central Insecticide Board of India now require GLP compliance while other regulatory agencies in the ministry of environment & forests, industrial chemicals, petroleum, etc are in the process of adopting similar stance. Compliance to GLP principles is, therefore no longer voluntary. It is now essential for the Indian test facilities, to assert their credibility and competitiveness in the testing business and to provide test data that can be mutually acceptable by the regulatory agencies thereby helping the Indian industry to globally position their equally credible products. Learning from the experience of OECD member countries, the strategy for GLP implementation in India has to be straightforward and tough. The principles of GLP have to be adopted as law with provision of punitive action against defaulters. The cadre of GLP inspectors has to be expanded and preferably full-time GLP inspectors have to be appointed with legal authority to inspect Indian test facilities. Regulatory agencies should insist on GLP compliance of reports submitted for their consideration and a national panel of toxicologists may be recognized by the National GLP Compliance Monitoring Authority of India to seek advice on advanced/alternative technologies and improved protocols for non-clinical safety/toxicity studies. The subtle differences that exist between test guidelines of OECD, Schedule-Y and Goitonde Committee may also be harmonized for global acceptability of protocols and test reports. The time has come for a formal education and training in Toxicology as a specialty subject instead of on-job conversion of allied graduates as toxicologists. Implementation of GLP is a capital- intensive proposition involving major up-gradation of infrastructure and analytical facilities, personnel hiring and training, documentation, quality assurance, etc. The Indian test facilities may, therefore require time and tax incentives to become GLP compliant before the enforcement of GLP Law as done in other countries. Considering the overall benefit to our industry and country, the best strategy for GLP implementation is to make a joint effort of all parties concerned.

Medical devices regulation and its status in India

Bhuvaneshwar GS

Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, India.

Today, the term 'medical devices' includes more than 900 product groups covering 50 clinical specializations encompassing a huge variety and range. Internationally, medical devices as a product class started getting regulated only around the 1970s with widespread public and political attention due to reports of device related safety issues. USA was the first country to legislate on devices regulation in 1976. The one introduced by the European Union during 1990's is a comprehensive system gaining world-wide acceptance. However, India is yet to have a full-fledged regulatory mechanism. The Global Harmonization Task Force (GHTF) was formed in 1992 to encourage convergence in regulatory practices for ensuring the safety, effectiveness and quality of medical devices, while promoting technological innovation and facilitating international trade. This harmonized approach consists of a risk based classification system, applying levels of regulation depending on the degree of risk posed by the medical device. Regulation is implemented through conformity assessment to quality assurance systems thereby ensuring that only good quality and safe products are placed on the market. In the post-market phase, a vigilance system collects and analyses adverse events to ensure that unsafe products are rectified, improved or even removed. In India, the term "DEVICES" was introduced in the Drug and Cosmetics Act in its 1982 amendments. Based on this, about 15 products, like syringes, needles, blood bags, contraception devices, etc. have been notified for regulation. However, the drug regulatory authority is unable to regulate all medical

devices as the approach to medical device regulation cannot be the same as that of drugs. The lack of a regulatory authority in India affects not only the quality and safety, but also development and manufacturing. These issues and also the need for a modified GLP system in the safety testing of biomaterials and medical devices are discussed in the presentation.

LEAD PAPERS

Quality assurance and Good Laboratory Practices

Deepak K Agarwal

Industrial Toxicology Research Centre, Mahatma Gandhi Marg, Lucknow

Quality assurance refers to a defined system established to assure *test facility management* of compliance with the principles of GLP. It should be documented and implemented by appropriately trained individuals designated by and directly responsible to the *management*. *QA-personnel* should not be involved in the conduct of studies being assured but be familiar with their procedures. At small *test facilities*, QA functions may be performed by individuals working in different departments/studies or by out-side agency, however, it is prudent to have at least one individual exclusively for co-ordination of QA-function. The responsibilities of QA include, but are not limited to (1) maintaining copies of all *study plans* and *standard operating procedures (SOPs)* in use at the *test facility*, reviewing/verifying that they contain all information required for GLP compliance and identifying critical phases for *inspection* (2) accessing an up-to-date copy of the *master schedule* for planning QA activities, assessing QA workload and evaluating extent of overlap between regulatory and non-regulatory studies through sharing common work areas and facilities (3) conducting *inspections* (study-based, process-based and facility-based) (4) *auditing final reports* for accuracy and completeness (5) promptly reporting the results of *inspection/audit* to the *management*, *study director* and *principal investigator(s)* and (6) preparing a signed *QA-statement* for inclusion in the *final report*. To effectively discharge its responsibilities, QA must also develop its own *SOPs*, inspection checklists, and plans of activity, documents, records etc for the *management* to evaluate their efficiency and effectiveness as well as for reconstruction of QA-activity by *GLP compliance monitoring authority*.

Discovery and regulatory toxicology of new pharma-ceuticals

Shingatgeri VM

Ranbaxy Research Laboratories, Gurgaon 122001.

Current focus of leading pharmaceutical industry is making an early decision on safety of a new chemical entity (NCE) by subjecting them through high throughput toxicology assays and *in vivo* screens, which together called as discovery /predictive toxicology. A successful discovery toxicity screen requires appropriate strategy backed by high throughput and miniaturized predictive assays so that minimum test material and reagents is used without compromising the quality of results. Whereas the regulatory toxicology phase of pharmaceutical development involves assessment of the potential toxic or adverse effect of drug candidates, their excipients and degradants using relevant *in vivo* animal models and *in vitro* technologies. The objective is to predict organ toxicity, decide first human dose and dosing regimens, escalation scheme, and finally mimic the schedule, duration, formulation and route as that proposed for the clinical trial. As toxicities are identified it is necessary to define the therapeutic index between toxic and efficacious exposures and to provide a safety assessment or risk-benefit analysis relative to the therapeutic benefit of the drug candidate. Toxicology evaluations that enable and support human clinical assessment include acute, repeated dose and chronic toxicities, evaluation of potential effects on vital organs (safety pharmacology), the conduct of reproductive and developmental toxicity studies, and an assessment of the genotoxicity and carcinogenicity potential. Additional information that contributes to the overall safety assessment includes characterization of metabolism, full metabolite identification, full CYP characterization, CYP induction studies, drug transporter assessments, full protein binding and elucidation of binding site, red blood cell distribution, and assessment of tissue distribution. Since regulatory toxicity studies contribute in critical decision-making process with regard to progression or discontinuation of a compound the assays/tests must comply with the standard scientific protocols, regulatory guidelines and good laboratory practices.

Role of management of test facility in GLP studies

BalaKrishna Murthy P

International Institute of Biotechnology and Toxicology, Padappai-601301

It is well known fact that the acceptability of safety studies to regulatory agencies depends upon the use of valid testing protocols and the application of principles of Good laboratory Practices. To implement the GLP in

any test facility, management is the key player and has the responsibility for the organization and functions of the test facility in accordance with the principles of GLP. The management must ensure that each element of a test facility must comply with the principles of good laboratory practices and should define the individual role and responsibility in implementing GLP. Management of the test facility must ensure that the adequate, valid and approved Standard Operating Procedure for the execution of the each and every task adopting GLP under the regulatory environment. All the studies carried out in a test facility must approved by the management of a test facility and proper study plan should exist before its execution. There should be adequate facilities arranged for the completion of study, by a study director, designated by the test facility management. Quality assurance programme in a test facility, implementing GLP, will ensure the proper conduct of the study. Management has the responsibility of ensuring the QA personnel in accordance with principles of GLP. Management of a test facility must ensure that resources for the implementation of GLP such as Archives, Test substance controller must exists and the individual designated for these are working inline with the compliance of GLP principles.

Conduct of non- regulatory work in a GLP compliant test facility: the issues

Geetha Rajashekhar

Toxicology Department, Rallis India Ltd, Rallis Research Centre, Bangalore 560 058

Many test facilities include in their scope of work regulatory studies aimed at dossier preparation /submission in addition to non-regulatory studies (e.g. testing or exploratory work). As the Principles of Good Laboratory Practice (GLP) apply only to regulatory studies, there is often confusion regarding the way in which such a situation is to be handled. While everyone would like to keep the two worlds separate from each other, with constraints on man, materials and time being the order of the day, this is not always possible. The same people, equipment and facilities are involved in both kinds of work. In order to understand the issues involved in such a situation, the implications of being a GLP compliant test facility have first to be understood. GLP is a quality system, which is concerned with the organizational process and conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported. GLP, therefore, involves not just a study-based inspection of individual studies, but an inspection of systems in place to ensure that the activities necessary for a GLP compliant study are carried out to the same high standard time and time again. In addition, the problems which might arise by applying varying standards to regulatory and non-regulatory studies have also to be understood. If the non-regulatory studies are not conducted in accordance with standards comparable to GLP, this will have a negative impact on the GLP compliance of regulatory studies. Management of test facilities should be aware of all the implications of such a situation and should take a clear cut decision on whether to have separate facilities for GLP and for non-regulatory work or they should decide on the degree of separation required if the same facilities are used for both. Alternatively they should decide on whether to do all work in compliance with the requirements of GLP.

Principles of Good Laboratory Practice

Sankaranarayanan A

Torrent Research Centre, Bhat 382428, Gujarat, India

The Good Laboratory Practice (GLP) is a quality system concerned with the organizational process and conditions under which safety studies on pharmaceuticals are planned, performed, monitored, recorded, archived and reported. Adoption of such systems facilitates acceptance of the test data across the countries. The test facility management is responsible for compliance with the GLP on various activities of the facility by ensuring availability of qualified personnel, equipment and materials. It organizes the quality assurance programme (QAP) and designates the study directors. The study director functions as a single point of study control and is responsible for the over-all conduct of the study and documentation of the final report. The test facility would have a documented quality assurance programme to ensure that the studies are conducted in accordance with the principles of GLP. Its personnel would maintain study plans and SOPs in use, conduct inspections and communicate the inspection results to the study director and the management. To ensure the quality and integrity of the data generated, written standard operating procedures (SOP) would be prepared and approved by the facility management and would be followed. The study director would ensure that any deviations from the SOP would be documented. Before the initiation of the study, a study plan would be approved by the study director and verified by the QAP for GLP compliance. Any amendments, deviations from the plan would be acknowledged and documented by the study director. All data generated during the conduct of the study would be recorded directly and promptly with signatures etc. Final report would be prepared for each study with the signatures of study director, principal investigator and study scientists. On completion of the study all the relevant documents of the study are archived. Good laboratory practice as detailed above comprehensively covers all aspects of hazard assessment procedures with the purpose of ensuring generation of quality data. All

aspects of these tests are controlled and documented and hence are reliable and widely acceptable across the countries.

Session: Plant, Environmental pollution and occupational hazards, metal/microbial toxicity

LEAD PAPERS

Potential health risks related to tire-fire smoke

Kirpal S. Sidhu¹, Frederick L. Keeslar², Peter O. Warner³

¹Division of Environmental and Occupational Epidemiology, Michigan Department of Community Health, 3423 North Martin Luther King Jr. Blvd, PBox 30195, Michigan, 48909, ²Grand Traverse County Health Department, 2325 Garfield Road, N Traverse City, Michigan, 49686, ³Department of Occupational and Environmental Sciences, Wayne State University, Detroit, Michigan, 48201, USA

A tire-fire at a retreading location in Blair Township near Interlochen, Michigan was reported at 9.30am on December 29,1995. The company had stored over 700,000 petroleum based tires at this location. It took 22 days to 15 fire departments to stop the fire. The smoke was visible from 40 miles away from the location. Inside the tire piles the temperature reached up to 2400oF. Tire-fire smoke usually include inorganic and organic particulates, ash, arsenic, benzene, carbon monoxide, formaldehyde, lead, oxides of nitrogen, polycyclic aromatic hydrocarbons, phenol, sulfur dioxide, zinc, etc. The above mentioned environmental contaminants in low concentrations were found at or near the tire-fire location in outdoor air, groundwater and snow. No contaminants were detected in indoor environments (residential areas) about one mile away from the tire-fire location. Reported concentrations and potential health risks of the released contaminants are briefly discussed. The tire-fire smoke is an irritant to eye, nose, and the respiratory tract. Because there is evidence that tire fire smoke causes adverse health effects in humans,exposure to this source of air pollution should be regulated and minimized.

Asthma and respiratory diseases due to environmental air pollution

Sahu AP, Kumar D and Paul BN

Preventive Toxicology Division, Industrial Toxicology, Research Centre, Lucknow-226 001, India

Science has progressed extensively and had also created the adverse impact to the environment and animal kingdom. The travelling distances were reduced due to the availability of the fast running automobiles but polluted our clean air. This air pollution to human exposure causes the increasing trend of asthma and respiratory diseases world over. In comparison to the developed nations, in India, we have major air pollution from the outdoor environmental pollution from motorized vehicles and industries. The air quality standards are variable in different part of the world. With the understanding of the air pollution, emission, exposure and effects on the susceptible population depends on the associated factors such as socioeconomic status, race or gender. These factors may play a role in susceptibility to particulate matter (PM) related effects to the other organs and lungs responsible for the resultant tissue damage. The translocations of these air toxics from lungs via the blood or lymph to other body organs is also not well known. Besides, susceptible populations of asthma, the other respiratory diseases are chronic bronchitis, pulmonary fibrosis, and chronic obstructive pulmonary disease (COPD). People with asthma may be especially susceptible to the various components present in the PM and can be exacerbated extensively due the enhanced exposure to PM. In asthmatic condition, the inflamed bronchiolar epitheliums are not able to trap the fine and ultra fine particulate matter and they are carried with air stream into the deeper part of the lung. Thus, the whole distal lungs along with the conducting and respiratory airways are associated with impact of the air toxics in the combined deleterious effects of the existing asthmatic condition. Moreover, presently little pharmacological cure is available for these ailments. We have conducted experiment in which the PM collected from the Lucknow city was exposed to the normal mice and mice with asthmatic condition induced by the allergen - Ovalbumin. The bronchoalveolar lavage fluid (BALF) cells of the asthmatic mice were of larger size while the BALF cells of the combined group showed the heavy deposition of the PM. The lungs of the asthmatic mice showed hyper reactive bronchiolar epithelium and damage in the lung parenchyma and cellular debris in the alveoli. The effects of the PM on the mouse model of asthma showed enhanced effects on bronchiolar epithelium, which was highly, inflamed thus narrowing the passage of the airways. The results of the study showed that PM is injurious to health and can exacerbate the asthmatic conditions.

Selenium: physiological, clinical and toxicological aspects

Sandhu HS

Department of Pharmacology & Toxicology, College of Veterinary Science, Punjab Agricultural University, Ludhiana 141 004, India

Selenium (Se) is a naturally occurring non-metallic trace element that belongs to group VIa of the periodic table. First considered as a poison, now it has gained physiological and pharmacological interest as well. Presently it is appreciated as a powerful catalytic element constituting the active centre of a number of eukaryotic proteins (Behne and Kyriakopoulos 2001). Trace amounts of selenium in the diet are required to prevent certain deficiency diseases in both animals and humans. However, selenium in twice the required concentration becomes toxic. The two faces of selenium, required nutrient and potent toxin, make it a particularly important trace element in the health of both animals and man (Lemly 1997). Properties and Sources: The metalloid selenium has four natural oxidative states-elemental selenium (0), selenide (-2), selenite (+4) and selenate (+6). The alkali selenites and selenates are soluble in water and therefore have greater bio-availability compared with water insoluble elemental selenium or selenides. Selenium has a strong tendency to complex with heavy metals and it easily replaces sulphur to form a large number of organic selenium compounds (Barceloux 1999). Dietary selenium consists mainly of selenoaminoacids and analogues such as L-selenomethionine from cereal grains and animal proteins or L-selenocysteine from animal's meat, poultry, fish, and dairy products. The organic forms of selenium, usually present in plants, are more toxic than inorganic selenium.

Strategies on the treatment of cyanide poisoning

Bhattacharya R

Division of Pharmacology and Toxicology, DRDE, Jhansi road, Gwalior -474 002, (M.P), India

Cyanide is an extremely toxic poison and its toxicity has been implicated in suicidal and homicidal attempts through ingestion of cyanide salts. Occupational exposures, smoke inhalation are the other important sources of cyanide poisoning. Of even greater importance is the possible use of cyanide in chemical warfare and terrorism. Therefore, cyanide poses threat from both military and civil point of view. There are a number of effective antidotes for the treatment of cyanide poisoning, including the amyl nitrite and the combination of sodium nitrate and sodium thiosulphate. These antidotes were developed some 80 years back with the primary aim to antagonize the inhibitory effects of cyanide on cellular respiration. Although these antidotes have certain limitations, they continued to be the treatment of choice for cyanide poisoning world over. There have been recent developments which suggest that the mechanism of cyanide toxicity proposed several years ago may be viewed from a different perspective with respect to the toxicodynamic basis for the antidotal effect. The recent work on development of mechanistic based cyanide antidotes were prompted by the fact that the toxic effects of cyanide were found to involve more than a single biochemical lesion and more complex effects. Some of the other approaches to counter cyanide toxicity included reactivation of inhibited cytochrome oxidase by methemoglobin formers (4-dimethyl aminophenol, p-aminopropiophenone, p-amino octanyl phenone, p-amino heptanoyl phenone etc), complexing of cyanide by cobalt compounds (hydroxycobalamin, cobalt EDTA, etc), scavenging by cyanohydrin formers (sodium pyruvate, α -ketoglutarate, etc), thiol detoxification by enzymes (rhodanese) or thiosulfates, thiosulfonates, persulfides etc. In addition to hyperbaric oxygen, various other pharmacological agents like antioxidants, free radical scavengers, cardio protective and neuroprotective agents, Ca^{2+} modulators, anticonvulsants, etc. have also been evaluated as anti-cyanide compounds. The most promising approach to medical management of cyanide poisoning by the combination of thiosulfate and α -ketoglutarate, limitations and future challenges have also been discussed here.

RESEARCH PAPERS

Arsenic induced tissue oxidative stress and its response to co-administration of taurine and mono isoamyl DMSA in rats

Kannan GM and Flora SJS

Defence Research and Development establishment, Gwalior

Arsenic contamination in natural water is a World wide problem and has been reported in recent years from several parts of world including India. Arsenic induced oxidative stress in blood and other soft tissue has been postulated to be one of the possible mechanisms of arsenic induced toxic effects. Chelation has been recognized to be the medical treatment for chronic arsenic poisoning. However, mono therapy with these chelating drugs is compromised with many drawbacks like inability to provide clinical relief and turn over in altered biochemical variables. The present study was planned to investigate beneficial effects of combined

administration of an antioxidant (taurine) combination with mono isoamyl DMSA, on parameters indicative of tissue oxidative stress and have metabolism, post arsenic exposure. Male rats were exposed to 25 ppm sodium arsenite for four weeks and then treated with 15 mg/kg MiADMSA, orally once daily for consecutive five days either individually or in combination with taurine (50 or 100 mg/kg), IP. Arsenic exposure leads to significant inhibition of blood ALAD activity, marginal depletion of GSH and significant increase in blood ZPP level. These changes were accompanied by alteration in number of other clinical hematological variables (like WBC, RBC, PLT and hemoglobin etc.). Hepatic SOD activity and GSH level also showed a decrease, while, catalase and GSSG level increase significantly on arsenic exposure. Kidney GSH level decreased while, GSSG level increased significantly accompanied by an increase in tissue arsenic level. MiADMSA was marginally effective while taurine was ineffective when given alone in influencing hematological variables but was significantly effective in reducing tissue oxidative stress. However, when given in combination with MiADMSA, the beneficial effects were more pronounced compared to mono therapy. A dose of 100 mg/kg of taurine provided better recovery than 50 mg/kg. Co-administration of taurine with MiADMSA, had no additional beneficial effects on tissue arsenic mobilization over the effects of MiADMSA. The present study thus recommends use of an antioxidant (like taurine) during chelation with potent thio chelators to get optimum effects of chelation treatments.

Pesticide residues in milk - a study from Bundelkhand

Subir K Nag and Mukesh K Raikwar

Plant Animal Relationship Division, Indian Grassland and Fodder Research Institute, Jhansi 284 003, UP

Pesticides are toxic xenobiotics, which can adversely affect the biological systems in a number of ways. They may be present as residues in animal feed, fodder and water, and after entering into the animal body they are distributed in different organs, tissues and also translocated in milk in case of milch animals. Because of special position of milk in the diet of infants and children the presence of significant amount of residues in milk is undesirable. That is why the residue limits of milk and dairy products tend to be more severe than those for other products. With this background we collected bovine milk samples from Datia, Dabra, Gwalior, Orai and Jalaun of Bundelkhand region and monitored them for detecting residues of different pesticides like organochlorines (16 including isomers and metabolites), organophosphate (9), carbamate (1) and synthetic pyrethroids (7). About 83% samples were contaminated with organochlorines where as synthetic pyrethroids were detected in 73% samples. Organophosphate residues were present in only 23% samples and comprised mainly of chlorpyrifos and its methyl derivative, malathion and phosphamidon. In some places they were totally absent and in others only few of them were detected in very less conc. Carbofuran (carbamate) could not be detected in any sample. Organochlorine (DDT, HCH, and endosulfan) residues although were present in number of samples but their conc. was less and mostly within the respective maximum residue limits (MRL). Contamination with pyrethroids was caused primarily by cypermethrin which was present in 61% samples all having conc. above its MRL. Others like cyfluthrin, fenvalerate, fluvalinate and cyhalo-thrin were also detected in some samples.

Assessment of organochlorine pesticides in the blood of thyroid patients and their influence on calcium level in the blood serum

Ila solanki, John PJ, Soni IP, Saxena GN and Bhatnagar P

Environmental toxicology unit, Deptt. of Zoology, University of Rajasthan, SMS Medical College Hospital, Jaipur

Organochlorine compounds, due to their strong lipophilic nature and environmental stability tend to accumulate and get magnified in the ecosystem. Consequently, the animals at high tropic levels in the food chain accumulate the highest concentration of these pesticides. The present study was, therefore, conducted to assess the magnitude of organochlorine pesticide burden in the blood of thyroid patients who visited the S.M.S. medical college, Jaipur. Further, an attempt has been made to evaluate the influence of these pesticides upon the calcium level of euthyroid, hypothyroid and hyperthyroid subjects. The blood samples of 200 randomly selected patients were collected from thyroid clinic, SMS Medical College, Jaipur. The samples were subjected to pesticide extraction and calcium content estimation. The pesticides detected were Hexachlorohexane (HCH) and its isomers, dieldrin, heptachlor and dichloro diphenyl trichloro-ethane (DDT) and its metabolites. There appeared to be no positive correlations between the pesticide residues and calcium levels in the blood of the thyroid subjects.

Session: Research Papers for Young Scientist Award

Protective effect of *Emilia sonchifolia* Linn. (DC.) on per chlorate induced toxicity in experimental animal models

Gayatri Devi D and Annie Abraham

Department of Biochemistry, University of Kerala, Karia-vattom, Thiruvananthapuram

All organisms capable of handling oxygen are readily exposed to reactive oxygen species, the free radicals-hydroxy radical (OH.), super oxide anion (O₂⁻), hydroperoxy radical (H₂O) etc., which are formed as the byproducts of many biochemical reactions such as the electron transport chain, catabolic steps in mitochondria and microsomal reactions. These reactive oxygen species will result in the peroxidation of lipids, leading to tissue damage. This oxidative stress plays an important role in cell death associated with many diseases like cancer, ageing, coronary heart diseases, cataract etc. Certain chemicals like CCl₄, H₂O₂ etc. are found to enhance the lipid peroxidation process. In the present study, perchlorate ion (ClO₄⁻) is proved to be a potent inducer of lipid peroxidation *in vivo*. In order to determine the specific effect on lipid peroxidation, 0.2% of sodium perchlorate was administered to female Albino rats (Sprague dawley strain) along with laboratory diet and ethanolic extract of *Emilia sonchifolia* by gastric intubation, for a period of 30 days. At the end of the experimental period, the animals were sacrificed and analyzed by the different parameters. The parameters studied include the activity of superoxide dismutase (SOD) and catalase, the antioxidant enzymes and alkaline phosphatase, glutamate oxaloacetate transaminases (GOT) and glutamate pyruvate transaminase (GPT)-the enzymes to assess hepatic toxicity. The peroxidation status was determined by measuring the concentration of malonaldehyde (MDA) and reduced glutathione (GSH). The results indicate that the ethanolic extract of *Emilia sonchifolia* is a protective agent against the toxicity induced by sodium perchlorate.

***In vitro* study to distinguish between cytotoxicity and cytocompatibility of Titanium using human umbilical vein endothelial cells**

Krishna Prasad C. and Lissy K.Krishnan

Thrombosis Research Unit, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum

Evaluation of the *in vitro* cytotoxicity of a biomaterial is the initial step of biocompatibility study. The protocols developed by USP and that are described in ISO10993 being a qualitative analysis using immortal cells, *in vitro* survival of specific cells of human origin might give valuable information. Titanium (Ti) metal is widely used to make cardiovascular devices such as endovascular stent and endothelial cells are one of the specific types of cells that interact with such implants. Objective of this study is to screen Ti for its ability to support cell attachment, migration, proliferation and viability using human umbilical vein endothelial cells (HUVECs). A parallel study with mouse fibroblast cell line L-929 is done to identify the difference in cell-specific behavior on the same material. The cell spreading was assessed by actin staining with Texas Red Phalloidin, anti-PCNA antibody was used for proliferation assay, and vibrant apoptosis assay kit was used for cell viability. It is observed that proliferation of HUVECs was significantly low on bare Ti compared to the L-929 cells. Most of the HUVECs turned apoptotic by 72 h in culture where as virtually no L-929 cells were apoptotic. Further to identify if the surface properties of Ti affects cell behavior, we used fibrin composite coating on the material surface for growing endothelial cells. While spreading of cells on bare Ti was very low, on modified surfaces, the cells have spread well, proliferated and remained viable. The results suggest that while bare Ti is not cytotoxic with respect to the L929 cell growth, it has not supported cell spreading proliferation and survival of endothelial cells. Therefore, the endothelial cell culture gives a better picture about the cytocompatibility of Ti compared to the cytotoxicity assay using L929 culture alone. Since coating of surface with a thin layer of proteins could improve cell growth, proliferation and survival of HUVEC, probably the EC is affected by surface characteristics of bare Ti, such as wettability, surface charge, surface free energy and topography but not due to cytotoxicity of the bulk material. The study results also suggest that similar approaches may reduce the number of *in vivo* animal experiments required to establish implant non-toxicity.

Concomitant administration of zinc, copper and selenium protects arsenic intoxication in rats

Manoj Modi, Richa Gupta, Prasad GBKS and Flora SJS

Division of Pharmacology and Toxicology, Defence Research and Development establishment, Jhansi Road, Gwalior and SOS in Biochemistry Jiwaji University, Gwalior

Arsenic is a wide spread environmental toxicants that may cause neuropathy skin lesions vascular lesions and cancer upon prolonged exposure. There are millions of people at risk in world because they drink water containing carcinogenic amount of arsenic. Improving nourishment like supplementation of micro nutrient,

vitamins and amino acid etc., could be able to half the risk in those who were previously the poorly nourished. Micro nutrients can affect toxicity of metals by interacting with metals at the primary site of actions. Micronutrients can also modify the body's response to toxic metals by altering their metabolism and transport. Role of essential metals in reducing the impact of metals is extensive and consistently support the conclusion that injection of metals like calcium, iron, zinc etc., may greatly reduce the metal toxicity while, animal raised on a diet low in these metals have much higher metal concentrations. Interaction of arsenic and selenium promotes the biliary excretion of exogenous selenium and selenite also augments the excretion of arsenic in to bile. Although there are number of reports available about the interaction of trace metals with number of toxic metals but there are relatively very few studies available with arsenic. The present study was thus planned to investigate the effect of concomitant administration of zinc, copper and selenium with arsenic on some arsenic sensitive biochemical variables and mobilization of arsenic from blood and soft tissue interacts. Trace metal administration particularly zinc with arsenic decrease the blood and liver uptake of arsenic and reduced the arsenic induced inhibition of blood d aminolevulinic acid dehydratase (ALAD) activity. Zinc and selenium administration also provides significant protection to the liver injury caused by arsenic. No significant protection, however, was provided by copper supplementation. The results suggest the potential role of concomitant zinc administration during arsenic exposure in animals.

Hepatoprotective effect of emodin against sub chronic injury induced by acetaminophen and carbon tetrachloride

Monika Bhadauria and Sangeeta Shukla

SOS in Zoology, Jiwaji University, Gwalior, MP

Fulminant hepatic failure, caused by a number of etiologies, produces considerable morbidity and mortality throughout the world. Herbs have a long history of medicinal use within the folk and traditional system. In realization of the interest in herbal medicines and natural products have great potential in the emerging hepatoprotective agents. In this context we have selected Emodin, an active principle of plant *Ventilago maderaspatana* (Rhamanaceae, commonly known as Raktavalli). Adult female albino rats weighing 120+10 g were randomly divided in to five groups of five animals each. Group 1 served as normal control. Groups 2 and 3 were administered acetaminophen (20mg/kg, po) and carbon tetrachloride (0.15ml/kg, ip) for 21 days following 5 days rest and served as experimental controls. Groups 4 and 5 were administered with aqueous extract of emodin at the dose of 30 mg/kg (po) for 5 days after 21 day of toxicant administration. Animals of all the groups were sacrificed after 24 hours of last treatment. Acetaminophen and carbon tetrachloride caused a sharp elevation in the activity of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (SALP) and lactate dehydrogenase (LDH). Hepatic reduced glutathione level (GSH) showed marked depletion on the contrary, hepatic lipid peroxidation (LPO) was enhanced significantly. Hepatic microsomal drug metabolizing enzymes (Aniline hydroxylase and Amidopyrine N-demethylase) showed inhibition in the activity. Treatment with emodin for 5 days significantly recovered the alteration induced by toxicant in the markers of oxidative stress and markers of liver function test. Improvement in the histoarchitecture of liver and kidney also supported the biochemical studies. Ultra structure of liver also substantiated these findings, concluding that emodin has potential hepatoprotective activity.

Male reproductive toxicity of *Stevia rebaudiana* (bert.) in rats

Prasad S, Jayakumar K, Honnegowda, Narayana K and *Suguna Rao

Deptt. of Pharmacology and Toxicology, *Deptt. of Veterinary Pathology, Veterinary College, UAS, Bangalore-560 024

The ambiguity about the safety of *Stevia rebaudiana* (Bert.), a natural non-sucrose sweetener has prompted us to undertake this study. Aqueous extract of *Stevia rebaudiana* was fed orally to 3 groups of adult male Wistar albino rats for a period of 65 days at the doses of 100, 1000 and 5000 mg/kg body weight. A control group was also maintained. The results revealed decrease in the epididymal sperm count, plasma testosterone concentration and decrease in organ body weight ratio of testis and cauda epididymis in the group fed with 5000mg/kg body weight as compared to saline fed control group. There were no significant changes in the organ-body weight ratio of seminal vesicles and prostate gland in any of the 3 stevia administered groups as compared to saline control group. This study revealed the anti-fertility effect of *stevia rebaudiana* (Bert.) in adult male Wistar albino rats.

Therapeutic efficacy of tiferron in combination with propolis or piperine against beryllium induced toxicity in rats

Satendra Kumar Nirala, Mathur R and Mathur A*

SOS in Zoology, Jiwaji University, *KRG College, Gwalior

The study aims first time to evaluate therapeutic efficacy of Tiferron (sodium-4,5- dihydroxy- 1,3-benzene disulphonate) in combination with 'propolis' (Honey bee's product) or piperine (active principle of *Piper longum*) against toxicological consequences of beryllium exposure in female albino rats of Sprague dawely strain. Animals were exposed to beryllium (as beryllium nitrate) at a dose of 1 mg/ 2ml/kg (*i.p*) once a day for 28 consecutive days followed by therapy with tiferron (300 mg/2ml/kg, *i.p*), Propolis (200 mg/5ml/kg, *po*) and piperine (10 mg/5ml/kg, *po*) individually and in combination for 5 consecutive days after toxicant administration. Data were statistically analyzed through student's test followed by one way ANOVA and the values were considered to be significant at $P<0.05$. Results of blood biochemistry revealed significant depletion in blood sugar level, serum alkaline phosphatase, albumin and urea whereas non significant fall in Hb and total serum protein contents was also noticed. Significant elevation was noticed in AST, ALT, LDH, bilirubin, creatinine and γ -GT. Tissue biochemistry revealed severe alterations in liver and kidney. Significant fall in total protein and glycogen contents, succinic dehydrogenase, alkaline phosphatase, adenosine triphosphatase and glucose-6-phosphatase was noticed in both the organs. On the contrary, significant elevation in acid phosphatase, triglycerides, total and esterified cholesterol level were also recorded in both organs. Significant rise in lipid peroxidation and decrease in reduced glutathione in both liver and kidney showed oxidative stress induced due to beryllium exposure. Although tiferron and propolis exhibited therapeutic efficacy against beryllium induced toxicity yet tiferron in combination with propolis or piperine exerted statistically more beneficial effects in respect to control to reverse alterations in different variables altered due to beryllium intoxication. Mobilization study of beryllium showed that combination of tiferron and propolis significantly reduced beryllium burden in liver, kidney, blood, brain and bones without any significant changes in essential metals. Photo micrographic observations of liver and kidney also supported the data concluding that the combination of tiferron either with propolis or with piperine can be expected as better choice in treatment of beryllium induced toxicity.

Arsenic induced oxidative stress and its response to few thiol chelators in rats

Smrati Bhadauria, Dhaked RK and Flora SJS

Division of Pharmacology and Toxicology & Biotechnology, Defence Research and Development Establishment, Jhansi Road, Gwalior-474 002, India

Arsenic and its compounds cause adverse health effects in humans. Exposure to arsenic may occur from natural or industrial sources. The treatment regimen that is in use at present employs administration of thiol chelators, such as meso 2,3-dimercaptosuccinic acid (DMSA) and sodium 2,3-dimercaptopropane 1-sulfonate (DMPS), which facilitate its excretion from the body. However, these chelating agents are compromised with number of limitations due to their lipophobic nature, particularly in case of chronic poisoning. In chronic cases of poisoning arsenic has gained access into the cell and it is mandatory for the drug to cross cell membrane to chelate intracellular arsenic. In order to address this problem effectively, analogs of DMSA having lipophilic character were examined against chronic arsenic poisoning in experimental animals. In the present study, therapeutic efficacy of meso 2,3-dimercaptosuccinic acid (DMSA) and sodium 2,3-dimercaptopropane 1-sulfonate (DMPS), mono-isoamyl DMSA (MiADMSA) was compared in terms of reducing arsenic burden, as well as turnover in the clinical and biochemical variables particularly indicative of oxidative stress. Adult male Wistar rats were given 100ppm arsenic for 10 weeks followed by chelation therapy with the above chelating agents at a dose of 50mg/kg (orally) once daily for 5 consecutive days. Arsenic exposure resulted in marked elevation in reactive oxygen species (ROS) in blood, inhibition of ALAD activity and depletion in GSH. These changes were accompanied by significant decline in haemoglobin counts. MiADMSA was the most effective chelator in reducing ROS in red blood cells, restoring blood ALAD and elevating haemoglobin counts. Brain superoxide dismutase (SOD) and glutathione peroxidase (GPx) decreased while ROS and TBARS increased significantly following arsenic exposure. There was a significant increase in the activity of glutathione-transferase (GST) with a corresponding decline in its substrate i.e. Glutathione. Among all three chelators, MiADMSA showed maximum reduction in the level of ROS in brain. Additionally, administration of MiADMSA was most effective in counteracting arsenic induced inhibition in brain ALAD, SOD and GPx activity. Based on these results we propose MiADMSA to be a potential future drug of choice for the treatment of chronic arsenic poisoning.

Evaluation of a potential corneal culture system for assessing ocular irritation of biomaterials as an *in vitro* alternative

Viji Mary Varghese, Mohanan PV, Usha Vasudev and Kumary TV

Sree Chitra Tirunal Institute for Medical Sciences and Technology, BMT Wing, Thiruvananthapuram

The development and evaluation of different *in vitro* assays to reduce animal experiments has been a major focus of many researchers. Cytotoxicity tests have been proposed as alternatives for Draize eye irritation test for

different chemicals. In the present study, a potential corneal culture system for assessing ocular irritation of biomaterials as an *in vitro* alternative was evaluated by assessing specific cytotoxicity of materials for ophthalmic application using SIRC (well known established cell line from the cornea of rabbit eyes). General cytotoxic response was evaluated using L-929 cells based on ISO and USP standards. The results were comparable to that of *in vivo* Draize eye irritation tests of the same materials. This system can serve as a first screen to avoid *in vivo* testing of severe ocular irritants.

Session: Agrochemicals and pesticides, Metabolic/residual studies,

LEAD PAPERS

Metabolic disposition studies: a tool for toxicity evaluation

Singh RL

Department of Biochemistry, Dr. Ram Manohar Lohia Avadh University, Faizabad 224 001

The majority of xenobiotics (foreign chemical) that enter the body tissues are lipophilic, a property that enables them to penetrate lipid membranes and to be transported by lipoproteins in the body fluids. The metabolism of xenobiotics, carried out by a number of relatively non-specific enzymes, usually consists of two phases. During phase I reactions, a polar group is introduced into the molecule to render the xenobiotic a suitable substrate for phase II reactions. In phase II, the altered compound combines with an endogenous substrate to produce a water-soluble conjugation product that is readily excreted. It is a general belief that the chemicals are made non-toxic out of metabolic reactions. But in a number of cases the products of xenobiotic metabolism are more toxic than the parent compound. A number of different techniques have been devised to study the metabolism using whole animal, whole organ, tissue slices and cell cultures. The selection of model system depends on the type of xenobiotic in question. These studies provide information on the absorption, biotransformation, disposition and excretion of the xenobiotics in living system. The data may be extrapolated to know the likely impact of the foreign chemical on human beings.

Toxic effects of dimethoate on collagen metabolism in rats

Neelakanta Reddy P

Biological Sciences Division, Central Leather Research Institute, Council of Scientific & Industrial Research, Adayar, Chennai 600 020, India

The toxic effects of three different doses of dimethoate on the collagen metabolism in the tissues of female albino rats were studied by measuring the specific and total activities of 3H- hydroxyproline in the dermal and uteral collagen fractions and in urine. Compared to controls, the total activity of 3H-hydroxyproline in the soluble collagen and in the urine at 12 h after the administration of 3H-proline was significantly lower in the higher dose of dimethoate treated groups. The urinary excretion of hydroxyproline and the total activity of urinary 3H- hydroxyproline measured after 28 days of injection of labeled proline were decreased in higher doses of dimethoate treated animals but the excretions of urinary 3H-hydroxyproline were decreased in lower doses of dimethoate treated animals. The results of the present investigation clearly indicate that the synthesis of collagen is decreased in the higher doses of dimethoate treated animals compared to lower doses of dimethoate treated animals. In addition, the rates of catabolism of both soluble and insoluble collagens were decreased in higher doses of dimethoate treated rats. It concludes that the lower doses of dimethoate treated rats were less affected than the higher doses of dimethoate treated rats.

Immunotoxicity studies of imidacloprid in rats

Gatne MM, Ramesh, Bhoir PS and Deore MD

Department of Pharmacology and Toxicology, Bombay Veterinary College, Mumbai 400 012

Imidacloprid is an acaricide used in agriculture as well as in veterinary practice. After its use as spot-on formulation, treated animals get exposed to low levels for prolonged period. To assess the effect of continuous exposure to imidacloprid on immune system the study was conducted on 40 rats (Sprague dawley strain) divided in four equal groups. Three groups of rats (10 each) were exposed to oral imidacloprid (technical grade) at doses (mg/kg) of 16, 48 and 160 respectively for consecutive 28 days and the fourth group was kept as control. There was progressive and proportional decrease in Haemagglutinating antibody titre (HAT) and delayed type of hypersensitivity (DTH) response in treated rats. Phagocytic index as well as leucocytic migration was also reduced. Immunotoxic effect was evident at 160 mg/kg. It is suggested to monitor immunological parameters in animal patients undergoing imidacloprid treatment.

Session : Pesticides and the environment

A study on the effects of acute oral toxicity of haloquinol (Chlorohydroxyquinoline) in poultry broilers

Shiva Kumar, Kavitha Rani B, Rajeevalochana D, Brij Mohan and Radhakrishna PM

R & D Center, Vetcare, Yelahanka New Town, Bangalore

Acute oral toxicity of a non-antibiotic antimicrobial, haloquinol (chlorohydroxyquinoline) was studied in poultry broilers. Haloquinol used in the present study is a broad spectrum antimicrobial having antibacterial, antifungal and antiprotozoal activity and commonly used in poultry rearing. Twelve groups of poultry broilers (aged 7 days) consisting of 10 birds in each group were used as one batch for estimating the LD50 value in poultry broilers. The median lethal dose was estimated according to the method of Finney (1971) using haloquinol BP 80. The oral LD50 values determined in poultry broilers were 2183.33 mg/kg. The clinical signs of toxicity noted were in appetite, ruffling, sleepy nature of the birds., which further manifested into anorexia, leg weakness and weight loss. The postmortem findings showed evidence of death due to rupture of liver. The other lesions were extensive subcutaneous haemorrhages on thighs and wings, clotted blood in the peritoneal cavity, ascites, rickets, pale musculature and fragile skeletal structures.

Safety evaluation of genetically modified/engineered drugs: challenge of toxicology in twenty first century

Deepak K Agarwal

Industrial Toxicology Research Centre, Lucknow

The advent of genetically modified/engineered drugs, also called biotechnology derived pharmaceuticals, has ushered-in a new era of medicine. Their safety, as for conventional drugs and biologics, has to be ensured so that intended application does not entail any untoward effect on the life and health of the target population. The conventional approaches to toxicity testing of pharmaceuticals are however, not considered appropriate for GM-drugs due to their unique structural and biological properties including high degree of specificity to the target species, immunogenicity and unpredicted pleiotropic activities. Safety issues include reactivity from host-cell derived materials, excessive and undesirable pharmacological activity, potential tumorigenicity, etc though genotoxicity and intrinsic chemical toxicity may not be major concerns. The available guidelines for non-clinical safety evaluation of GM-drugs are applicable only on case-by-case basis and the major considerations revolve around the selection of a relevant test system (*in vivo* or *in vitro*) in which the test material is pharmacologically active due to the expression of the receptor or an epitope as in the target species. Transgenic/gene knockout animals or animal models of disease, though acceptable alternatives in absence of a relevant test system, are scarce and expensive. Study plan for safety/toxicity evaluation of GM-drugs also follow a different criteria. The pharmacokinetic and pharmacodynamic profile of test material play an important role in selecting the type and number of relevant test animals, test dose, route and frequency/duration of exposure. Observations include local tolerance and immunogenicity with specific consideration on safety pharmacology, presence of an organic linker molecule that may induce a genotoxic response and/or the mitogenic stimulation of pre-existent transformed cells and clonal expansion leading to neoplasia. The new generation toxicologist must therefore, demonstrate capability to address contemporary challenges and answer future emergencies through continuous development and application of new knowledge towards safety/toxicity evaluation of GM-drugs.

POSTER PRESENTATIONS

Effect of cytochrome P 450 activity on the levels of chloropyrifos and 3,5,6-trichloro- pyridinol in rat liver and serum

Verma RS and Nalini Srivastava

School of Studies in Biochemistry, Jiwaji University, Gwalior

Phosphorothionates exert their main toxicological effects through inhibition of acetylcholine-esterase for which they are pseudosubstrates. The parent phosphorothionates are weak acetyl-cholinesterase inhibitor, they can be activated by cytochrome P 450 dependent reactions to oxons which are more potent anticholinesterase compounds. Chlorpyrifos (O, O' diethyl 3,5,6-trichloro-2- pyridyl phosphorothionate) is an effective OP pesticides widely used in farms, office and homes. CPF undergoes oxidative desulfuration or dearylation by hepatic microsomal cytochrome P 450 (CYP)-mediated monooxygenase reaction to CPF oxon or desethyl CPF, which is further transformed in to major excretory form, 3,5,6-trichloropyridinol (TCP). Present study was designed to investigate the effect of CYP induction on the rate of CPF metabolism. Male Wistar strain was used for present study. Animals were divided in to two groups, each group was further subdivided into three sub

groups. Animals of one group were given phenobarbitone while other received 50 mg and 100 mg CPF/Kg bodyweight for three days and the third subgroup served as control. In CPF exposed rats, levels of CPF were than the rats receiving similar dose of CPF along with phenobarbitone. Phenobarbitone (PB) treatment elevated CYP activity causing faster metabolism of CPF. On PB treatment, the level of CPF was decrease in both liver and serum where as increase was observed in the levels of TCP in liver and serum at both doses.

Acute oral effects of *Acorus calamus* on lipid peroxidation and glutathione levels in different organs of rat

Mahaboob M, Rahman MF and Grover P

Toxicology Unit, Biology Div., Indian Institute of Chemical Technology, Hyderabad-500 007

The aim of this study was to evaluate acute oral toxicity of *Acorus calamus* and its effects on lipid peroxidation and antioxidant enzyme activity in different dose levels were administered orally once to Wistar albino rats (120-150) of either sex and kept for a week under observation. Acute oral treatment caused no mortality .but produced symptoms only in high dose treated rats. On 7th day, all the animals survived in the high dose (2000 mg/kg) that were killed and their liver, kidney and brain were dissected out for biochemical assays. The malondialdehyde (MDA) level a marker of lipid peroxidation and reduced glutathione content of these tissues increased significantly in male and female treated rats in comparison to control. The results showed that the acute oral treatment of *Acorus calamus* might induce oxidative stress in these organs due to lipid peroxidation at the high dose level.

Toxicity study of *Semecarpus anacardium* on histopathology of kidney and some of its enzymes in albino rat

Deshmukh PB and Choudhari CV

Science College, Nanded 431605, Maharashtra.

Acute and sub-chronic toxicity study of Bhallataka (*Semecarpus anacardium*) was conducted in groups (six animals each) of albino rats (Wistar strain) of either sex. SAE from ripen nut was orally administered to albino rats along with feed. After treatment of three sub lethal doses @ 250mg, 500mg and 750mg/kg b wt for 7 days and two sub lethal doses @ 83.33mg and 166.66mg/kg wt for 21 days, results were compared with control group. Acute and sub-chronic study revealed adverse effects on GOT, GPT, LDH and SDH activities of liver. SAE was found to have adverse effects on activity levels of GOT, GPT, LDH & SDH of kidney. These observations have been supported by histopathological study.

Hepatotoxicity induced by percutaneously administered sulphur mustard in mice

Usha Arora

Defence Research and Development Establishment, Jhansi Road, Gwalior 474 002

The Chemical Weapons Convention (CWC) prohibits the production, storage, transport and use of chemicals on enemy forces. One such chemical is sulphur mustard (SM), commonly known as mustard gas that causes serious blisters on contact with human skin. SM was administered precutaneously at a single dose of 20 LD50 (162mg/kg) in mice and were sacrificed at 1, 6 and 10 days later. In another study mice were administered with 2 LD50 (16.2mg/kg) daily for 4 days and were sacrificed on day 7. The lethal administration of SM caused proliferation of bile duct. The portal area and hepatic vanule have come closer after SM exposures were surrounded by newly constructed bile ducts. In the portal triad area particularly hyperplastic bile ducts were interspersed with collagen fibers, fibroblast and polymorphoneutrophils. Polymorpho neutrophils and hyper active Kupffer cells were present in dilated sinusoids. The plasma membrane of hepatocytes was highly convoluted in the intact area of hepatocyte under transmission electron microscope. In conclusion, percutaneously administered acute exposure of SM can induce mild to moderate effects on liver on day 10th while repeated exposure of low doses causes cumulative and irreversible.

Chlorpyrifos induced alterations in the levels of hydrogen peroxide, nitrate and nitrite

Anugya Mehta, Verma RS and Srivastava

School of Studies in Biochemistry, Jiwaji University, Gwalior 474 011

Chlorpyrifos (O,O'-diethyl-3,5,6-trichloro-2-pyridyl phosphorothionate), an organophosphorous compound, extensively used as a pesticide and termicide for agricultural and domestic purposes throughout the world. Like other OP compounds, it exhibits its neurotoxicity by inhibiting acetylcholinesterase. Besides being anticholine agents, OP compounds exert their toxicity by adversely affecting other pathways. Ops are known to

produce oxidative stress, which may also have role on its overall pesticidal action. Present study was aimed to see the effect of CPF on the levels of hydrogen peroxide, nitrate and nitrite. Reactive oxygen species such as hydrogen peroxide, hydroxyl radicals, nitric oxides and their metabolites are the subject of research because they also affect the central nervous system under various neurological conditions. Male rats of Wistar strain were used for this study. Animals were given both acute and sub-acute exposure of CPF and were sacrificed. The results of present study showed an increase in the levels of hydrogen peroxide, nitrate and nitrite in brain and liver of rats on CPF exposure. The results of present study showed an increase in the levels of hydrogen peroxide, nitrate and nitrite in brain and liver of rats on CPF exposure. The results clearly indicate the involvement of reactive oxygen species in the toxicity of CPF. Results will be discussed at the time of presentation.

Chloropyrifos and methyl parathion induced alterations in the levels of glutathione in different regions of rat brain: attenuation by antioxidant vitamins

Verma RS, Anugya Mehta and Srivastava N

School of Studies in Biochemistry, Jiwaji University, Gwalior 474 011

Chloropyrifos (O,O'-diethyl 3,5,6-trichloro-2-pyridyl phosphorothionate CPF) and methyl parathion (O,O'-dimethyl-O-p-nitro phenyl phosphorothionate, MPT) are organophosphorous (OP) pesticides used for killing a number of pests in farms and homes. OP pesticides including CPF and MPT exert their toxicity mainly by inhibiting acetylcholinesterase (AChE). These pesticides also induced oxidative stress inhibiting both enzymatic (i.e. superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase) and non enzymatic antioxidants. In the present study it was found that both CPF and MPT also affects glutathione metabolism in different regions of rat brain. It was observed that reduced glutathione (GSH) levels were decreased while oxidized glutathione (GSSG) levels were increased in both CPF and MPT exposed rats resulting in significantly marked decrease in GSH/GSSG ratio. The pretreatment of vitamins (Vit A, Vit E and Vit C) for one month leads to the restoration of GSH/GSSG ratio to normal. Results will be discussed at the time of presentation.

Biochemical and histopathological studies of amitraz induced toxicity in rats.

Misha Nath, Jayakumar K, Prasad S, Narayana K and Honnegowda

Department of Pharmacology and Toxicology, Veterinary College, Hebbal, Bangalore 560 024

Amitraz is a formamidine pesticide of growing commercial importance in the control of demodectic mange and cattle ticks. This study was done to investigate the sub-acute toxicity effects of amitraz on biochemical parameters in rats. Rats were divided into four groups each group consisting of six rats of either sex. Amitraz was administered by oral gavage at the dose of 0 (control), 5 (low dose), 15 (intermediate dose) and 50 mg/kg (high dose) body weight daily for a period of 28 days. The biochemical parameters *viz.*, serum BUN, AST, ALT and glucose were estimated on Day 7, 14, 21 and 28 of the experiment. Serum BUN, AST and ALT concentrations were increased in intermediate and high dose groups. An increase in the glucose concentration was observed in the high dose group. Histological studies indicated hydropic degeneration in liver. The present study indicated the possible hepatotoxic effects of amitraz in rats.

Alterations in the levels of glutamate metabolism in different brain regions during acute and sub-acute phosphomidon treatment in rats with reference to behavioural tolerance

Venkateswara Rao P, Chetan PS and Rajendra W

Division of Molecular Biology, Department of Zoology, SV University, Tirupati

This present study was carried out to evaluate the functional significance of glutamate metabolism in different regions of rat brain during acute and sub-acute phosphomidon treatment with reference to behavioral tolerance. Wistar strain male albino rats (150±20g) were divided into 6 groups having six in each group. Acute (1day) and Sub-acute (1day, 7days, 15 days) doses were administered by gavage, and after specific time intervals in different brain regions were isolated. The levels of glutamic acid decarboxylase activity (GAD), glutamate dehydrogenase activity (NAD-GDH) glutamine synthetase activity (Gln. syn) were estimated. GDH and glutamate synthetase activities were inhibited in almost all regions of brain during different dosing regimen when compared to glutamic acid decarboxylase (GAD). GAD activity showed an interesting trend during sub-acute phosphomidon treatment. GAD activity was significantly inhibited in all the brain regions except in cerebral cortex where the inhibition is non-significant after acute treatment. The elevated GAD levels in specific brain regions after 1 day and 7 day sub acute treatment suggest the involvement of GABAergic mechanism during behavioral tolerance to OP compounds. The oxidative deamination of glutamate was lowered in brain suggesting a reduced utilization of glutamate towards oxidative deamination. Decreased GDH activity and glutamate synthetase and

elevated GAD activity in specific brain regions suggest that glutamate might have been channeled towards the synthesis of GABA during OP-induced behavioural tolerance.

Multifunctional alveolar epithelial model-alternate *in vitro* system to study effect of aerosolized toxins/ drugs

Maya Nandkumar A

Division of Microbiology, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum 695012.

Inhalation represents one of the major routes by which the body can be exposed by accident or design to foreign materials. Once having entered the respiratory tract inhaled materials may be readily absorbed or may react directly with the alveolar epithelium. Also an increasing number of drugs and chemicals are being identified which produce lung damage on systemic administration. Lung tissue can be specifically targeted by xenobiotics, as the concentration of oxygen available is 100 times greater than in other tissues. The mechanism underlying these cytotoxic drug induced pulmonary toxicity is poorly understood and animal models are inadequate. A heterotypic *in vitro* system, which mimics the complex cellular interplay, seen *in vivo* will be ideal in such a situation. The system we report here is capable of maintaining the differentiated phenotype of alveolar type II pneumocytes for a prolonged period of 70 days in culture. The methodology adopted was to partially purify alveolar epithelial cells from Wistar rats seed them on dishes grafted with poly (*N*-isopropylacrylamide). On monolayer formation transfer undamaged monolayers to fresh culture dishes eliminating enzymatic dissociation. The coating of poly(*N*-isopropyl acrylamide) facilitates transfer of monolayer by lowering of temperature which results in phase transition of the PIPAAm making it become highly hydrophilic and causes cell detachment. The non-enzymatic transfer maintains the cell-cell junctions and the extra cellular matrix intact and this facilitates the maintenance of cell morphology and the differentiated characters like the ability to express surfactant proteins like SP-A, SP-B and SPC which are otherwise lost at an early stage in culture. It was observed that alveolar epithelial cells close to fibroblasts showed higher viability suggesting that these interactions might be related to juxtacrine signaling mechanism. The presence of fibroblasts in the system proved essential and promoted the inter-cellular interactions to maintain the harmonized lung cell function. Such a functional system will help in elucidating the series of events either in lung repair, death or abnormal state and help in understanding the role of various cytokines such as tumour necrosis factor, interleukin 1 etc which are involved.

Heavy metal (Cd and Pb) contamination of Pondicherry region (India): two year study report from two different areas at three different seasons

Aruna Devy R and Anisa B Khan

Ecology and Environmental Science Department, Pondicherry University, Pondicherry 605 014

Water used for irrigation, soil where edible crops and vegetables were cultivating and plants like paddy and vegetables were collected from agricultural and industrial areas of Pondicherry region at three different seasons from 1999 to 2001. From the extracts Cd and Pb concentration present in the different samples were analyzed by using atomic absorption spectrophotometer, GBC-902. Cd concentration in the water samples ranges from 0.2 ± 0.06 mg/l to 0.6 ± 0.08 mg/l and Pb ranges from 0.1 ± 0.08 mg/l to 0.8 ± 0.11 mg/l. Soil samples collected from agricultural area shows a highest Cd concentration of 2.0 ± 0.23 mg/kg whereas the highest concentration of 17.9 ± 0.16 mg/kg Pb was observed from industrial area. A high concentration of 2.3 ± 0.24 mg/kg Cd and 27.2 ± 0.18 mg/kg Pb was observed in the root part of paddy plant whereas grain accumulates the lowest concentration of 0.2 ± 0.31 mg/kg Cd and 8.5 ± 0.22 mg/kg Pb. Among the vegetables, root of spinach accumulates the highest concentration of 4.3 ± 0.12 mg/kg Cd and lowest concentration of 0.1 ± 0.2 mg/kg Cd in the fruit of urinal. Highest Pb concentration of 21.4 ± 0.14 mg/kg was seen in the root of ladyfinger and lowest concentration of 0.6 ± 0.12 mg/kg was observed in the fruit of chilly. Study reveals that Cd concentration was more in agricultural area and Pb concentration was more in industrial area.

Effect of oil refinery pollution on internal health status of Indian buffaloes

Ram Naresh, Neeru, Swarup D and Patra RC

Division of Medicine, Indian Veterinary Research Institute, Izatnagar 243 122, Bareilly, UP

Environmental pollution is matter of serious concern in faster developing economies like India. The present study was carried out in 3 km area of Mathura oil refinery (Uttar Pradesh) to assess the effect of refinery pollution on buffaloes. At the time of survey the refinery was processing 8031 million metric ton of crude oil and per hour gas emission was 550 kg. Apparently healthy buffaloes were randomly selected for the study. A group

of healthy buffaloes was also selected from an area of 10-15 km far from refinery as non-pollutant control. Five ml of blood was collected in heparinised vials from selected animals. The samples were processed within an hour of collection. The plasma glucose, total protein, total globulin and total albumin were estimated from the plasma to assess the internal health status of the animals. The plasma level of glucose (65.61 ± 2.63 mg/dl), total protein (6.75 ± 0.43 mg/dl), total globulin (3.75 ± 0.41 mg/dl) and total albumin (3.61 ± 0.18 mg/dl) was significantly ($P < 0.05$) lower in buffaloes within 3km radius of Mathura oil refinery than the buffaloes of 10-15 km away from the refinery. The mean plasma glucose, total protein, total globulin and total albumin values in buffaloes reared 10-15 km far from refinery, were 75.72 ± 3.91 , 8.28 ± 0.39 , 6.17 ± 0.54 and 2.73 ± 0.01 mg/dl respectively. It is evident from the findings that internal health status of the animals in refinery area is affected sub clinically. Further studies should be carried out in detail in refinery areas to observe their other impact on animal health.

Study on the morphological and physiobiochemical changes in evergreen plants in the polluted areas of Thiruvananthapuram, Kerala

Jissy Jyothi S, Shaji PK* and Jaya DS

Department of Environmental Sciences, University of Kerala, Kariavattom and *Environmental Resource Research Centre, Peroorkada, Thiruvananthapuram, Kerala

Air pollution is a public health and environmental quality problem. Generally, there are more man made pollutants in the air near major cities and heavy industrial areas. Exposure of plants to air pollutants induces many changes in physiological and biochemical processes. The present study was conducted on evergreen plants *Alstonia scholaris*, R.Br., *Mangifera indica*, L. and *Polyalthia longifoli*, (Sonner). These plants are exposed to urban man-made pollutants in Thiruvananthapuram city. The morphological and physio-biochemical changes in the leaves of evergreen plants in the industrial area and in the high vehicular intensity areas, Palayam and Karamana of Thiruvananthapuram city were studied. *Alstonia scholaris*, R.Br. and *Mangifera indica*, L. showed slight chlorosis with chlorotic lesions. The air pollution tolerance index [APTII] of plants, which determines the tolerance as well as sensitivity levels of plants was evaluated by measuring the chlorophyll content, ascorbic acid content and relative water content in the leaves of the selected plants. The biochemical parameters like foliar protein, sulphate and malondialdehyde also showed changes in the evergreen plants due to various air pollution stresses. The study on the per oxidation changes in evergreen plants due to air pollution showed that there is considerable reduction in the foliar malondialdehyde content. It was also observed that there is accumulation of heavy metal, lead (Pb) in the leaves of some evergreen plants collected from heavy vehicular intensity areas of Thiruvananthapuram city.

Comparative study of the estimation of the acute oral toxicity in Wistar rats using OECD guidelines 420, 423 and 425

Ravi GS, Mohan Kumar SB, Prakash PJ and Nanjundappa K

Toxicology Department, Rallis Research Centre, Bangalore

Chemicals of different toxic levels i.e., hexaconazole (low toxic), chlorpyrifos (moderately toxic) and monocrotophos (highly toxic) were tested in Wistar rats as per the following OECD Guidelines: OECD 420-fixed dose procedure (FDP), OECD 423 acute toxic class method (ATCM) and OECD 425-up-and-down procedure (UDP). The suspension of each chemical in deionized water was administered once orally to rats fasted over night (16-18hours) at a dose volume of 5 ml/kg body weight and the animals were observed for 15 days. As per the results obtained from the present study, the chemicals are classified as per the globally harmonized Classification system as follows, hexaconazole: category 5, category 5 (>2000-5000), LD50 value greater than 2000 mg/kg, chlorpyrifos: category 4, category 4(>300-2000), estimated LD50 550 and monocrotophos: category 2, category 2 (>5-50) and estimated LD50 31 as per OECD 420, 423 and 425 guidelines respectively.

Modulatory effect of cyclophosphamide on urinary bladder antioxidants of mice

Kanchan Bhatia, Rehman H, Ali M, Manpreet Kaur, Atif F and Raisuddin S

Department of Medical Elementology and Toxicology, Jamia Hamdard (Hamdard University), New Delhi 110 062

Cyclophosphamide (CP), an alkylating agent is widely used in the chemotherapy of a variety of cancers. Its intake is associated with hematopoietic depression, nausea, vomiting, alopecia, hemorrhagic cystitis, cardiac damage, gonadotropy and carcinogenicity. CP induced urotoxicity and nephrotoxicity are also major cause of concern. The urological side effects of CP include dysuria, urinary frequency, hemorrhagic cystitis, bladder fibrosis, necrosis, contracture and vesico-metral flux and invasive bladder cancer. CP treatment also results in the formation of several reactive oxygen species (ROS), which result in per oxidative tissue damage. Role of

tissue antioxidants becomes important in prevention of such damage. In this study effect of sub chronic CP treatment on antioxidants of urinary bladder of mice was evaluated. Mice were treated with intraperitoneal semilog doses (5.6, 10, 18, 32 mg/kg bwt daily for 10 days) of CP showed a significant decrease in the activities of antioxidant enzymes viz, glutathione peroxidase (GPx), glutathione-s-transferase (GST), glutathione reductase (GR) and catalase (CAT) when compared to respective controls. Levels of reduced glutathione (GSH) were also decreased and a significant dose dependent increase was observed in lipid peroxidation (LPO). Thus, CP induced oxidative stress in the bladder and the antioxidant status was altered which is due to the presence of acrolein that is a toxic metabolic product of CP. Based on these findings, we now plan to evaluate efficacy of certain herbs used in traditional systems of medicine for their protective role against CP-induced oxidative stress and also for their antioxidant potential.

The use of Zoo kept birds as monitor for metal pollution in Jaipur, Rajasthan 302 004

Bhumesh Singh Badouria, Bakre PP, Pradeep Bhatnagar

Environmental Biology Laboratory, Deptt.of Zoology, University of Rajasthan, Jaipur. 302 004

Feathers of wild and domestic birds have been often used as an indicator tissue for pollutant metal analysis. Analysis of feathers gives indication with regard to the long-term heavy metal pollutant exposure of any place. The aim of present investigation is to draw a profile of metal pollution in feathers of Zoo kept birds. Jaipur Zoo is located in the Ram Niwas Bagh Complex, close to the walled city. Two important roads, which pass through the garden, are very close to the Zoo. Both roads have heavy automobile traffic density. Approximately 30,000 vehicles (mainly cars and two wheelers) pass daily emitting heavy automobile exhaust. The cages of bird section of Zoo are barely 100 feet away from the roads mentioned above. Thus the birds that are being kept in the cages are exposed to automobile exhaust fumes for at least 15 hours daily (the garden remains closed from 11pm to 8am). To ascertain the levels, to which Zoo birds are exposed to the exhaust, feathers were collected from all cages. All the samples are being analyzed for heavy metals with double beam atomic absorption spectrophotometer (AAS). The extent of environmental pollution caused by automobile exhaust in the area is discussed in the paper.

Toxicity studies of *Cassia spectabilis* DC in rabbits

Shridhar NB and Narayana K

Department of Pharmacology and Toxicology, Veterinary College, Hebbal, Bangalore-560024.

A deer that consumed the leaves of *Cassia spectabilis* DC died at Bangalore. To assess the toxic nature, 6 rabbits were fed 50 g/kg leaves of *Cassia spectabilis* for 5 days. On Day 5, all rabbits died. There was no change in blood serum creatinine, BUN and total protein concentrations in these rabbits. At necropsy, there was catarrhal gastroenteritis and pericardial petechia. Degenerative changes were noted in liver and myocardium. The present study indicates the toxic nature of this plant.

Effect of Ginkgo biloba alcoholic extract on hepatic oxidative stress and toxicity induced by sulphur mustard

Gautam Anshoo, Vijayaraghavan R, Pant SC, Kumar O, Singh Seema and Satish HT

DRDE, Gwalior-474002

Sulphur mustard is a chemical warfare agent of the blistering agent category. It is a frequently used as chemical warfare agent and is also a threat by the terrorist groups. SM forms sulphonium ion in the body that alkylates DNA and several other macromolecules, and induces oxidative stress. There are no satisfactory antidotes for SM toxicity. *Ginkgo biloba* has wide variety of flavanoids and other natural compounds, which has wide pharmacological actions and has been reported to reduce oxidative stress. This study was aimed to evaluate the protective effect of alcoholic extract of *Ginkgo biloba* against SM. Protective efficacy of *Ginkgo biloba* (500, 1000 and 2000 mg/kg, p.o) was studied by administering as three doses in Swiss mice against SM. The first dose was at simultaneous with SM exposure, two more doses on the frequent days. SM was administered (in PEG 300) percutaneously at varying doses for survival and protection studies. SM was also administered at a dose of LD50 (19.3 mg/kg) with and without *Ginkgo biloba* treatment and various biochemical markers were estimated 7 days after SM administration. The animals administered with SM died at various days depending upon the dose. The body weight decreased significantly. *Ginkgo biloba* protected the mice significantly, in a dose dependent manner. A significant decrease in reduced as well as oxidized glutathione and an increase in malonaldehyde, WBC count, RBC count and stress markers, catalase and glutathione reductase was recovered by *Ginkgo biloba* 2000 mg/kg doses significantly protected the biochemical markers because of the presence of

active flavanoids. The present study shows that percutaneous administration of SM induces oxidative stress and *Ginkgo biloba* can protect it.

Effect of long acting oxytetracycline on cell mediated immune status in rats

Ravikumar C*, Honnegowda, Jayakumar K, Prasad S, Krishnappa G and Narayana K

Deptt. of Pharmacology and Toxicology, Veterinary College, *Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore 560 024

The present study was conducted to evaluate the effect of long acting Oxytetracycline on non-specific immune status in Wistar albino rats. Inject able solution of long acting Oxytetracycline was used in the experiment. Rats were separated into four experimental groups consisting of ten animals with equal number of male and female rats in each group. Group I (saline control), Group II (pyrrolidone control), Group III (long acting Oxytetracycline low dose at 20mg/kg), Group IV (long acting Oxytetracycline high dose at 40mg/kg). The long acting Oxytetracycline was administered as single intra-muscular injection on day 1. Immunological parameters like total leucocyte count (TLC), absolute lymphocyte count (ALC), total serum protein (TSP), serum albumin (SA), total serum globulin concentration (TSG), albumin-globulin ratio (A: G), total serum immunoglobulin concentration (TIG), phagocytic index (PI) were estimated on day Zero (immediately before administering long acting oxytetracycline or vehicle) and then Day 1, 7, 14, 21, 28, 35 and 42 after administration of the drug. Dinitrochlorobenzene (DNCB) skin sensitivity test was also done. The parameters studied did not differ significantly ($P>0.05$) between long acting Oxytetracycline treated and control groups. From the present study it is concluded that, the long acting Oxytetracycline did not depress or stimulate non-specific immune status in rats

Effect of an anti-hypertensive compound 93/478 on peri and post-natal development in rats

Mathur SK, Sinha N, Singh G and Srivastava S

Division of Toxicology, Central Drug Research Institute, Lucknow

An anti hypertensive agent of a-blocker group, coded as 93/478, was developed at C.D.R.I. and studied for its effect on late gestation, parturition, lactation and post-partum growth of dams and their offsprings till weaning. The compound was administered to three groups of pregnant rats, each having 20 animals, at the daily oral doses of 15.0, 45.0 and 75 mg/kg body weight from 15th day of gestation through 21st day post-partum, as per OECD guidelines. Another group of 20 pregnant animals, which served as control, was run simultaneously and treated with vehicle for the same period. On careful observations and analysis of data, generated in the study, it was observed that the compound 93/478 did not exert any significant effect on the parameters, studied, except a few incidence of prolonged gestation among the dams of different groups and delayed post-partum growth parameters viz. opening of eyes and appearance of fur coat etc. in a few pups from different dams in various groups, including controls. Random neo-natal mortalities during, the weaning period, among the pups from dams of various groups were also recorded. The details of the results will be presented and discussed during the symposium.

Reproductive toxicity: pendimethalin

Ganiger S, Raghunath Reddy KR, Krishnappa H, Santhosh Kumar DP and Ramesh E

Toxicology Department, Rallis Research Centre, Bangalore-560 058

A two-generation reproduction toxicity study was carried with Pendimethalin in Wistar rats. Four groups comprising of one concurrent control and three doses (200, 1000 and 4000 ppm in food) were included in the study. Each group consisted 30 male and 30 female rats. The parameters like clinical signs, body weight and food consumption during different phases of reproduction, oestrous cycle length, pre-coital interval, gestation length, pups survivability, postnatal developmental observations, fertility indices of sires and dams, sperm evaluation, organ weights and organ weight ratios, gross pathological and histopathological findings in both the generations were evaluated as per OECD Guideline No.416, adopted on January 22, 2001. Daily administration of Pendimethalin at 4000 ppm dose level for two generations resulted in decrease in body weights and food consumption during different phases of reproduction in both the generations, mean number of pups in P generation and mean weight of pups in F1 generation. It also resulted in higher percentage of post implantation loss and lower percentage of live pups born in P generation. At 200 and 1000 ppm dose levels, there were no treatment related effects in the parental animals and no gross or microscopic changes observed in any of the organs and none of the reproductive parameters were affected and there were no effects on offspring. It was concluded that No Observed Effect Level (NOEL) for reproductive toxicity in rats in this study was 1000 ppm for two successive generations, which is equivalent to 66.1, and 98.2 mg/kg b wt/day for male rats and 96.0 and 114.6 mg/kg b wt/day for female rats for P and F1 generations, respectively.

Effects of pyrethroids on different microorganisms with special reference to mutagenic studies

Sonia Sethi, Pradeep Bhatnagar, Nupur Mathur

Environmental Toxicology Unit, Department of Zoology, University of Rajasthan

Pyrethrins are naturally occurring compounds with insecticidal properties that are found in pyrethrum extract from certain chrysanthemum flowers. The pyrethrins are often used in household insecticides and products on pests or livestock. Pyrethroids are manufactured chemicals that are very similar in structure to pyrethrins, but are often more toxic to insects as well as to mammals, and last longer in environment than the pyrethrins. Pyrethrins and Pyrethroids bind strongly to soil and are eventually degraded in soil and water. In the present work accumulation and degradation of Cypermethrin, Fenvalerate, Resmethrin and Tetramethrin by certain microorganisms were studied.

Effect of chloroform fraction of *Mucuna pruriens baker* on the reproductive organs and sperm count in albino rats

Chandrasekharan Nair AM and Gopakumar N

Veterinary College, Mannuthy, Thrissur, Kerala

The alcoholic extract from defatted powder of *Mucuna* seeds was fractionated with chloroform. The chloroform fraction was then fed to two batches of rats (T1&T2) at the rate of 0.088 and 0.176mg respectively/rat/day for 30 days. Another group (T3) was administered with testosterone propionate 0.15 mg /rat at five day intervals. A control was also kept without any drug (group C). From each group half the number of animals was sacrificed on 20th and 30th day of experiment. The body weight of the animals at the beginning and end of the experiment were recorded. The weight of the testicles, prostate and seminal vesicles, as well as total spermatozoa in the epididymis was also noted at the end of the experiment. The epididymis of the left and right testicles, cleaned of extraneous matter and taken in 1% sodium citrate solution, was squeezed thoroughly with a sharp blade and forceps for counting the spermatozoa. The spermatozoa count was taken using haemocytometer. The total spermatozoa count was significantly higher than the control in both the groups treated with *Mucuna* extract. However, both these treatments were found to be at par with that of testosterone treated group. Between the two doses of *Mucuna* tried, the lower dose of 0.088 mg showed the highest spermatozoa count. The histological examination of the testicles also supported this. No significant difference was observed between treatment durations of 20 and 30 days. The weight of the seminal vesicles and prostate of the treated groups also showed a significantly higher value than the control. However, in testosterone treated group the weight of testicle was reduced.

Flow cytometric (FACS) analysis of Tolbutamide induced apoptosis in rat embryos

Gyanendra Singh, Neeraj Sinha and Mathur SK

Division of Toxicology, Central Drug Research Institute, Lucknow-226001.

Apoptosis or programmed cell death is a genetically programmed, highly conserved, intricate mechanism of cellular suicide, that can be triggered by a variety of physio-logical & pathophysiological conditions and which plays a crucial role in the morphogenesis and appropriate functional connectivity in multicellular organism. A slight alteration in this process can result in birth defect. Literatures advocate the use of flow cytometric analysis as a wonderful tool to detect apoptosis. In this study, we have used flow cytometer to detect apoptosis that might have induced by Tolbutamide (Tb) while studying the teratogenicity of Tb by rat whole embryo culture at day 11. Our studies revealed the dose dependent increase in apoptosis when the cultured embryos were exposed to Tb *in vitro* at 10, 100 and 1000 mg /ml culture concentration. In cell cycle analysis, DNA histograms obtained from nuclei of 12th day embryo revealed a decrease in G0/G1 phase, a decrease in S phase and an increase in G2/M phase, which reflects the cell death by the process of PCD specifically. In addition to the determination of early apoptosis by the phosphatidylserine translocation with the Annexin-V assay, late apoptosis was determined by propidium iodide staining of isolated nuclei. At 24 hrs of treatment, increased number of sub G0 cells (indicator of apoptosis) appeared at all conc. however, their number at conc. 10mg/ml culture was low. Analysis of distribution of the cells across the cell cycle in control rat clearly indicates normal distribution of the cells. Giving *in vivo* single exposure of Tb to the 11th day embryos at dose levels 500 & 1000mg/kg b wt also confirmed this. The work will be discussed in details during the session.

Eighteen-month study on pendimethalin in Swiss albino mice

Krishnappa H, Malleappa HN, Vishwanath BN, Stanley A and Geetha Rajashekhar

Toxicology Department, Rallis Research Centre, Bangalore

An eighteen month study on Pendimethalin was carried out in Swiss albino mice. Four groups comprising of one concurrent control and three doses (100, 700 and 400ppm doses) were included in the study. Each group consisted of 50 male and 50 female mice. The parameters like clinical signs, growth, body weight, food consumption, examination of tumour or tumour prone tissues, haematology, ophthalmological examination, organ weights and their ratio's, necropsy and histopathological examination were carried out as per OECD Guideline No.451, adopted on 12th May, 1981. Daily oral administration of Pendimethalin at 4000ppm dose for 18 months resulted in significant decrease in body weight gains, food consumption and neutrophil percentage without appreciable changes in the other parameters. The Pendimethalin was found to be non-carcinogenic to Swiss Albino mice at all the doses tested. It was concluded that the evaluated No Observed Adverse Effect Level (NOAEL) for general effects of Pendimethalin in Swiss albino mice was 700 ppm in food which is equivalent to 108.8, 121.3 and 115.0 mg/kg b wt/day for males, females and combined sex, respectively.

Effect of molluscicides, Nicotinilide and Niclosamide on the osmoregulation of freshwater snail *Lymnaea luteola*, the vector of animal schistosomiasis.

Sukumaran D, Parashar BD, Prakash AO and Shriprakash.

DRDE Gwalior, School of Studies in Zoology, Jiwaji University, Gwalior 4764 06, India.

World Health Organization considers schistosomiasis as one of the major target diseases. Snails are known to act as intermediate host in transmitting both human and animal schistosomiasis. Molluscicides are the chemicals used to kill snails for controlling schistosomiasis. In India, freshwater snail *Lymnaea luteola* is known to transmit animal schistosomiasis and cercarial dermatitis in human. Studies were carried to find out the effect of molluscicides nicotinilide and niclosamide on osmoregulation balance of *L. luteola* snails. The snails were exposed to both molluscicides at different LC90 concentration for different durations till moribund stage. Organ/body weight ratio and wet/dry weight ratio of different parts of the snails was calculated to identify the specific portion of snails affected by these molluscicides. Changes in levels of sodium and potassium ions in haemolymph of *L. luteola* snails were also estimated. Results show that both nicotinilide and niclosamide affect mainly head-foot region and increase fluid accumulation in all tissues. A significant reduction was observed in levels of sodium and potassium ions in the haemolymph of the *L. luteola* snails exposed to LC90 concentration till moribund stage. The studies have indicated that both molluscicides play a vital role in disturbing osmoregulation of *L. luteola* snails leading to the accumulation of fluid in body tissues that leads to the mortality of snails. Nicotinilide is found more effective than niclosamide in disrupting the osmoregulation balance of the *L. luteola* snails.

Effect of the insect repellent, n, n- diethylphenyl lactamide on the respiration of mice.

Seema Singh, Anshoo Gautam, Shri prakash, Sekhar K and Vijayaraghavan R

Defense Research and Development Establishment, Gwalior

N, N- Diethylphenyl lactamide (DEPA) is a long acting and broad-spectrum insect repellent. The respiratory effect of DEPA aerosol was studied in mice, using a head only exposure assembly. DEPA was aerosolized using a compressed air nebuliser and mice were exposed for 4 h. The particle size of the aerosol was determined using a cascade impactor and the concentration was determined gas chromatographically. The respiration of the mice was monitored using an on line computer program, capable of recognizing the breathing pattern as sensory irritation, airway obstruction and pulmonary irritation. DEPA aerosols decreased the normal pattern of breathing and increased the breathing pattern characteristic of airway obstruction. The respiratory frequency and tidal volume decreased at a concentration of 0.43g/m³ of DEPA. There was no change in the respiratory frequency at 0.20g/m³ but the tidal volume decreased. The mice died only during exposure at high concentrations and there were no delayed deaths. The estimated LC50 of DEPA aerosols were 0.91g/m³. This study shows that DEPA is a moderately toxic chemical through inhalation and may cause airway obstruction after prolonged use.

Toxicity studies on *Diospyros montana* Roxb. on fish

Puttappa DR, Shridhar NB, Pradeep Kumar GB, Jayakumar K, Balgangadhar BR, Rao GPS and Narayana K

Department of Veterinary Pharmacology & Toxicology, Veterinary College, Bangalore-560024

Since time immemorial man has used various techniques to catch fish for food purpose. Besides modern technology many of these techniques are still followed in various parts of the world. *Diospyros montana* is one such plant used for catching fish by local folklore in Western Ghats especially in villages around Sagar taluk, Shimoga district, Karnataka. This study was conducted to evaluate fishicidal property of *Diospyros montana* in zebra-fish (*Brachydanio rerio*). Range finding test was conducted using fresh leaves where each concentration of 50, 25, 12.5, 6.25, 5, 2.5, 1 and 0.5g/L of fresh crushed leaves was tested on a single fish along with one control

without leaves. Fish were observed for 24 hrs and death if any, was recorded. After 24 hours fish survived only in the last two concentrations tested. For LC50 study concentration a range from 2.25 to 0.5g/L was considered. Five concentrations were employed for the study ie, 2.25, 2.0, 1.5, 1.0, and 0.5g/L. Each concentration was tested on 10 fishes in 4 liters of water and observed for 48hours. The mortality in different concentrations were as follows, 100% in 2.25 and 2.0g/L, 60% in 1.5g/L, 20% in 1g/L and no death in 0.5g/L. 1.37g/L was the LC50 obtained. In the present study *Diospyros montana* was found to be toxic to fish. Use of this plant as a good fishicide for controlling predatory fishes in shrimp culture and fisheries needs to be explored.

Toxicity studies of *Dichapetalum gelonioides* in rats

Jayanna E, Shridhar NB, Narayana K and Sathyanarayana ML

Department of Pharmacology and Toxicology, Veterinary College, University of Agricultural Sciences, Bangalore

Toxic nature of aqueous and methanol extracts of plant *Dichapetalum gelonioides* was evaluated in Wister albino rats. The aqueous extract of the leaves was given orally (n=6) at a dose starting from 0.5-16 g/kg b wt serially. To study the effect of methanol extract, the rats were divided into five groups (n=6). To group I-M (vehicle control) gum acacia suspension was given. The methanol extract was given orally at a dose of 2 (group II-M) or 3 (group III-M) or 4 (group IV-M) or 5 g/kg body weight (group V-M). The rats were observed for clinical signs and mortality for 14 days. No apparent clinical sign or mortality was noted in any of the rats given the aqueous extract. The methanol extract -rats showed dullness, frequent urination, jumping like frog, polypnoea and death. The phytochemical analysis of methanol extract of dried leaves revealed the presence of steroids, glucosides and terpenoids. The fluoride content in dried plant material was 0.005% by mass. In conclusion, methanol extract induced moderate toxicity and death in rats. The aqueous extract did not induce any toxicity. The oral LD50 of methanol extract was 3.5 g/kg body weight.

Acute toxicity of pendimethalin to the earthworm, *Eisenia foetida foetida*

Gireesh Kamath H, Indrani BK and Shivaram S

Rallis India Limited, Rallis Research Centre, Peenya Industrial Area, Bangalore 560 058

Earthworms (*Eisenia foetida foetida*) were exposed to different concentrations of technical grade pendimethalin in an artificial soil to determine the acute toxic effects. Lethal concentration (LC50) values for 14 days were determined after exposure. The toxic signs exhibited by the earthworms and mortality was recorded on the 7th and the 14th day post-exposure. Chloroacetamide was used as a positive control. The LC50 (14 day) of pendimethalin was found to be 4.19 mg/kg artificial soil (dry weight) with 95% confidence limits of 3.56 to 4.92 mg/kg artificial soil (dry weight). The NOEC (no observed effect concentration) was observed at less than 0.75 mg/kg artificial soil (dry weight).

Monitoring of pesticide burden in acute lymphoid leukemia (ALL) patients

Vijayalekshmi Gupta, Malhotra H, Pradeep Bhatnagar, John PJ, Inderpal Soni

Environmental Toxicology Unit, Department of Zoology, University of Rajasthan, SMS Medical College and Hospital, Jaipur 4, Rajasthan

The intensive use of persistent organochlorine insecticides both in agriculture and medical parasitology has lead to widespread contamination of the environment. Their residues are found at every level of the food chain. Human beings are placed at top of most of the food chains. Therefore, monitoring of organochlorine pesticide residues has always been important for assessment of human exposure to these pesticides and their relationship with the occurrence of certain diseases. Cancer experts believe that risk of haemolymphoid malignancies increases when the body's immune system gets affected or suppressed. Studies reveal that the pesticides especially organochlorines are immune-suppressor. The present study was, therefore, planned to study the correlation between the presence of pesticide residues in the human blood and occurrence of acute lymphoid leukemia. The blood of 25 patients suffering from ALL was collected and pesticides were extracted by method given by Bush *et al.* (1984). The extracted samples were analyzed by gas chromatograph for the presence of organochlorine pesticide residues. Besides this, detail information regarding the family history of any major disease, economic status, dietary habits, occupational exposure to pesticides etc. was also sought. The residues of DDT and its metabolites, aldrin, dieldrin, heptachlor, endosulfan and HCH & its isomers (a,β,γ,d) were found in the patients. An attempt has been made to establish correlation between pesticide burden and other factors (social/ economic) with the occurrence of ALL.

Evaluation of repeated dose 28 day oral administration of technical grade cypermethrin for target organ toxicity in male rats

Suhas YS, Jayakumar K, Sebastian VJ, Prasad S, Honnegowda and Narayana K

Department of Pharmacology and Toxicology, Veterinary College, UAS, Bangalore 560 024

Effect of repeated dose 28-day oral toxicity of technical grade cypermethrin was evaluated for target organ toxicity in male Wistar albino rats. Technical grade cypermethrin was administered to three test groups *viz.*, 3mg/kg body weight to group-II, 9mg/kg to group-III and 30mg/kg to group-IV. Group-I served as control group. The technical grade cypermethrin was dissolved in Tween-80 and water and administered orally daily for a period of 28 days. Blood samples were collected without adding any anticoagulant on Days 7, 14, 21 and 28 to study various serum biochemical parameters such as aspartate-amino-transferase (AST), alanine-amino-transferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), Creatinine (Creat), glucose (Glu) and total serum proteins (TSP). At the end of study on Day 28, the animals were weighed and humanely sacrificed for gross pathology and histopathological studies. There was a significant ($P < 0.05$) increase in serum Creatinine (Creat) and serum glucose (Glu) concentrations on Day 7, 14, 21 and 28. Animals did not show any gross pathological changes. The organ to body ratios of liver, spleen, kidneys, lungs, intestines and brain did not show any significant change. Histopathology of liver, kidneys and brain did not show any histopathological changes. Thus, from the present study it was concluded that technical grade cypermethrin at the doses and duration employed might be slightly nephrotoxic.

Investigations on the novel biological activities of plumbagin against insects

Kokate SD

Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Mumbai 85

Development of new botanicals and related synthetic compounds has prime position in integrated pest management. During the screening of several plants for bioactivity plumbagin from *Plumbago capensis* showed bioactivities against mosquito, *Culex pipiens quinquefasciatus* and cotton bug *Dysdercus koenigii*. A dose of 3ppm exhibited ovicidal action against the eggs of mosquito. Only 30% eggs could complete their embryogenesis at 2ppm dose level. Six ppm was found to be sufficient for 100% kill of larvae. Possession of hormonal activity was indicated by the development of few larval-pupae intermediates at low doses. Adults emerged out of the treated pupae at high doses died soon as compared to control. A dose of 10 ppm was required for 100% adult mortality. Adults showed significant knockdown property when released in treated interiorly glass flasks with plumbagin. Treatment of 0 - 4 h old *D. koenigii* adults with 5 to 10 μ g of plumbagin did not affect their mating or the frequency of egg laying. In both untreated as well as treated adults mating generally commenced within 24-48 h. The first batch of eggs was deposited within 6 to 7 days of adult emergence. Gross morphological deformities in testes as well as associated organs have been observed. Beaded ovaries were evident as early as 3-5 days after treatment with 5 μ g of this compound. However with advance in time, the ovaries assumed a clumped appearance suggesting some kind of abnormality.

Effect of curcumin and silymarin on chromium induced hepato-renal damage in rats

Joshua Allan, J., Venkateswaran, K.V., Balachandran, C1 and Kannappan, M.

Department of Pharmacology and Toxicology, Centralised Clinical Laboratory, Madras Veterinary College, Chennai-7

Environmental pollution causes public health concern and an attempt was made to find out the toxic effects of potassium dichromate, a hexavalent chromium compound due to its prominent role and to study the influence of Curcumin and Silymarin during its toxic manifestations. Potassium dichromate, a hexavalent form of chromium was orally administered to male albino Wistar rats at the dose of 10 mg/kg b wt of chromium for six weeks. Curcumin and Silymarin were given at the rate of 100 mg/kg and 20 mg/kg b wt simultaneously through oral route to evaluate their effects on chromium toxicity. At the end of six weeks animals were sacrificed. Liver was collected for chromium estimation, blood was collected for estimation of serum enzyme levels and liver and kidney were subjected to histopathological examination. In potassium dichromate treated group, the liver showed high chromium levels and there was marked damage to liver and kidney. Serum enzyme levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphates, lactate dehydrogenase and gamma glutamyl-S-transferase were significantly elevated. Curcumin and Silymarin when administered, individually and in combination with hexavalent chromium compound resulted in marked reduction in chromium concentration in liver, accompanied with reduced structural damage in liver and kidney and alleviated the enzyme levels during toxic induction. The effects were pronounced when the plant active principles were combined. The study revealed the hepato-renal protective effect of Curcumin and Silymarin from the deleterious effects of potassium dichromate.

Documentation of tissues in histopathology for regulatory toxicology

Yogananda moolemath

Department of zoology, City College, Bangalore- 560 082.

There is a need for a good documentation system because traceability and sampling of right tissue is important. In many regulatory toxicological studies the number of animals and tissues taken for histopathology evaluation is large. These tissues collected at necropsy would undergo a series of processes before its finally made into a slide. During the entire process there would not only be mixing up of tissues but also tissue loss. Unless a good documentation system is followed, which is simple, systematic and accurate.

Effects of acute oral toxicity of Haloquinol (Chlorohydroxyquinoline) in poultry broilers

Shiva Kumar, Kavitha Rani.B,D. Rajeevalochana, Brij Mohan and P.M.Radhakrishna

R&D Center, Vetcare, Yelahanka New Town, Bangalore-64

Acute oral toxicity of a non-antibiotic antimicrobial, Haloquinol (chlorohydroxyquinoline) was studied in poultry broilers. Haloquinol used in the present study is a broad spectrum antimicrobial having Antibacterial, Antifungal, and Antiprotozoal activity and commonly used in poultry rearing. Twelve groups of poultry broilers (aged 7 days) consisting of 10 birds in each group were used as one batch for estimating the LD 50 value in poultry broilers. The median lethal dose was estimated according to the method of Finney (1971) using Haloquinol BP 80. The oral LD 50 values determined in poultry broilers were 2183.33 mg/kg. The clinical signs of toxicity noted were inappetance, ruffling, and sleepy nature of the birds. which further manifested into anorexia, leg weakness and weight loss. The postmortem findings showed evidence of death due to rupture of liver. The other lesions were extensive subcutaneous hemorrhages on thighs & wings, clotted blood in the peritoneal cavity, ascites, rickets, pale musculature and fragile skeletal structures.