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Toxicity Testing: GLP Test Facility

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CSIR-IITR, Lucknow is the only multidisciplinary research institute in the field of toxicology in South East Asia with the motto:

"Safety to environment & health and service to industry".

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- Regulatory Toxicology
- Nanotherapeutics & Nanomaterial Toxicology
- Systems Toxicology & Health Risk Assessment

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- Indian Factories Act (Drinking Water)
- Bureau of Indian Standards (Synthetic Detergents)
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- CD-Strip for detection of butter yellow, an adulterant in edible oils
- Arsenic Detection Kit

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Guest Editorial

New paradigms in Regulatory Toxicology

Capacity building to test safety of new chemical entities, pharmaceuticals, cosmetics and agrochemicals for potential adverse effects on human health and environment has been the focus of regulatory toxicologists. In this process national and international guidelines have been developed to perform toxicity testing as per the standard protocols for greater reproducibility of results. As newer chemicals, smart materials and nano products are being developed, their safety assessment becomes extremely important as they find place not only in consumer products but impact the environment also. The challenge is also to generate safety data for traditionally used herbal medicines which may be a mixture of plant materials or herbo-mineral products. It is important to perform such studies under Good Laboratory Practice (GLP) norms developed by OECD. CSIR-IITR has the distinction of being the only CSIR laboratory to have GLP certification for toxicity testing. The toxicity tests being performed under the facility are acute toxicity, sub-acute toxicity, micronuclei assay, chromosomal aberration test and Environmental studies on aquatic and terrestrial organisms. A growing trend world-wide is to reduce and refine the use of animals. There is a paradigm shift in regulatory toxicology towards adoption of an integrated approach of safety assessment of chemicals/ products based on *in silico* studies, followed by confirmation using *in vitro* and *in vivo* studies. Extensive efforts are being made to replace use of animals for safety assessment studies. In this direction alternate to animal models are being developed and validated which can give specific information. Cell based assays; 2D and 3D cell cultures; organoids on chip; artificial skin etc are giving new direction and meaningful results. In the area of cosmetics, it has now become mandatory to use alternate to animal models for testing their safety. Automated high throughput screens have reduced use of animals and empowered the toxicologists to generate safety data in short time. Thus, as with industrialization newer chemicals are being introduced, regulatory toxicologists are empowered today with a plethora of information and new tools to generate quick and reliable safety data.

P Kakkar

CSIR-IITR Research Highlights

Systems Toxicology and Health Risk Assessment

Photosensitized rose bengal-induced phototoxicity on human melanoma cell line under natural sunlight exposure

Srivastav AK, Mujtaba SF, Dwivedi A, Amar SK, Goyal S, Verma A, Kushwaha HN, Chaturvedi RK, Ray RS. J Photochem Photobiol B. 2016 Mar;156:87-99.

Rose Bengal (RB) is an anionic water-soluble xanthene dye, which used for many years to assess eye cornea and conjunctiva damage. RB showed strong absorption maxima (λmax) under visible light followed by UV-B and UV-A. RB under sunlight exposure showed a time-dependent photodegradation. Results show that photosensitized RB generates $^1O_2$ via Type-II photodynamic pathway and induced DNA damage under sunlight/UV-R exposure. 2'dGuO degradation, micronuclei formation, and single- and double-strand breakage were the outcome of photogenotoxicity caused by RB. Quenching studies with NaN_3 advocate the involvement of $^1O_2$ in RB photogenotoxicity. RB induced linoleic acid photoperoxidation, which was parallel to $^1O_2$-mediated DNA damage. Oxidative stress in A375 cell line (human melanoma cell line) was detected through DCF-DA assay. Photosensitized RB decreased maximum cellular viability under sunlight followed by UV-B and UV-A exposures. Apoptosis was detected as a pattern of cell death through the increased of caspase-3 activity, decreased mitochondrial membrane potential, and PS translocation through inner to outer plasma membrane. Increased cytosolic levels of Bax also advocate the apoptotic cell death. Authors propose a p53-mediated apoptosis via increased expression of Bax gene and protein. Thus, the exact mechanism behind RB phototoxicity was the involvement of $^1O_2$, which induced oxidative stress-mediated DNA and membrane damage, finally apoptotic cell death under

natural sunlight exposure. The study suggests that after the use of RB, sunlight exposure may avoid to prevent from its harmful effects.

Increased serum urea and creatinine levels correlate with decreased retinal nerve fibre layer thickness in diabetic retinopathy

Srivastav K, Saxena S, Mahdi AA, Kruzliak P, Khanna VK. Biomarkers. 2015;20(6-7):470-3

Correlation of increased levels of serum urea and creatinine with retinal nerve fibre layer (RNFL) thinning on spectral domain optical coherence tomography (SD-OCT) was studied in diabetic retinopathy (DR). Sixty consecutive cases and 20 healthy controls were included. Cases were divided into three groups: without DR, non-proliferative DR with macular oedema and proliferative DR with oedema. Serum urea and creatinine were measured using a standard protocol. Average (RNFL) was measured using SD-OCT. Increased severity of DR was associated with decrease in levels of serum urea and serum creatinine levels. RNFL thinning correlated positively with increase in serum urea and creatinine levels.

Benzophenone 1 induced photogenotoxicity and apoptosis via release of cytochrome c and Smac/DIABLO at environmental UV radiation

Amar SK, Goyal S, Dubey D, Srivastav AK, Chopra D, Singh J, Shankar J, Chaturvedi RK, Ray RS. Toxicol Lett. 2015 Dec 15;239(3):182-93.

Solar UV radiation is main factor of photocarcinogenesis, photoageing, and phototoxicity; thus, protection from UV radiation is major concern. Sunscreens containing UV filters are suggested as sun safe practices, but safety of UV filters remains in controversies. Benzophenone-1 (BP1) is commonly used in sunscreens as UV blocker. Authors assessed the photogenotoxicity and apoptotic parameters in human keratinocytes (HaCaT cells) by western blot, immunocyt-ochemistry, flowcytometry, comet assay and TEM imaging. The results exposed that BP1 photosensitized and generated intracellular ROS (2.02 folds) under sunlight/UVR. Decrease in cell viability was recorded as 80.06%, 60.98% and 56.24% under sunlight, UVA and UVB, respectively. Genotoxic potential of BP1 was confirmed through photomicronuclei and CPDs formation. BP1 enhanced lipid peroxidation and leakage of LDH enzyme (61.7%). Apoptotic cells were detected by AnnexinV/PI staining and sub G1 population of cell cycle. BP1 induced up regulation of apoptotic proteins Bax/Bcl2 ratio, Apaf-1, cytochrome c, Smac/DIABLO and cleaved caspase 3 was noticed. Down regulation of pro caspase 3 was inhibited by Z-VAD-fmk (inhibitor of caspase). Thus, study established the involvement of BP1 in photogenotoxicity and apoptosis via release of cytochrome c and Smac/DIABLO. These findings suggest sunscreen user to avoid BP1 in cosmetics preparation for its topical application.

Ethosuximide induces hippocampal neurogenesis and reverses cognitive deficits in an amyloid-β toxin-induced Alzheimer rat model via the phosphatidylinositol 3-kinase (PI3K)/Akt/Wnt/β-catenin pathway

Tiwari SK, Seth B, Agarwal S, Yadav A, Karmakar M, Gupta SK, Choubey V, Sharma A, Chaturvedi RK. J Biol Chem. 2015 Nov 20;290(47):28540-58.

Neurogenesis involves generation of new neurons through finely tuned multistep processes, such as neural stem cell (NSC) proliferation, migration, differentiation, and integration into existing neuronal circuitry in the dentate gyrus of the hippocampus and subventricular zone. Adult hippocampal neurogenesis is involved in cognitive functions and altered in various neurodegenerative disorders, including Alzheimer disease (AD). Ethosuximide (ETH), an anticonvulsant drug is used for the treatment of epileptic seizures. However, the effects of ETH on adult hippocampal neurogenesis and the underlying cellular and molecular mechanism(s) are yet unexplored. Herein, authors studied the effects of ETH on rat multipotent NSC proliferation and neuronal differentiation and adult hippocampal neurogenesis in an amyloid β (Aβ) toxin-induced rat model of AD-like phenotypes. ETH potently induced NSC proliferation and neuronal differentiation in the hippocampus-derived NSC *in vitro*. ETH enhanced NSC proliferation and neuronal differentiation and reduced Aβ toxin-mediated toxicity and neurodegeneration, leading to behavioral recovery in the rat AD model. ETH inhibited Aβ-mediated suppression of neurogenic and Akt/Wnt/β-catenin pathway gene expression in the hippocampus. ETH activated the PI3K·Akt and Wnt·β-catenin transduction pathways that are known to be involved in the regulation of neurogenesis. Inhibition of the PI3K·Akt and Wnt·β-catenin pathways effectively blocked the mitogenic and neurogenic effects of ETH. *In silico* molecular target prediction docking studies suggest that ETH interacts with Akt, Dkk-1, and GSK-3β. Findings suggest that ETH stimulates NSC proliferation and differentiation *in vitro* and adult hippocampal neurogenesis via the PI3K·Akt and Wnt·β-catenin signaling.

Expression of CCK receptors in carcinoma gallbladder and cholelithiasis: a pilot study

Faridi MS, Jaiswal MS, Goel SK. J Clin Diagn Res. 2015 Jul;9(7):PC04-7.

Gastrin and cholecystokinin (CCK) receptors are trophic for various gastrointestinal malignancies. Their role in gallbladder cancer has not been widely studied. To identify expression of CCK-A and CCK-B receptors in the tissue and blood of patients suffering from

carcinoma (CA) gallbladder and gallstone disease and to compare expression of CCK A and B receptors in the gall bladder tissue and blood of healthy individuals and patients of CA gallbladder, and gallstone diseases. Forty nine subjects of both genders were recruited, comprising of 22 patients of CA gall bladder, 19 cases of cholelithiasis and, 8 normal gallbladders obtained from patients operated for trauma of the biliary system or Whipple's procedure. RNA extraction and cDNA formation for CCK-A and CCK-B receptors were carried out. Real Time PCR was performed on cDNA and threshold cycle (Ct) value of each sample was obtained and ΔCt was calculated. Chi-square test for comparing two groups and ANOVA test for comparing multiple groups were applied and if $p<0.05$ then Dunnett-C test was performed. Both CCK-A and CCK-B receptors were expressed irrespective of its origin in all tissues and blood samples studied; be it normal, Cholelithiasis or CA gallbladder and there was no difference among them ($p>0.05$). This preliminary study showed higher expression of CCK-A receptors in patients of cholelithiasis and decreased expression of CCK-A receptors in patients of CA gallbladder as compared to normal gallbladder although it did not rise to statistical significance.

Similarities in lindane induced alterations in protein expression profiling in different brain regions with neurodegenerative diseases

Mudawal A, Singh A, Yadav S, Mishra M, Singh PK, Chandravanshi LP, Mishra J, Khanna VK, Bandyopadhyay S, Parmar D. Proteomics. 2015 Nov;15(22):3875-82.

Previous studies have reported that lindane, an organochlorine pesticide induces oxidative stress in rat brain that may lead to neurodegeneration. However, as the proteins involved in lindane induced neurodegeneration are yet to be identified, the present study aims to identify the proteins that may regulate lindane induced neurotoxicity. The data showed that repeated exposure of lindane (2.5 mg/kg) for 21 days to adult rats significantly increased the reactive oxygen species and lipid peroxidation in different brain regions. Proteomic study revealed that lindane induces major dysregulation in the ubiquitin proteasome pathway. Alterations in the expression of molecular chaperones in brain regions and an increase in the expression of α-synuclein in substantia-nigra and corpus-striatum and amyloid precursor protein in hippocampus and frontal-cortex suggests the accumulation of proteins in these brain regions. Western blotting also revealed alterations in the dopaminergic and cholinergic pathways in hippocampus and substantia-nigra isolated from lindane treated rats. Neurobehavioural data indicating alterations in learning and working memory, conditioned avoidance response and motor function, supports the proteomic data. The data suggest that repeated exposure of lindane to adult rats induces alterations, which are similar to that seen in neurodegenerative diseases.

Noncoding RNAs: possible players in the development of fluorosis

Daiwile AP, Sivanesan S, Izzotti A, Bafana A, Naoghare PK, Arrigo P, Purohit HJ, Parmar D, Kannan K . Biomed Res Int. 2015;2015:274852.

Fluorosis is caused by excess of fluoride intake over a long period of time. Aberrant change in the Runt-related transcription factor 2 (RUNX2) mediated signaling cascade is one of the decisive steps during the pathogenesis of fluorosis. Up to date, role of fluoride on the epigenetic alterations is not studied. In the present study, global expression profiling of short noncoding RNAs, in particular miRNAs and snoRNAs, was carried out in sodium fluoride (NaF) treated human osteosarcoma (HOS) cells to understand their possible role in the development of fluorosis. qPCR and *in silico* hybridization revealed that miR-124 and miR-155 can be directly involved in the transcriptional regulation of Runt-related transcription factor 2 (RUNX2) and receptor activator of nuclear factor κ-B ligand (RANKL) genes. Compared to control, C/D box analysis revealed marked elevation in the number of UG dinucleotides and D-box sequences in NaF exposed HOS cells. Herein, authors report miR-124 and miR-155 as the new possible players involved in the development of fluorosis. Authors report that the alterations in UG dinucleotides and D-box sequences of snoRNAs could be due to NaF exposure.

Involvement of cathepsin B in mitochondrial apoptosis by p-phenylenediamine under ambient UV radiation

Goyal S, Amar SK, Dubey D, Pal MK, Singh J, Verma A, Kushwaha HN, Ray RS. J Hazard Mater. 2015 300:415-25.

Paraphenylenediamine (PPD), a derivative of paranitroaniline has been most commonly used as an ingredient of oxidative hair dye and permanent tattoos. Authors have studied the phototoxic potential of PPD under ambient ultraviolet radiation. PPD is photodegraded and form a novel photoproduct under UV exposure. PPD shows a concentration dependent decrease in cell viability of human Keratinocyte cells (HaCaT) through MTT and NRU test. Significant intracellular ROS generation was measured by DCFDA assay. It caused an oxidative DNA damage via single stranded DNA breaks, micronuclei and CPD formation. Both lysosome and mitochondria is main target for PPD induced apoptosis which was proved through lysosomal destabilization and release of cathepsin B by immunofluorescence, real time PCR and western blot analysis. Cathepsin B process BID to active tBID which induces the release of cytochrome C

from mitochondria. Mitochondrial depolarization was reported through transmission electron microscopy. The cathepsin inhibitor reduced the release of cytochrome C in PPD treated cells. Thus study suggests that PPD leads to apoptosis via the involvement of lysosome and mitochondria both under ambient UV radiation. Therefore, photosensitizing nature of hair dye ingredients should be tested before coming to market as a cosmetic product for the safety of human beings.

Activation of autophagic flux against xenoestrogen bisphenol-a-induced hippocampal neurodegeneration via AMP kinase (AMPK)/mammalian target of rapamycin (mTOR) pathways

Agarwal S, Tiwari SK, Seth B, Yadav A, Singh A, Mudawal A, Chauhan LK, Gupta SK, Choubey V, Tripathi A, Kumar A, Ray RS, Shukla S, Parmar D, Chaturvedi RK. J Biol Chem. 2015; 290(34):21163-84.

The human health hazards related to persisting use of bisphenol-A (BPA) are well documented. BPA-induced neurotoxicity occurs with the generation of oxidative stress, neurodegeneration, and cognitive dysfunctions. However, the cellular and molecular mechanism(s) of the effects of BPA on autophagy and association with oxidative stress and apoptosis are still elusive. Authors observed that BPA exposure during the early postnatal period enhanced the expression and the levels of autophagy genes/proteins. BPA treatment in the presence of bafilomycin A1 increased the levels of LC3-II and SQSTM1 and also potentiated GFP-LC3 puncta index in GFP-LC3-transfected hippocampal neural stem cell-derived neurons. BPA-induced generation of reactive oxygen species and apoptosis were mitigated by a pharmacological activator of autophagy (rapamycin). Pharmacological (wortmannin and bafilomycin A1) and genetic (beclin siRNA) inhibition of autophagy aggravated BPA neurotoxicity. Activation of autophagy against BPA resulted in intracellular energy sensor AMP kinase (AMPK) activation, increased phosphorylation of raptor and acetyl-CoA carboxylase, and decreased phosphorylation of ULK1 (Ser-757), and silencing of AMPK exacerbated BPA neurotoxicity. Conversely, BPA exposure down-regulated the mammalian target of rapamycin (mTOR) pathway by phosphorylation of raptor as a transient cell's compensatory mechanism to preserve cellular energy pool. Moreover, silencing of mTOR enhanced autophagy, which further alleviated BPA-induced reactive oxygen species generation and apoptosis. BPA-mediated neurotoxicity also resulted in mitochondrial loss, bioenergetic deficits, and increased PARKIN mitochondrial translocation, suggesting enhanced mitophagy. These results suggest implication of autophagy against BPA-mediated neurodegeneration through involvement of AMPK and mTOR pathways. Hence, autophagy, which arbitrates cell survival and demise during stress conditions, requires further assessment to be established as a biomarker of xenoestrogen exposure.

Association of resistin with insulin resistance and factors of metabolic syndrome in north Indians

Singh AK, Tiwari S, Gupta A, Shukla KK, Chhabra KG, Pandey A, Pant AB. Indian J Clin Biochem. 2015; 30(3):255-62.

Metabolic syndrome (MetS) is a cluster of interrelated common clinical disorders. The role of resistin in insulin sensitivity and MetS is controversial till date. So, the aim of the present study was to investigate the relationship of plasma resistin levels with markers of the MetS in Indian subjects. In a case control study, total 528 subjects were selected for the study. 265 (194 male and 71 female) were cases (with MetS) and 263 (164 male and 99 female) were controls (without MetS). Required anthropometric measurements and calculations were carried out accordingly. All the Biochemical estimations were carried out according to standard protocol. Resistin level was measured by the standard protocol (By ELISA i.e. enzyme linked immunosorbent assay) as illustrated in the kit. Insulin level was also measured by the standard protocol as illustrated in the kit and insulin resistance was calculated by the standard procedures. Plasma resistin levels were significantly higher in cases compared with controls (male = 13.05 ± 4.31 vs. 7.04 ± 2.09 ng/ml; $p \leq 0.001$ and female = 13.53 ± 4.14 vs. 7.42 ± 2.30 ng/ml; $p \leq 0.001$). Plasma resistin levels were well correlated with waist circumference, glucose, triglycerides, waist/hip ratio, systolic and diastolic blood pressure, high density lipoprotein, total cholesterol, serum low density lipoprotein, serum very low density lipoprotein, insulin and insulin resistance. Plasma resistin levels were elevated in presence of the MetS and were associated with increased metabolic risk factors.

Inhibitory effects of bisphenol-a on neural stem cells proliferation and differentiation in the rat brain are dependent on Wnt/β-catenin pathway

Tiwari SK, Agarwal S, Seth B, Yadav A, Ray RS, Mishra VN, Chaturvedi RK. Mol Neurobiol. 2015; 52(3):1735-57.

Neurogenesis, a process of generation of new neurons, occurs throughout the life in the hippocampus and sub-ventricular zone (SVZ). Bisphenol-A (BPA), an endocrine disrupter used as surface coating for packaged food cans, injures the developing and adult brain. However, the effects of BPA on neurogenesis and underlying cellular and molecular mechanism(s) are still unknown. Herein, authors studied the effect(s) of prenatal and early postnatal exposure of low dose BPA on Wnt/β-catenin signaling pathway that controls

different steps of neurogenesis such as neural stem cell (NSC) proliferation and neuronal differentiation. Pregnant rats were treated with 4, 40, and 400 µg BPA/kg body weight orally daily from gestational day 6 to postnatal day 21. Both *in vivo* and *in vitro* studies showed that BPA alters NSC proliferation and differentiation. BPA impaired NSC proliferation (5'-bromo-2'-deoxyuridine (BrdU(+)) and nestin(+) cells) and neuronal differentiation (BrdU/doublecortin(+) and BrdU/neuronal nuclei (NeuN(+)) cells) in the hippocampus and SVZ as compared to control. It significantly altered expression/protein levels of neurogenic genes and the Wnt pathway genes in the hippocampus. BPA reduced cellular β-catenin and p-GSK-3β levels and decreased β-catenin nuclear translocation, and cyclin-D1 and TCF/LEF promoter luciferase activity. Specific activation and blockage of the Wnt pathway suggested involvement of this pathway in BPA-mediated inhibition of neurogenesis. Further, blockage of GSK-3β activity by SB415286 and GSK-3β small interfering RNA (siRNA) attenuated BPA-induced down regulation of neurogenesis. Overall, these results suggest significant inhibitory effects of BPA on NSC proliferation and differentiation in the rat via the Wnt/β-catenin signaling pathway.

Cyclosporine a and MnTMPyP alleviate α-synuclein expression and aggregation in cypermethrin-induced parkinsonism

Agrawal S, Dixit A, Singh A, Tripathi P, Singh D, Patel DK, Singh MP. Mol Neurobiol. 2015; 52(3):1619-28.

Cypermethrin induces the mitochondrial dysfunction and oxidative damage to the nigrostriatal dopaminergic neurons leading to parkinsonism in rats. Despite α-synuclein aggregation is reported to be critical in Parkinson's disease, its role and alliance with the mitochondrial dysfunction and oxidative damage leading to cypermethrin-induced parkinsonism have not yet been deciphered. The present study aimed to examine the effect of cypermethrin on the expression and aggregation of α-synuclein and its subsequent connection with oxidative damage and mitochondrial dysfunction leading to the nigrostriatal dopaminergic neurodegeneration in the presence or absence of a mitochondrial membrane transition pore opening inhibitor, cyclosporine A and a superoxide dismutase/catalase mimetic, manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride (MnTMPyP). The expression of α-synuclein, 3-nitrotyrosine (3-NT), 4-hydroxynonenal (4-HNE)-modified proteins, mitochondrial dysfunction-dependent apoptotic proteins, nitrite content, lipid peroxidation (LPO) and number of tyrosine hydroxylase (TH)-positive neurons were estimated in the substantia nigra and dopamine content in the striatum of control and treated rats employing standard procedures. Cypermethrin augmented the expression of α-synuclein, 3-NT, 4-HNE-modified proteins, caspase-3, mitochondrial Bax and cytosolic cytochrome-c along with nitrite and LPO and reduced the expression of cytosolic Bax, mitochondrial cytochrome-c, dopamine and number of TH-positive neurons. Cyclosporine A or MnTMPyP alleviated the expression and aggregation of α-synuclein along with indicators of the mitochondrial dysfunction, oxidative damage and dopaminergic neurodegeneration. The results demonstrate that cypermethrin induces α-synuclein expression and aggregation while cyclosporine A or MnTMPyP rescues from α-synuclein over-expression and aggregation along with the mitochondrial dysfunction and oxidative damage leading to parkinsonism in rats.

Lead intoxication synergies of the ethanol-induced toxic responses in neuronal cells--PC12

Kumar V, Tripathi VK, Jahan S, Agrawal M, Pandey A, Khanna VK, Pant AB. Mol Neurobiol. 2015; 52(3):1504-20.

Lead (Pb)-induced neurodegeneration and its link with widespread neurobehavioral changes are well documented. Experimental evidences suggest that ethanol could enhance the absorption of metals in the body, and alcohol consumption may increase the susceptibility to metal intoxication in the brain. However, the underlying mechanism of ethanol action in affecting metal toxicity in brain cells is poorly understood. Thus, an attempt was made to investigate the modulatory effect of ethanol on Pb intoxication in PC12 cells, a rat pheochromocytoma. Cells were co-exposed to biological safe doses of Pb (10 µM) and ethanol (200 mM), and data were compared to the response of cells which received independent exposure to these chemicals at similar doses. Ethanol (200 mM) exposure significantly aggravated the Pb-induced alterations in the end points associated with oxidative stress and apoptosis. The finding confirms the involvement of reactive oxygen species (ROS)-mediated oxidative stress, and impairment of mitochondrial membrane potential, which subsequently facilitate the translocation of triggering proteins between cytoplasm and mitochondria. Authors further confirmed the apoptotic changes due to induction of mitochondria-mediated caspase cascade. These cellular changes were found to recover significantly, if the cells are exposed to N-acetyl cysteine (NAC), a known antioxidant. Data suggest that ethanol may potentiate Pb-induced cellular damage in brain cells, but such damaging effects could be recovered by inhibition of ROS generation. These results open up further possibilities for the design of new therapeutics based on antioxidants to prevent neurodegeneration and associated health problems.

Food, Drug and Chemical Toxicolgy

Sulfur alleviates arsenic toxicity by reducing its accumulation and modulating proteome, amino acids and thiol metabolism in rice leaves

Dixit G, Singh AP, Kumar A, Dwivedi S, Deeba F, Kumar S, Suman S, Adhikari B, Shukla Y, Trivedi PK, Pandey V, Tripathi RD. Sci Rep. 2015; 5:16205.

Arsenic (As) contamination of water is a global concern and rice consumption is the biggest dietary exposure to human posing carcinogenic risks, predominantly in Asia. Sulfur (S) is involved in di-sulfide linkage in many proteins and plays crucial role in As detoxification. Present study explores role of variable S supply on rice leaf proteome, its inclination towards amino acids (AA) profile and non protein thiols under arsenite exposure. Analysis of 282 detected proteins on 2-DE gel revealed 113 differentially expressed proteins, out of which 80 were identified by MALDI-TOF-TOF. The identified proteins were mostly involved in glycolysis, TCA cycle, AA biosynthesis, photosynthesis, protein metabolism, stress and energy metabolism. Among these, glycolytic enzymes play a major role in AA biosynthesis that leads to change in AAs profiling. Proteins of glycolytic pathway, photosynthesis and energy metabolism were also validated by western blot analysis. Conclusively S supplementation reduced the As accumulation in shoot positively skewed thiol metabolism and glycolysis towards AA accumulation under AsIII stress.

Suppression in PHLPP2 induction by morin promotes Nrf2-regulated cellular defenses against oxidative injury to primary rat hepatocytes

Rizvi F, Mathur A, Krishna S, Siddiqi MI, Kakkar P. Redox Biol. 2015; 6:587-98.

Recent advances indicate a possible role of phytochemicals as modulatory factors in signaling pathways. Authors have previously demonstrated PHLPP2-mediated suppression of Nrf2 responses during oxidant attack. The present study was designed to explore Nrf2-potentiating mechanism of morin, a flavonol, via its possible role in intervening PHLPP2-regulated Akt/GSK3β/Fyn kinase axis. Efficacy of morin was evaluated against oxidative stress-mediated damage to primary hepatocytes by tert-butyl hydroperoxide (tBHP) and acetaminophen. The anti-cytotoxic effects of morin were found to be a consequence of fortification of Nrf2-regulated antioxidant defenses since morin failed to sustain activities of redox enzyme in Nrf2 silenced hepatocytes. Morin promoted Nrf2 stability and its nuclear retention by possibly modulating PHLPP2 activity which subdues cellular Nrf2 responses by activating Fyn kinase. Pull-down assay using morin-conjugated beads indicated the binding affinity of morin towards PHLPP2. Molecular docking also revealed the propensity of morin to occupy the active site of PHLPP2 enzyme. Thus, dietary phytochemical morin was observed to counteract oxidant-induced hepatocellular damage by promoting Nrf2-regulated transcriptional induction. The findings support the novel role of morin in potentiating Nrf2 responses by limiting PHLPP2 and hence Fyn kinase activation. Therefore, morin may be exploited in developing novel therapeutic strategy aimed at enhancing Nrf2 responses.

Expression of CCK receptors in carcinoma gallbladder and cholelithiasis: a pilot study

Faridi MS, Jaiswal MS, Goel SK. J Clin Diagn Res. 2015; 9(7):PC04-7.

Gastrin and cholecystokinin (CCK) receptors are trophic for various gastrointestinal malignancies. Their role in gallbladder cancer has not been widely studied. To identify expression of CCK-A and CCK-B receptors in the tissue and blood of patients suffering from carcinoma (CA) gallbladder and gallstone disease and to compare expression of CCK A and B receptors in the gall bladder tissue and blood of healthy individuals and patients of CA gallbladder, and gallstone diseases. Forty nine subjects of both genders were recruited, comprising of 22 patients of CA gall bladder, 19 cases of cholelithiasis and, 8 normal gallbladders obtained from patients operated for trauma of the biliary system or Whipple's procedure. RNA extraction and cDNA formation for CCK-A and CCK-B receptors were carried out. Real Time PCR was performed on cDNA and threshold cycle (Ct) value of each sample was obtained and ΔCt was calculated. Chi-square test for comparing two groups and ANOVA test for comparing multiple groups were applied and if $p<0.05$ then Dunnett-C test was performed. Both CCK-A and CCK-B receptors were expressed irrespective of its origin in all tissues and blood samples studied; be it normal, Cholelithiasis or CA gallbladder and there was no difference among them ($p>0.05$). This preliminary study showed higher expression of CCK-A receptors in patients of

cholelithiasis and decreased expression of CCK-A receptors in patients of CA gallbladder as compared to normal gallbladder although it did not rise to statistical significance.

Circulating miRNAs revealed as surrogate molecular signatures for the early detection of breast cancer

Mishra S, Srivastava AK, Suman S, Kumar V, Shukla Y. Cancer Lett. 2015; 369(1):67-75.

The miRNAs have well studied roles in cancer. Here, authors identified altered miRNA expression by global miRNA profiling in peripheral blood mononuclear cells (PBMCs) of breast cancer (n = 15) and healthy subjects (n = 15), and further validated the selected miRNAs in PBMCs (n = 45), blood plasma (n = 45) and breast tissue samples (n = 09). The expression of altered miRNAs was also evaluated in PBMCs among early stage (n = 32), advanced stage (n = 13), triple positive (n = 5) and triple negative (n = 5) breast cancer patients. Results showed differential pattern of expressions of these miRNAs in multiple cohorts, however in early stage breast cancer, miR-106a-5p and miR-454-3p were upregulated ($p < 0.05$), miR-195-5p and miR-495 were downregulated ($p < 0.05$) in PBMCs. In addition, these miRNAs were also significantly associated with cancer and ErbB signaling pathways. Multiple regression analysis and receiver-operative curve (ROC) analysis of miR-195-5p and miR-495 with area under curve (AUC) of 0.901 showed best discriminating combination for early stage breast cancer detection. In summary, the present study delineated the importance of miR-195-5p and miR-495 miRNAs as prospective circulating surrogate molecular signatures for early detection of breast cancer.

Differential susceptibility of brain regions to tributyltin chloride toxicity

Mitra S, Siddiqui WA, Khandelwal S. Environ Toxicol. 2015; 30(12):1393-405.

Tributyltin (TBT), a well-known endocrine disruptor, is an omnipresent environmental pollutant and is explicitly used in many industrial applications. Previously authors have shown its neurotoxic potential on cerebral cortex of male Wistar rats. As the effect of TBT on other brain regions is not known, authors planned this study to evaluate its effect on four brain regions (cerebellum, hippocampus, hypothalamus, and striatum). Four-week-old male Wistar rats were gavaged with a single dose of TBT-chloride (TBTC) (10, 20, and 30 mg/kg) and sacrificed on days 3 and 7, respectively. Effect of TBTC on blood-brain barrier (BBB) permeability and tin (Sn) accumulation were measured. Oxidative stress indexes such as reactive oxygen species (ROS), reduced and oxidized glutathione (GSH/GSSG) ratio, lipid peroxidation, and protein carbonylation were analyzed as they play an imperative role in various neuropathological conditions. Since metal catalyzed reactions are a major source of oxidant generation, levels of essential metals like iron (Fe), zinc (Zn), and calcium (Ca) were estimated. Authors found that TBTC disrupted BBB and increased Sn accumulation, both of which appear significantly correlated. Altered metal homeostasis and ROS generation accompanied by elevated lipid peroxidation and protein carbonylation indicated oxidative damage which appeared more pronounced in the striatum than in cerebellum, hippocampus, and hypothalamus. This could be associated to the depleted GSH levels in striatum. These results suggest that striatum is more susceptible to TBTC induced oxidative damage as compared with other brain regions under study.

Environmental Toxicology

Novel LinA type 3 δ-hexachlorocyclohexane dehydrochlorinase

Shrivastava N, Prokop Z, Kumar A. Appl Environ Microbiol. 2015; 81(21):7553-9.

LinA is the first enzyme of the microbial degradation pathway of a chlorinated insecticide, hexachlorocyclohexane (HCH), and mediates the dehydrochlorination of α-, γ-, and δ-HCH. Its two variants, LinA type 1 and LinA type 2, which differ at 10 out of 156 amino acid residues, have been described. Their activities for the metabolism of different HCH isomers differ considerably but overall are high for γ-HCH, moderate for α-HCH, low for δ-HCH, and lacking for β-HCH. Here, authors describe the characterization of a new variant of this enzyme, LinA type 3, whose gene was identified from the metagenome of an HCH-contaminated soil sample. Its deduced primary structure in the region spanning amino acid residues 1 to 147 of the protein exhibits

17 and 12 differences from LinA type 1 and LinA type 2, respectively. In addition, the residues GIHFAPS, present at the region spanning residues 148 to 154 in both LinA type 1 and LinA type 2, are deleted in LinA type 3.The activity of LinA type 3 for the metabolism of δ-HCH is several orders of magnitude higher than that of LinA type 1 or LinA type 2 and can be useful for improvement of the metabolism of δ-HCH.

Nucleoplasmic bridges and tailed nuclei are signatures of radiation exposure in *Oreochromis mossambicus* using erythrocyte micronucleus cytome assay (EMNCA)

Anbumani S, Mohankumar MN. Environ Sci Pollut Res Int. 2015; 22(23):18425-36.

Gamma radiation-induced genetic perturbations in aquatic vertebrates is largely unknown at low-dose rate, especially in the wake of a nuclear disaster and/or other environmental outbreaks. Freshwater fish, *Oreochromis mossambicus* subjected to low-dose rate (2 mGy/min) at 2.5-, 5-, and 10-Gy doses, were analyzed for "exposure signatures" in blood samples drawn on days 3, 6, 12, 18, and 30, respectively. Significant dose-dependent increments in micronuclei frequency and other anomalies such as nucleoplasmic bridges and tailed nuclei were observed and exhibit a strong positive correlation, suggesting that they could be used as prospective signatures of radiation exposure. Similarly increased incidence of apoptosis and DNA repair machinery circuits at high and low doses were noted. This work highlighted "cytogenetic signatures" in fish and the sensitivity of these endpoints toward low-dose rate of radiation exposure.

Regulatory Toxicology

Efficacy of etidronic acid, BioPure MTAD and SmearClear in removing calcium ions from the root canal: An *in vitro* study

Yadav HK, Tikku AP, Chandra A, Yadav RK, Patel DK. Eur J Dent. 2015; 9(4):523-8.

The purpose of this study was to quantify the amount of calcium ions removed from the root canal by etidronic acid (HEBP), BioPure MTAD, and SmearClear using atomic absorption spectrophotometer. Fifty (n = 50) freshly extracted human mandibular premolar teeth were collected and decoronated at the cementoenamel junction. The canals were prepared in a crown down fashion using the rotary system and copiously irrigated with 1.0% sodium hypochlorite. All specimens were rinsed with the deionized water. Based on the type of chelating agent used, the samples (n = 10) were randomly divided into five (four test and one negative control) groups. Accordingly, Group I - 9% HEBP, Group II - 18% HEBP, Group III - SmearClear, Group IV - BioPure MTAD, and Group V - normal Saline. Subsequent to irrigation, the solution was collected in a test tube and subjected to atomic absorption spectrophotometer for the quantification of calcium ions removed from the root canal. The mean concentration of calcium ions removed from the root canal (mean ± standard deviation) in all groups (I-V) were 13.32 ± 0.54 µg/ml, 16.36 ± 0.27 µg/ml, 20.04 ± 0.24 µg/ml, 18.15 ± 0.39 µg/ml, and 8.74 ± 0.49 µg/ml, respectively. SmearClear was the most effective agent for the removal of calcium ions from the root canal. Hence, its combined use with an organic solvent can be recommended for efficient smear layer removal.

Ultrasound-assisted dispersive liquid-liquid microextraction followed by GC-MS/MS analysis for the determination of valproic acid in urine samples

Jain R, Gupta MK, Chauhan A, Pandey V, Reddy Mudiam MK. Bioanalysis. 2015; 7(19):2451-9.

Valproic acid (VPA) is an anticonvulsant drug used for the treatment of epilepsy and bipolar disorder. A method based on simultaneous derivatization and dispersive liquid-liquid microextraction followed by GC-MS/MS analysis has been developed for the determination of VPA in urine samples. This optimized and validated method shows good linearity with R(2) value of 0.999. LOD and LOQ of VPA was found to be 0.4 ng $ml^{-1}$and 1.4 ng ml^{-1}, respectively. Recovery of VPA was found to be in the range of 80 to 92%. The developed method can find its wide applicability for the routine analysis of VPA in toxicological and clinical laboratories.

Imprinted nanospheres based on precipitation polymerization for the simultaneous extraction of six urinary benzene metabolites from urine followed by injector port silylation and gas chromatography-tandem mass spectrometric analysis

Chauhan A, Bhatia T, Gupta MK, Pandey P, Pandey V, Saxena PN, Mudiam MK. J Chromatogr B Analyt Technol Biomed Life Sci. 2015;1001:66-74

In the present communication, uniformly sized molecularly imprinted polymer (MIP) as nanospheres were synthesized based on precipitation polymerization using dual-template imprinting approach and used it as sorbent for solid phase extraction of six urinary benzene metabolites (UBMs). This approach in combination with injector port silylation (IPS) has been used for the quantitative determination of these UBMs by gas chromatography-tandem mass spectrometry. The MIP was synthesized by using t,t-muconic acid (t,t-MA) and 1,2,4-trihydroxybenzene (THB) as templates, methacrylic acid (MAA) as a monomer, ethyleneglycoldimethacrylate (EGDMA) as crosslinker, acetonitrile and dimethylsulphoxide as a porogen and azobisisobutyronitrile (AIBN) as an initiator. The factors affecting the performance of polymer and IPS were investigated and optimized for the simultaneous determination of UBMs in urine. Binding study of imprinted and non-imprinted polymer (NIP) shows that, MIP possesses higher affinity in comparison to NIP for these analytes. Under the optimum conditions, the method developed was found to be linear with regression coefficients falls in the range of 0.9721-0.9988 for all the analyzed metabolites. The percent recovery of the metabolites analyzed in urine was found to be in the range of 76-89%, while the limit of detection and limit of quantification were found to be in the range of 0.9-9.1ngmL^{-1}and 2.8-27ngmL^{-1}respectively. The validated method was successfully applied to the real urine samples collected from different groups (kitchen workers, smokers and petroleum workers) and found that the developed method has been promising applications in the routine analysis of urine samples of benzene exposed population.

Modeling the binding affinity of structurally diverse industrial chemicals to carbon using the artificial intelligence approaches

Gupta S, Basant N, Rai P, Singh KP. Environ Sci Pollut Res Int. 2015; 22(22):17810-27.

Binding affinity of chemical to carbon is an important characteristic as it finds vast industrial applications. Experimental determination of the adsorption capacity of diverse chemicals onto carbon is both time and resource intensive, and development of computational approaches has widely been advocated. In this study, artificial intelligence (AI)-based ten different qualitative and quantitative structure-property relationship (QSPR) models (MLPN, RBFN, PNN/GRNN, CCN, SVM, GEP, GMDH, SDT, DTF, DTB) were established for the prediction of the adsorption capacity of structurally diverse chemicals to activated carbon following the OECD guidelines. Structural diversity of the chemicals and nonlinear dependence in the data were evaluated using the Tanimoto similarity index and Brock-Dechert-Scheinkman statistics. The generalization and prediction abilities of the constructed models were established through rigorous internal and external validation procedures performed employing a wide series of statistical checks. In complete dataset, the qualitative models rendered classification accuracies between 97.04 and 99.93%, while the quantitative models yielded correlation (R(2)) values of 0.877-0.977 between the measured and the predicted endpoint values. The quantitative prediction accuracies for the higher molecular weight (MW) compounds (class 4) were relatively better than those for the low MW compounds. Both in the qualitative and quantitative models, the Polarizability was the most influential descriptor. Structural alerts responsible for the extreme adsorption behaviour of the compounds were identified. Higher number of carbon and presence of higher halogens in a molecule rendered higher binding affinity. Proposed QSPR models performed well and outperformed the previous reports. A relatively better performance of the ensemble learning models (DTF, DTB) may be attributed to the strengths of the bagging and boosting algorithms which enhance the predictive accuracies. The proposed AI models can be useful tools in screening the chemicals for their binding affinities toward carbon for their safe management.

Predicting toxicities of diverse chemical pesticides in multiple avian species using tree-based QSAR approaches for regulatory purposes

Basant N, Gupta S, Singh KP. J Chem Inf Model. 2015; 55(7):1337-48.

A comprehensive safety evaluation of chemicals should require toxicity assessment in both the aquatic and terrestrial test species. Due to the application practices and nature of chemical pesticides, the avian toxicity testing is considered as an essential requirement in the risk assessment process. In this study, tree-based multispecies

QSAR (quantitative-structure activity relationship) models were constructed for predicting the avian toxicity of pesticides using a set of nine descriptors derived directly from the chemical structures and following the OECD guidelines. Accordingly, the Bobwhite quail toxicity data was used to construct the QSAR models (SDT, DTF, DTB) and were externally validated using the toxicity data in four other test species (Mallard duck, Ring-necked pheasant, Japanese quail, House sparrow). Prior to the model development, the diversity in the chemical structures and end-point were verified. The external predictive power of the QSAR models was tested through rigorous validation deriving a wide series of statistical checks. Inter-correlation analysis and PCA methods provided information on the association of the molecular descriptors related to MW and topology. The S36 and MW were the most influential descriptors identified by DTF and DTB models. The DTF and DTB performed better than the SDT model and yielded a correlation (R(2)) of 0.945 and 0.966 between the measured and predicted toxicity values in test data array. Both these models also performed well in four other test species (R(2) > 0.918). ChemoTyper was used to identify the substructure alerts responsible for the avian toxicity. The results suggest for the appropriateness of the developed QSAR models to reliably predict the toxicity of pesticides in multiple avian test species and can be useful tools in screening the new chemical pesticides for regulatory purposes.

Predicting aquatic toxicities of chemical pesticides in multiple test species using nonlinear QSTR modeling approaches

Basant N, Gupta S, Singh KP. Chemosphere. 2015; 139:246-55.

In this study, authors established nonlinear quantitative-structure toxicity relationship (QSTR) models for predicting the toxicities of chemical pesticides in multiple aquatic test species following the OECD (Organization for Economic Cooperation and Development) guidelines. The decision tree forest (DTF) and decision tree boost (DTB) based QSTR models were constructed using a pesticides toxicity dataset in *Selenastrum capricornutum* and a set of six descriptors. Other six toxicity data sets were used for external validation of the constructed QSTRs. Global QSTR models were also constructed using the combined dataset of all the seven species. The diversity in chemical structures and nonlinearity in the data were evaluated. Model validation was performed deriving several statistical coefficients for the test data and the prediction and generalization abilities of the QSTRs were evaluated. Both the QSTR models identified WPSA1 (weighted charged partial positive surface area) as the most influential descriptor. The DTF and DTB QSTRs performed relatively better than the single decision tree (SDT) and support vector machines (SVM) models used as a benchmark here and yielded R(2) of 0.886 and 0.964 between the measured and predicted toxicity values in the complete dataset (*S. capricornutum*). The QSTR models applied to six other aquatic species toxicity data yielded R(2) of >0.92 (DTF) and >0.97 (DTB), respectively. The prediction accuracies of the global models were comparable with those of the *S. capricornutum* models. The results suggest for the appropriateness of the developed QSTR models to reliably predict the aquatic toxicity of chemicals and can be used for regulatory purpose.

Metabolomics reveals the perturbations in the metabolome of *Caenorhabditis elegans* exposed to titanium dioxide nanoparticles

Ratnasekhar Ch, Sonane M, Satish A, Mudiam MK. Nanotoxicology. 2015; 9(8):994-1004.

The increasing use of nanotechnology in daily life can have many unintended effects and pose adverse impact on human health, environment and ecosystems. Wider application of engineered nanoparticles, especially TiO_2 nanoparticles (TiO_2 NP) necessitates the understanding of toxicity and mechanism of action. Metabolomics provides a unique opportunity to find out biomarkers of nanoparticles exposure, which leads to the identification of cellular pathways and their biological mechanisms. Gas chromatography mass spectrometry (GC-MS)-based metabolomics approach was used in the present study to understand the toxicity of sub-lethal concentrations (7.7 and 38.5 μg/ml) of TiO_2 NP (<25 nm) in well-known, soil nematode *Caenorhabditis elegans (C. elegans)*. Multivariate pattern recognition analysis reflected the perturbations in the metabolism (amino acids, organic acids, sugars) of *C. elegans* on exposure to TiO_2 NP. The biological pathways affected due to the exposure of TiO_2 NP were identified, among them mainly affected pathways are tricarboxylic acid (TCA) cycle, arachidonic acid metabolism and glyoxalate dicarobxylate metabolism. The manifestation of differential metabolic profile in organism exposed to TiO_2 (NP or bulk particle) was witnessed as an effect on reproduction. The present study demonstrates that metabolomics can be employed as a tool to understand the potential toxicity of nanoparticles in terms of organism-environment interactions as well as in assessing the organism function at the molecular level.

Nanotherapeutics and Nanomaterial Toxicology

PLGA-encapsulated tea polyphenols enhance the chemotherapeutic efficacy of cisplatin against human cancer cells and mice bearing Ehrlich ascites carcinoma

Singh M, Bhatnagar P, Mishra S, Kumar P, Shukla Y, Gupta KC. Int J Nanomedicine. 2015; 10:6789-809.

The clinical success of the applicability of tea polyphenols awaits efficient systemic delivery and bioavailability. Herein, following the concept of nanochemoprevention, which uses nanotechnology for enhancing the efficacy of chemotherapeutic drugs, authors employed tea polyphenols, namely theaflavin (TF) and epigallocatechin-3-gallate (EGCG) encapsulated in a biodegradable nanoparticulate formulation based on poly(lactide-co-glycolide) (PLGA) with approximately 26% and 18% encapsulation efficiency, respectively. It was observed that TF/EGCG encapsulated PLGA nanoparticles (NPs) offered an up to ~7-fold dose advantage when compared with bulk TF/EGCG in terms of exerting its antiproliferative effects and also enhanced the anticancer potential of cisplatin (CDDP) in A549 (lung carcinoma), HeLa (cervical carcinoma), and THP-1 (acute monocytic leukemia) cells. Cell cycle analysis revealed that TF/EGCG-NPs were more efficient than bulk TF/EGCG in sensitizing A549 cells to CDDP-induced apoptosis, with a dose advantage of up to 20-fold. Further, TF/EGCG-NPs, alone or in combination with CDDP, were more effective in inhibiting NF-κB activation and in suppressing the expression of cyclin D1, matrix metalloproteinase-9, and vascular endothelial growth factor, involved in cell proliferation, metastasis, and angiogenesis, respectively. EGCG and TF-NPs were also found to be more effective than bulk TF/EGCG in inducing the cleavage of caspase-3 and caspase-9 and Bax/Bcl2 ratio in favour of apoptosis. Further, *in vivo* evaluation of these NPs in combination with CDDP showed an increase in life span ($P<0.05$) in mice bearing Ehrlich's ascites carcinoma cells, with apparent regression of tumor volume in comparison with mice treated with bulk doses with CDDP. These results indicate that EGCG and TF-NPs have superior cancer chemosensitization activity when compared with bulk TF/EGCG.

Effect of graphene oxide on the conformational transitions of amyloid beta peptide: a molecular dynamics simulation study

Baweja L, Balamurugan K, Subramanian V, Dhawan A. J Mol Graph Model. 2015; 61:175-85.

The interactions between nanomaterials (NMs) and amyloid proteins are central to the nanotechnology-based diagnostics and therapy in neurodegenerative disorders such as Alzheimer's and Parkinson's. Graphene oxide (GO) and its derivatives have shown to modulate the aggregation pattern of disease causing amyloid beta (Aβ) peptide. However, the mechanism is still not well understood. Using molecular dynamics simulations, the effect of graphene oxide (GO) and reduced graphene oxide (rGO) having carbon:oxygen ratio of 4:1 and 10:1, respectively, on the conformational transitions (alpha-helix to beta-sheet) and the dynamics of the peptide was investigated. GO and rGO decreased the beta-strand propensity of amino acid residues in Aβ. The peptide displayed different modes of adsorption on GO and rGO. The adsorption on GO was dominated by electrostatic interactions, whereas on rGO, both van der Waals and electrostatic interactions contributed in the adsorption of the peptide. Authors study revealed that the slight increase in the hydrophobic patches on rGO made it more effective inhibitor of conformational transitions in the peptide. Alpha helix-beta sheet transition in Aβ peptide could be one of the plausible mechanism by which graphene oxide may inhibit amyloid fibrillation.

ZnO nanoparticles induced inflammatory response and genotoxicity in human blood cells: a mechanistic approach

Senapati VA, Kumar A, Gupta GS, Pandey AK, Dhawan A. Food Chem Toxicol. 2015; 85:61-70.

The wide application of zinc oxide nanoparticles (ZnO NPs) in cosmetics, paints, biosensors, drug delivery, food packaging and as anticancerous agents has increased the risk of human exposure to these NPs. Earlier *in vitro* and *in vivo* studies have demonstrated a cytotoxic and genotoxic potential of ZnO NPs. However, there is paucity of data regarding their immunomodulatory effects.

Therefore, the present study was aimed to investigate the immunotoxic potential of ZnO NPs using human monocytic cell line (THP-1) as model to understand the underlying molecular mechanism. A significant ($p < 0.01$) increase in pro-inflammatory cytokines (TNF-α and IL-1β) and reactive oxygen species (ROS) was observed with a concomitant concentration dependent (0.5, 1, 5, 10, 15 and 20 µg/mL) decrease in the glutathione (GSH) levels as compared to control. The expression levels of mitogen activated protein kinase (MAPK) cascade proteins such as p-ERK1/2, p-p38 and p-JNK were also significantly ($p < 0.05$, $p < 0.01$) induced. Also, at the concentration tested, NPs induced DNA damage as assessed by the Comet and micronucleus assays. Data demonstrated that ZnO NPs induce oxidative and nitrosative stress in human monocytes, leading to increased inflammatory response via activation of redox sensitive NF-κB and MAPK signalling pathways.

Chromium oxide nanoparticle-induced genotoxicity and p53-dependent apoptosis in human lung alveolar cells

Senapati VA, Jain AK, Gupta GS, Pandey AK, Dhawan A. J Appl Toxicol. 2015; 35(10): 1179-88.

Chromium oxide (Cr_2O_3) nanoparticles (NPs) are being increasingly used as a catalyst for aromatic compound manufacture, abrading agents and as pigments (e.g., Viridian). Owing to increased applications, it is important to study the biological effects of Cr_2O_3 NPs on human health. The lung is one of the main exposure routes to nanomaterials; therefore, the present study was designed to determine the genotoxic and apoptotic effect of Cr_2O_3 NPs in human lung epithelial cells (A549). The study also elucidated the molecular mechanism of its toxicity. Cr_2O_3 NPs led to DNA damage, which was deduced by comet assay and cytokinesis block micronucleus assay. The damage could be mediated by the increased levels of reactive oxygen species. Further, the oxygen species led to a decrease in mitochondrial membrane potential and an increase in the ratio of BAX/Bcl-2 leading to mitochondria-mediated apoptosis induced by Cr_2O_3 NPs, which ultimately leads to cell death. Hence, there is a need of regulations to be imposed in NP usage. The study provided insight into the caspase-dependent mechanistic pathway of apoptosis.

Green synthesis of silver and titanium dioxide nanoparticles using *Euphorbia prostrata* extract shows shift from apoptosis to G0/G1 arrest followed by necrotic cell death in *Leishmania donovani*

Zahir AA, Chauhan IS, Bagavan A, Kamaraj C, Elango G, Shankar J, Arjaria N, Roopan SM, Rahuman AA,Singh N. Antimicrob Agents Chemother. 2015; 59(8):4782-99.

The aim of the present study was to synthesize silver (Ag) and titanium dioxide (TiO_2) nanoparticles (NPs) using green synthesis from aqueous leaf extract of *Euphorbia prostrata* as antileishmanial agents and to explore the underlying molecular mechanism of induced cell death. *In vitro* antileishmanial activity of synthesized NPs was tested against promastigotes of *Leishmania donovani* by alamar Blue and propidium iodide uptake assays. Antileishmanial activity of synthesized NPs on intracellular amastigotes was assessed by Giemsa staining. The leishmanicidal effect of synthesized Ag NPs was further confirmed by DNA fragmentation assay and by cell cycle progression and transmission electron microscopy (TEM) of the treated parasites. TEM analysis of the synthesized Ag NPs showed a spherical shape with an average size of 12.82 ± 2.50 nm, and in comparison to synthesized TiO_2 NPs, synthesized Ag NPs were found to be most active against Leishmania parasites after 24 h exposure, with 50% inhibitory concentrations (IC50) of 14.94 µg/ml and 3.89 µg/ml in promastigotes and intracellular amastigotes, respectively. A significant increase in G0/G1 phase of the cell cycle with a subsequent decrease in S (synthesis) and G2/M phases compared to controls was observed. The growth-inhibitory effect of synthesized Ag NPs was attributed to increased length of S phase. A decreased reactive oxygen species level was also observed, which could be responsible for the caspase-independent shift from apoptosis (G0/G1 arrest) to massive necrosis. High-molecular-weight DNA fragmentation as a positive consequence of necrotic cell death was also visualized. Authors also report that the unique trypanothione/trypanothione reductase (TR) system of Leishmania cells was significantly inhibited by synthesized Ag NPs. The green-synthesized Ag NPs may provide promising leads for the development of cost-effective and safer alternative treatment against visceral leishmaniasis.

Metabolomics reveals the perturbations in the metabolome of *Caenorhabditis elegans* exposed to titanium dioxide nanoparticles

Ratnasekhar Ch, Sonane M, Satish A, Mudiam MK. Nanotoxicology. 2015; 9(8) :994-1004.

The increasing use of nanotechnology in daily life can have many unintended effects and pose adverse impact on human health, environment and ecosystems. Wider application of engineered nanoparticles, especially TiO_2 nanoparticles (TiO_2 NP) necessitates the understanding of toxicity and mechanism of action. Metabolomics provides a unique opportunity to find out biomarkers of nanoparticles exposure, which leads to the identification of cellular pathways and their biological mechanisms. Gas chromatography mass spectrometry (GC-MS)-based metabolomics approach was used in the present study to understand the toxicity of sub-lethal concentrations (7.7 and 38.5 µg/ml) of TiO_2 NP (<25 nm) in well-known, soil nematode *Caenorhabditis elegans (C. elegans)*. Multivariate pattern recognition analysis reflected the perturbations in the metabolism (amino acids, organic acids, sugars) of *C. elegans* on exposure to TiO_2 NP. The biological pathways affected due to the exposure of TiO_2 NP were identified, among them mainly affected pathways are tricarboxylic acid (TCA) cycle, arachidonic acid metabolism and glyoxalate dicarobxylate metabolism. The manifestation of differential metabolic profile in organism exposed to TiO_2 (NP or bulk particle) was witnessed as an effect on reproduction. The present study demonstrates that metabolomics can be employed as a tool to understand the potential toxicity of nanoparticles in terms of organism-environment interactions as well as in assessing the organism function at the molecular level.

Topic of Interest

Regulatory Toxicology Framework – Current Scenario and Compliance

Regulatory affairs as a discipline Dr Poonam Kakkar and Dr Akshay Dwarkanath evolved out of the necessity and desire of governments / agencies / departments to ensure the safety, efficacy and compatibility of various products, viz., pharmaceuticals and nutraceuticals, chemicals, cosmetics, agrochemicals, food and feed additives, medical devices, packaging materials etc. In addition, with increasing social responsibility and scrutiny of their activities and processes, the onus also lies on corporate bodies to ensure the safety of their products. Organizations involved in the discovery, testing, manufacture and marketing of products also need to ensure the safety of their products and contribute towards public health and welfare.

In the realm of health care, the regulatory frame work has a very specific role to play in pharmaceuticals, medical devices, biologics, functional foods etc. The ultimate goal is to ensure (a) protection of human health, (b) safety, efficacy and quality of drugs and (c) accuracy of product information. Regulatory professionals are the liaison between the manufacturer / organization and the regulator. They ensure compliance to all regulatory requirements, appropriateness and scientific validity of documents for regulatory approval like New Drug Application (NDA), Abbreviated New Drug Application (ANDA), Investigational New Drug Application (INDA), Drug Master File (DMF) etc. They also offer expertise in translating regulatory requirements into workable plans and advise organizations on the regulatory climate that would affect their proposed activities.

Broadly stated, toxicology studies explore the undesirable effects of natural or synthetic chemicals, drugs, poisons, toxicants, impurities etc on any living system. Mere compiling of this information does not meet the present day requirements of predictive toxicology. The process of gathering and evaluating existing toxicological information in order to establish concentration based standards of safe exposure to chemicals forms the basis of regulatory toxicology. In addition to evaluating existing information, regulatory toxicology also encompasses gathering toxicological information from animal experimentation that are used to determine the level of a chemical / toxicant that would cause illness or death. Various regulatory agencies use data from these types of experiments to formulate safety standards i.e. the level of a

chemical that an individual can be exposed to without the threat of an adverse / harmful effect.

Several legislative and regulatory changes over the last few years have prompted a re-evaluation of the requirements for toxicity testing and risk assessment. The passage of the Food Quality Protection Act in the United States of America is one such example that has mandated a more robust evaluation of health risk in children by the application of an additional 10 fold safety factor in cases of insufficient data generation. Similarly, following a review of its testing protocols, the US-Environment Protection Agency (USEPA) has increased both the duration of treatment and the toxicology endpoints in developmental toxicity studies to ensure a more thorough evaluation of effects on neurological, immune and reproductive systems. Several new testing guidelines have evolved to be used along with existing test protocols in carcinogenesis, neurotoxicity studies, endocrine disruption and pharmacokinetic studies. This has resulted in a rigorous evaluation of the adequacy of the methods used for safety endpoints and establishing reference values.

Regulatory requirements for biological implants, tissue engineering and regenerative medicine products

According to the Food Drugs and Cosmetics Act of the United States, a medical device is an instrument or apparatus, intended for use in the diagnosis or treatment of disease or is intended to affect the structure or function of the subject in which it is used. Such a device is not dependent on any chemical action or metabolic process to achieve its intended purpose. Similarly, tissue engineering / regenerative medicine products are innovative therapies being developed to address unmet clinical needs. By composition, these products are complex in both structure and function since they usually are combinations of cells, scaffolds and other factors. This challenges the product developer to ensure that the product is safe and effective prior to clinical trials in humans.

Human medical products that are regulated by the FDA include human drugs, tissues, biological products, and medical devices. World over, different regulatory agencies categorize medical devices based on type and duration of contact following similar benchmarks. Regulators insist on biocompatibility assessment prior to approval for clinical use. Prescribed guidelines include those developed by International Organization for Standardization (ISO) and FDA viz., "Biological Evaluation of Medical Devices," and "Use of International Standard ISO 10993, 'Biological Evaluation of Medical Devices'—Part 1: Evaluation and Testing" respectively. The FDA document largely accepts the ISO testing matrix, with the exception of an additional assessment based on guidelines published by the Organization for Economic Cooperation and Development (OECD).

Regulatory requirements for food / feed additives

In order to ensure the implementation of science based standards for food substances and to regulate their manufacture, storage, distribution, sale and import, the **Food Safety and Standards Authority of India (FSSAI) was e**stablished under Food Safety and Standards Act, 2006. This act consolidates several diverse acts & orders that were thus far handling food related problems across Ministries and Departments. The end goal of the act and the authority is to ensure availability of safe and wholesome food for human consumption. **Several a**cts like Prevention of Food Adulteration Act, 1954; Fruit Products Order, 1955; Meat Food Products Order, 1973; Vegetable Oil Products (Control) Order, 1947; De- Oiled Meal and Edible Flour (Control) Order, 1967; Milk and Milk Products Order, 1992 etc have been repealed after the introduction of the FSS Act, 2006. The Act also aims to establish a single reference point for all matters relating to food safety and standards, by moving from multi-level, multi- departmental control to a single line of command.

Regulatory requirements for herbal formulations / phytopharmaceuticals

Among the different traditional systems of medicine in India, Ayurveda, Yoga, Naturopathy, Unani, Siddha and Homeopathy are increasingly occupying a position of eminence in the health care industry of the country. Department of AYUSH in conjunction with the Drugs and Cosmetics Act, 1940 and its subsequent amendments in 2005, 2008 and draft amendment, 2015 primarily regulates herbal medicines in India. With its varied agro climatic zones, India is home to 6000 to 7000 species of flowering plants with medicinal usage. Among this huge repertoire of herbal diversity, about 960 species are in commercial use with annual consumption in excess of 100 metric tonnes. With a growing global resurgence of alternative health care systems, herbal trade world over is expected to reach 7 trillion USD by 2050.

Classification and categorisation of herbal drug products varies with functional foods, dietary

supplements and traditional medicines being grouped together in some countries. In addition, evaluation of herbal products is fraught with the inherent complexity of being a mixture of constituents. More often than not, the constituents responsible for the therapeutic effects are unknown which further complicates the stability of the product. There have been several recently introduced changes in regulations or newly introduced regulations compliance with the regulatory bodies in Europe, the United States and India. A combined effort by the Committee for Herbal Medicinal Products (HMPC) and Committee of European Medicines Agency (EMA) is underway for the development of guidelines for quality, nonclinical studies, clinical efficacy and safety. Medicines and Healthcare Products Regulatory Agency (MHRA, UK) has recently introduced the Traditional herbal medicines registration scheme (THMRS). Similarly the US FDA has also issued draft guidance for Industry on "Complementary and Alternative Medicine Products and Their Regulation". The Indian regulators have also recently amended the Drugs and Cosmetics Rules to control the quality, safety and efficacy of herbal drug products in India. However the regulations for herbal drug products in Europe and United States are more stringent than in India. Some of the important guidelines being followed internationally and in India are:

- Organization for Economic Co-operation and development (OECD) Principles of Good Laboratory Practices (GLP)
- International Committee on Harmonization (ICH)
- Schedule Y
- Occupational Safety and Health Administration
- FSSAI – Food Safety and Standards Authority of India
- EMA – European Medicines Agency

These principles were developed to ensure the generation of high quality and reliable test data pertaining to the safety of new drugs, food / feed additives, industrial chemical substances etc. They were created with the goal of harmonising testing procedures to ensure global acceptance of data. These guidelines encompass the collection, processing and evaluation of experimental toxicology data to permit toxicologically based decisions directed towards the protection of health against harmful effects of chemical substances. This also aids in the development of standard protocols and new testing methods to facilitate the decision-making processes.

With constant modernization of the global pharmaceutical industry, the regulation of pharmaceuticals is also keeping pace. Figure 1 describes the regulatory framework structure capable of evaluating, approving and regulating new chemical entities, household products, smart/engineered materials etc which is an obvious offshoot of the technological boom in the country. Fig 2 describes briefly the goals and challenges faced by the regulatory toxicologists The drug regulation system of the country is also being strengthened at a rapid pace. A lot still needs to be done to ensure effective implementation of the policies and processes that regulate the Drugs and chemicals industry.

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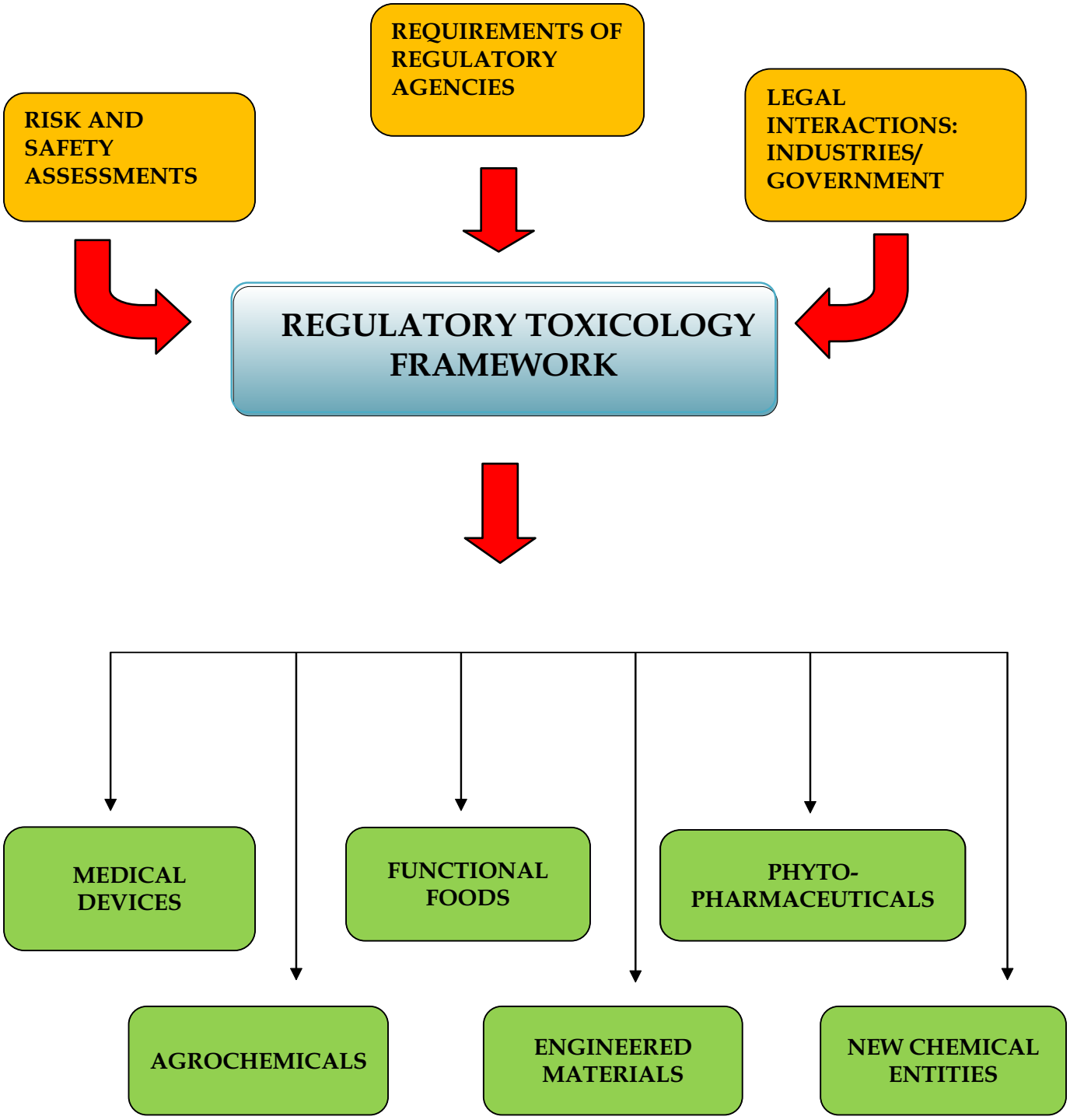
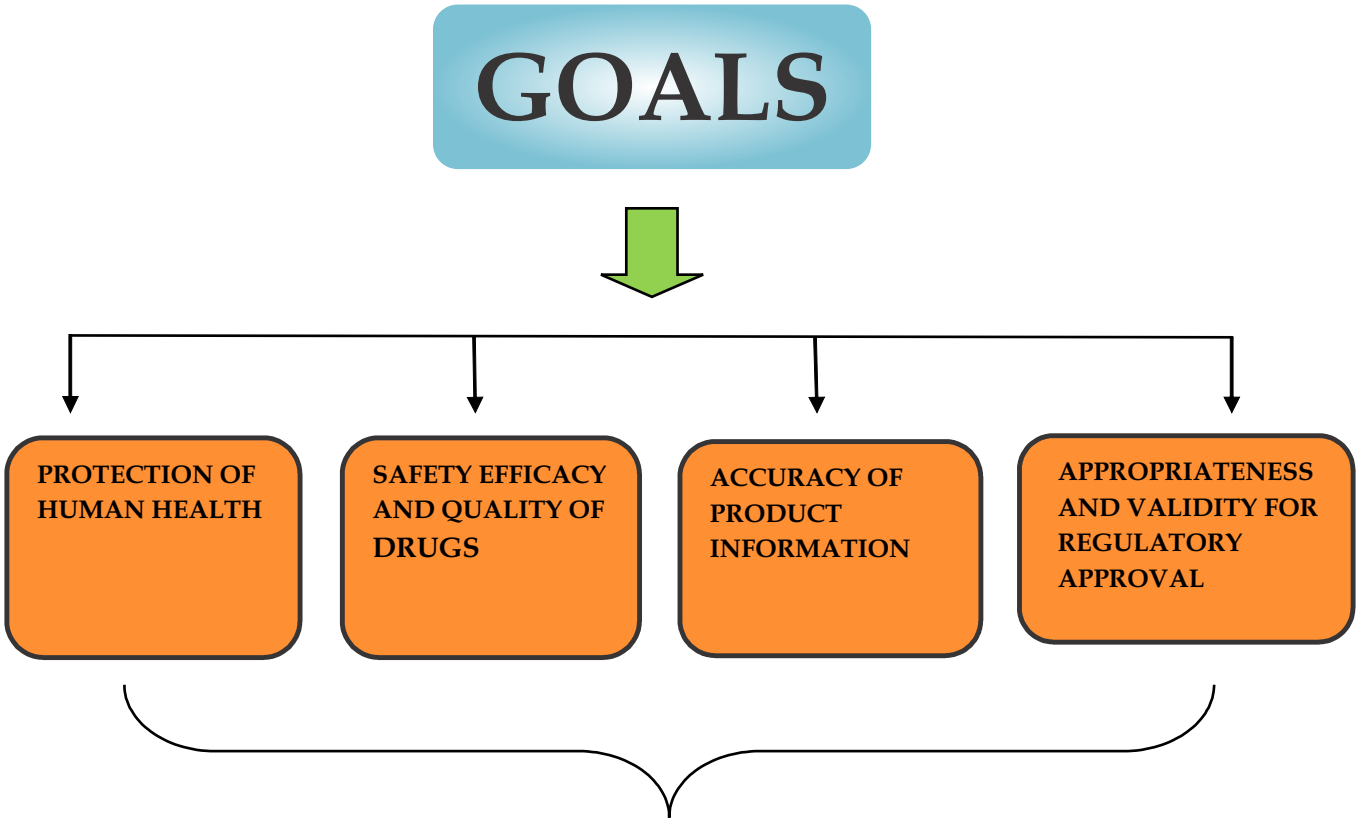

REQUIREMENTS OF REGULATORY AGENCIES
RISK AND SAFETY ASSESSMENTS
LEGAL INTERACTIONS: INDUSTRIES/ GOVERNMENT
REGULATORY TOXICOLOGY FRAMEWORK
MEDICAL DEVICES
FUNCTIONAL FOODS
PHYTO-PHARMACEUTICALS
AGROCHEMICALS
ENGINEERED MATERIALS
NEW CHEMICAL ENTITIES



CHALLENGES

- **NEW CHEMICAL ENTITIES:** chemical makeup, stability, solubility and bioavailability.
- **AGROCHEMICALS:** delivery and destiny at molecular level.
- **PHYTO-PHARMACEUTICALS:** diverse nature, unknown complex mixtures.
- **MEDICAL DEVICES:** structural and functional complexities and biocompatibility.
- **FUNCTIONAL FOODS:** safe and wholesome food availability.
- **ENGINEERED MATERIALS:** physicochemistry at nanoscale and biological

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Any feedback on this publication is welcome
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