Multidisciplinary research in toxicology for development without destruction at Industrial Toxicology Research Centre, Lucknow

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With rapid industrialization and agricultural expansion in India more and more chemicals are being manufactured and used every day. To consolidate the gains of our prosperity, the undesirable side-effects of increasing industrial and agricultural activities have to be simultaneously minimized so that the health, productivity and welfare of the people and our immense natural resources can be preserved. With this objective the Industrial Toxicology Research Centre (ITRC), at Lucknow, was founded on 4 November, 1965 and has dedicated itself to serve the needs of the nation in the area of chemical safety.

Aims and objectives

(i) To identify through systematic epidemiological surveys the health hazards to which industrial and agricultural workers are exposed in their work environment.
(ii) To undertake safety evaluation of chemicals used in industry, agriculture and day-to-day life
(iii) To conduct experimental studies on the mode of action of chemical pollutants and develop research capabilities in the relevant scientific disciplines
(iv) To develop suitable diagnostic tests and suggest remedial/preventive measures
(v) To collect and disseminate information on hazardous chemicals

Since occupational and environmental toxicology is a multifaceted area of biomedical sciences, ITRC over the years has been able to develop capabilities and train manpower in diverse areas such as industrial medicine, pathology, biochemistry, pharmacology, physiology, immunology, neurobiology, zoology, botany and chemistry. Recently, new vistas such as biotechnology, preventive toxicology, immunobiology, photochemistry, etc. have emerged as special areas. Using this multidisciplinary approach, in-depth toxicological research has been done on priority chemicals such as heavy metals, industrial dusts and fibres, plastics and polymers, hydrocarbons, pesticides, detergents, dyes and food additives. These activities are being carried out in 27 separate sections, each with independent R&D programmes along with intersectional multimodel collaborative projects. This approach has helped to develop complete toxicological information on a wide variety of chemicals under Indian context, along with the evaluations of units of excellence in specific areas such as neurotoxicology, developmental toxicology, immunotoxicology, pulmonary toxicology, preventive toxicology, epidemiology and environmental carcinogenesis.

In order to concentrate on specific issues in totality, from identification of problems and human risk assessment to elucidation of toxic mechanisms, and development of diagnostic, curative and preventive measures (by multipronged approach), pesticides, heavy metals and hydrocarbons have been identified as the areas of thrust, each with a definite programme to provide necessary information on all aspects of chemical safety.

Recently, various R&D sections have been listed under five broad groups, namely pesticide toxicology, preventive toxicology, systemic toxicology, environmental health and ecotoxicology, to add coherence and coordination in R&D efforts.

Pesticide toxicology

Chronic exposure of treated malnourished animals to hexachlorocyclohexane (HCH), one of the pesticides largely used in India, enhances cardiotoxicity and immunosuppression. HCH was not found to produce significant chromosomal changes in the bone marrow cells of animals. The course of delayed neurotoxicity of pesticides as well as changes in cerebral neurotoxic esterase were not modified by thiamine hydrochloride and pyridoxine which are important in neuronal metabolism. Endosulfan was found to change the animal behaviour because of significant changes in the concentrations of regional brain neurotransmitters. Ocular toxicity of endosulfan and HCH in rabbits have been demonstrated after chronic exposure in animals. The residual concentrations of these pesticides in per gram of brain lipids were about three times higher in the cerebral cortex and cerebellum than in the spinal cord and brain stem.

In a detailed study among workers exposed to DDT and organophosphates, prevalence of macular changes were noticed in workers exposed to organophosphates.

Systemic toxicology

The pathogenesis of early manganese poisoning was established and a simple method for early detection of poisoning has been evolved. Cases of manganese poisoning were detected in a health survey conducted among underground drillers in manganese mines at Balaghat and Ukua near Nagpur, Maharashtra. Increased levels of serum calcium were of great help in the detection of early manganese poisoning. Using the sophisticated microprocessor-controlled instrument 'Optovariceps' with video monitor for studying early behavioural abnormalities, ITRC's findings have shown that cadmium-induced changes in early locomotor activity and oxidative damage to the brain can be prevented by supplementation with vitamin E.

A central role for generation of active oxygen species and their scavenging leading to altered membrane functions and metabolic changes in the neurotoxicity of heavy metals was established.

Pretreatment with low dosage of
heavy metal may protect against acute toxicity, and sometimes develop cross
tolerance. Most of the metals induce de
novo synthesis of hepatic and/or renal metabolism-like proteins capable of
binding excess toxic metals. Nickel
induced metallothionein may be respon
sible for protection against the observed cadmium nephrotoxicity.

A neurotransmitter receptor-binding
screen to evaluate the toxicity of neurotoxicants has been developed using high-
affinity radioligand binding assay. Invol
vement of neurotransmitter receptors in the toxic action of styrene and acryla
mide was demonstrated. Phthalic acid esters were found to affect the xenobi
otic-metabolizing enzymes in routine
and dose-dependent manner and to interac
t with other xenobiotics at the pharma
cokinetics phase. The brain was found
to be equipped with the phase I and
phase II enzymes of xenobiotic metabolism. Brain microsomal aryl hyd
rocyanide hydroxylase was found to
have some similarities and some differ
ces with the hepatic enzymes.

Assessment of reproductive and deve
lopmental toxicity of chemicals, particu
larly in malnourished state, is being car
ried out. Developmental studies in rela
tion to behaviour and profile of neurochemicals are being evaluated after pre-
and postnatal exposure with chemi
cals.

An intestinal-loop technique has been
standardized to study the effect of wa
ter-borne industrial toxicants on the intes
tinal membrane and its constit
uents. An anorexic protein was found
to be released from intestines in membranes under stress conditions involving loss of
appetite.

Presence of particulate pollutants in the
occupational environment, leading to
conditions like pneumoconiosis, is a
major industrial hazard in India. Using suitably designed experimental models,
the cytotoxic and fibrogenic effects of
industrial dusts and fibres like silica,
mica, kaolin, talc and asbestos were
studied in detail and their mechanisms elucidated. The influence of super
imposed conditions such as protein malnutrition, ascorbic acid deficiency and pulmonary infection on the develop
ment of pneumoconiotic lesions was established and found to be of great significance in the Indian context.

Silicic acid, a constituent of noxious particulate air pollutants, was found, by
alkaline elution technique to cause DNA strand breaks in vitro.

Considerable new fundamental infor
mation regarding extracellular and extra
microsomal biotransformations such as
AHF induction in lungs, skin and brain has been obtained. MFO activity was
detected in brain mitochondria.

Studies on the mechanistic aspect of
nickel toxicity and its detoxication by
selective drugs have shown that cyclam
and cyclam-S are highly efficacious in
enhancing the excretion of this metal.

An all-glass chamber for exposing ani
mals to toxic gases for inhalation
-toxicity studies was developed. Extensive inhalation-toxicity studies were conducted with MIC to study the pathogenesis and
mechanism of toxicity.

Preventive toxicology

Benzene exposure to rats resulted in the
depletion of hepatic regulatory haem.
Administration of polyphenolic metabo
lites to mice for 45 days showed bone
marrow depressive effects. Disturbances
in lipid profile of brain and peripheral
nerves were observed after exposure to
neurotoxic hexacarbons. Various bio
chemical parameters can be used to
assess the hepatotoxicity of hydrocarbons.

ITRC studies have indicated for the
first time that the bacterial glycoprotein
protein A can abrogate the toxicity of a
number of industrial chemicals including those having carcinogenic ability. Protein
A has been found to be a potent immunomodulator and an interferon
inducer.

Vitamin B has been found to increase
the therapeutic index of calcium verse
inate, a popular chelating drug for metallic
intoxication. Vitamin B complex supple
mentation diminishes susceptibility to
lead and cadmium intoxication. 6-MFA,
an interferon inducer of fungal origin,
was found to reduce toxicity of benzene.

By dot blot immunohbounding assay
and Western blotting, a 15-fold induc
ion of phenobarbital type of cytochrome P-
450 was observed in lindane-exposed rat
liver without any effect on methylchlo
roanthrene type. The transcriptional events were followed by photobiotinylated
DNA probes.

Two bacterial strains (ACR-2 and
ACR-3) isolated from garden oil were
found to utilize acrylamide monomer.
Acrylic acid, the initial product, was
completely degraded while ammonia
was accumulated in the medium. These
strains were tentatively identified as
Pseudomonas species.

A usual microbial technology has
been developed for bioconversion of
distillery wastes to methane by cutting
down the incubation time from 19 days
to 11.5 days. Many distilleries can
utilize this technology for controlling
pollution.

Environmental health research

Protocols for testing tumour-initiating
and tumour-promoting potentials of
chemicals were developed. Butyric acid
was found to abolish TPA-induced
increase in skin ornithine decarboxylase,
an indicator of tumour formation.

Spleen lymphocytes of protein A-
treated tumour-bearing mice showed
cytotoxicity against $180$ tumour targets.
Tumour-promoting and tumour-initiating
effects and complete carcinogenicity of
mancozeb, a commonly used fungicide,
were detected for the first time.

A health survey in an Ordnance
Factory at Kanpur showed that 23% of
the workers were suffering from allergic
disorders. Immunotherapy given by
ITRC significantly improved the symp
matology and lung function in these
workers. Glass bangle workers at Firo
zabad showed a high prevalence of
respiratory morbidity (52.7%), especially
pulmonary tuberculosis (12.8%) and
chronic bronchitis (23.9%).

Studies on pesticide sprayers in Mal
habad mango orchards revealed an
overall morbidity of 41.7%, the respira
tory morbidity being maximum
(34.5%).

Food colours

A major programme on the evaluation of
the magnitude and usage pattern of
artificial synthetic colours in foodstuffs
was initiated. The first 10 years of a
detailed survey revealed that 70% of
foodstuffs contained nonpermissible syn
thetic colours and as many as 18
prohibited dyes were used quite com
monly. Another 10-year survey showed
that the usage pattern of nonpermissible
food colours has changed. About 40% of
foodstuffs in rural areas still contained
nonpermissible food colours. The im
provement in urban usage was due to
ITRC data which focused the attention of regulatory authorities on the need to take necessary steps.

A survey by ITRC, in collaboration with some departments of the Uttar Pradesh government, showed that out of 1100 samples of mustard oil collected from 57 districts of UP, 28.5% were adulterated, 24 samples contained synthetic cancer-producing colours such as butter yellow while 13 oils contained argemone alkaloids which produce paralytic syndrome.

A portable minicolumn kit for rapid identification of aflatoxin in food commodities under field conditions has been developed by ITRC.

A paralytic disease affecting the lower limbs was detected in many residents of villages around Unnao, UP. Although the exact cause could not be identified, 35% of the food samples contained 'matri dal' showing presence of B-Oxalyl aminoalanine. A large number of water and soil samples from the same region showed high content of manganese.

Detailed clinical and experimental studies were conducted on the toxicity of benzanthrone, ascorbic acid administration was found beneficial in protecting workers against skin lesions. This recommendation has now been followed by the industry.

Ecotoxicology

Ecotoxicology was developed as a multidisciplinary science for assessment of ecological impact of pollutants through multispecies and microcosm studies. Dermal toxicity of synthetic detergents was evaluated and national standards for consumer safety protection and test protocols were developed. The ecological impact of detergents was studied and based on their toxicity to various fauna and flora, ecological safe limits were prescribed.

Linear alkylbenzene sulfonate, a major ingredient of detergents, was found to produce marked phytotoxicity as evidenced by impaired CO₂ assimilation, oxygen evolution, δ'-thymidine incorporation and C chlorophyll synthesis. TEM showed thyroid membrane dissociation and SEM displayed significant changes in surface morphology of the alga Spirodela quadriculata.

Ultrastructural and biochemical studies on the damage to fish skin and gills by linear alkylbenzenesulphonate were carried out.

The phototoxicity of riboflavin was found to be due to formation of active oxygen species leading to damage to membranes and genetic elements as studied by ultrastructural and biochemical approaches.

The role of active oxygen species in the toxicity of many compounds like aniline, ether and detergents and the mechanisms of antioxidant defence were studied. The mechanism of induction of superoxide dismutase isozymes in pulmonary and neurotoxicity responses was elucidated.

Based on a survey of a large number of pesticides and dyes, toxicity under Indian conditions due to photochemical activation was established. These studies are being extended under CSIR's Biosphere and Geosphere Programme on ozone depletion.

As the referral laboratory of the Pollution Control Board, water and waste water are being analysed for regulatory purposes.

The incidence of brackishwater and the relation between heavy-metal pollution and development of antibiotic resistance has been studied. The involvement of plasmid-mediated antibiotic/metal-resistance mechanisms was resolved.

National mission programmes

Safe drinking water mission

Safety evaluation of drinking water was carried out in 13 problem districts of 11 states covering about 14 lakhs of people from 2636 villages of Uttar Pradesh, West Bengal, Meghalaya, Mizoram, Arunachal Pradesh, Sikkim, Orissa, Rajasthan, Jammu and Kashmir, Himachal Pradesh and Maharashtra. The studies involved physicochemical and bacteriological analysis of water samples from drinking water supply sources. A portable water-analysis kit has been developed for this purpose and the technology has been transferred to an entrepreneur for its manufacture and sale in various states of the country.

A model 'mobile water analysis laboratory' has also been developed for carrying out the water mission programme in villages. The Department of Rural Development, Government of India, seeing the usefulness of this mobile laboratory, has assigned the job to ITRC for the production of 17 such mobile laboratories for various states.

National edible oil mission

The safety of new and existing oil sources, quantification of pesticide residues in edible oils and the effect of pesticides on oilseed quality, germination and growth profile are also being investigated.

Water-related societal missions

Ganga river water quality analysis

The Government of India, conscious of environmental problems, has created the Central Ganga Authority with the aim of monitoring Ganga river water quality so that the use of this huge water resource may not pose any serious health hazard to the general population. ITRC has been entrusted with the responsibility of monitoring the Ganga river for presence of heavy metals and pesticides regularly every month at 27 different locations between Rishikesh (Uttar Pradesh) and Haldia (West Bengal). During three and a half years of study significantly high levels of a few metals and pesticides were found at some locations and the data have been reported to the Central Ganga Authority.

Indo-Dutch collaborative studies on aquatic ecotoxicology

The Yamuna, another important river of northern India, receives urban and industrial wastes from major cities like Delhi, Ghaziabad, Mathura, Agra and Allahabad, leading to serious large-scale ecological problems. Considering the seriousness of the problem, the Department of Environment and Forests, Government of India, through the Central Pollution Control Board, invited the support of experts from the Netherlands as a part of the Indo-Dutch bilateral agreement for cooperation. ITRC in collaboration with the Netherlands organized an intensive training programme on aquatic ecotoxicology and biomonitoring in 1988. A three-year programme on biological and environmental monitoring of the Yamuna has started in which samples of water and sediments are being collected from 15...
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sites on the Yamuna for analysis of hydrocarbons, pesticides, heavy metals, biodegradation capacity, physicochemical properties of water and sediment, and micro and macro flora and fauna.

All-India coordinated programme on toxicant exposure assessment

Heavy-metal contents of hundreds of samples of food, water, air, soil, biological samples and human blood, urine and hair from different parts of Uttar Pradesh and Bihar were quantified as a part of Department of Environment and Forests, Government of India-sponsored All-India programme. A similar study on pesticides has been initiated by ITRC for ICMR.

Toxicological evolution of the marine and estuarine environment on the northeast coast

In order to have a complete environmental-impact study on the pollution load in the coastal and estuarine regions of the Hooghly, the Department of Ocean Development, Government of India, has sponsored a project at ITRC. Detailed studies on pollution-load assessment, mapping of the locations, ecotoxicological surveys and human-health assessment are being carried out together with epidemiological health surveys related to pollution.

Aquatic ecotoxicological studies for water resources management

The Oil and Natural Gas Commission (ONGC) has made a contract with ITRC for detailed assessment of ecological impact of process water from oil fields in Gujarat. Thermal pollution of ecosystems by coolant water from superthermal plants is a serious problem in India. This problem has been referred by NTPC for finding a solution and designing national safety standards. At a time when water scarcity has surfaced at many places, the possibility of reusing industrial waste water for irrigation, aquaculture and industrial processes offers promise. The Central Pollution Control Board has given a contract to develop protocols for assessing the safety of water and the produce, to form national guidelines and to test the safety of effluent samples and food items. Analysis of fluoride levels in drinking water in several problem areas of the country has been undertaken.

Other activities

Information on toxicity of chemicals is regularly being disseminated to users in industries and government departments through the toxicity data unit and Distributed Information Centre on toxic chemicals of the Department of Environment at ITRC. Complete toxicological information on 230 important chemicals has been computerized and published as four volumes of ITRC Toxicity Hand Book Series.

As the national correspondent on toxicological matters, ITRC has been interacting with the International Programme on Chemical Safety of WHO, Geneva, and the International Register of Potentially Toxic Chemicals of WHO.

ITRC has been offering its services for the assessment and abatement of problems in occupational and environmental toxicology which are undertaken on sponsored basis. Toxicity evaluation is being done using standard protocols in conformity with WHO/OECD guidelines. It is planned to create a national facility for safety evaluation of chemicals.

A standard protocol based on leachable plastic materials was developed for evaluating the risk of using plastic containers for food, water, transfusion materials and biomedical implants. This has now been adopted by regulatory agencies.

Consultancy services are being provided on various aspects of predictive and preventive toxicology, management of occupational/environmental problems, design and fabrication of equipment, and hazard assessment through mathematical modelling.

A fully equipped environmental-monitoring facility, which is recognized as a state air laboratory, to undertake detailed quantification of pollution load, including gases, dusts, hydrocarbons, noise, etc., is available which has solved several problems throughout the country. As a part of this service, ITRC has prepared a document entitled 'Emergency preparedness plan' for the Hazira gas processing complex based on extensive surveys to appraise any possible chemical disaster and assessment of potential accident-prone situations and to suggest a strategy for its containment.

ITRC has entered into contracts for time-bound projects with ITI, Mankapur, and National Thermal Power Corporation, New Delhi, for conducting environmental impact assessment studies.

Immediately after the Bhopal gas tragedy ITRC's expertise was summoned for containment, emergency response and follow-up. Based on this experience and expertise ITRC has furnished necessary information on chemical accident prevention to regulatory agencies. Detailed multimodel experimental studies on the toxicology of MIC after inhalation were conducted as a project sponsored by the central commission.

Human resources development programme was undertaken as one of the important activities of ITRC since institutionalized formal multidisciplinary training in toxicology is yet to be developed in India.

Concluding remarks

It is gratifying that ITRC could contribute to be in the vanguard of national scientific activities especially in the area of development without destruction through chemical safety as evident from some of the salient services to the nation and science mentioned above. This has been possible only through the sustained support and encouragement of the people and the Government of India, CSIR, and the scientific community. The same commitment will continue and the capabilities, facilities and accomplishments will further enhance the recent trend of exponential expansion of the nation in coming years. With this firm determination, ITRC is entering its second quarter century.

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