# Disclaimer

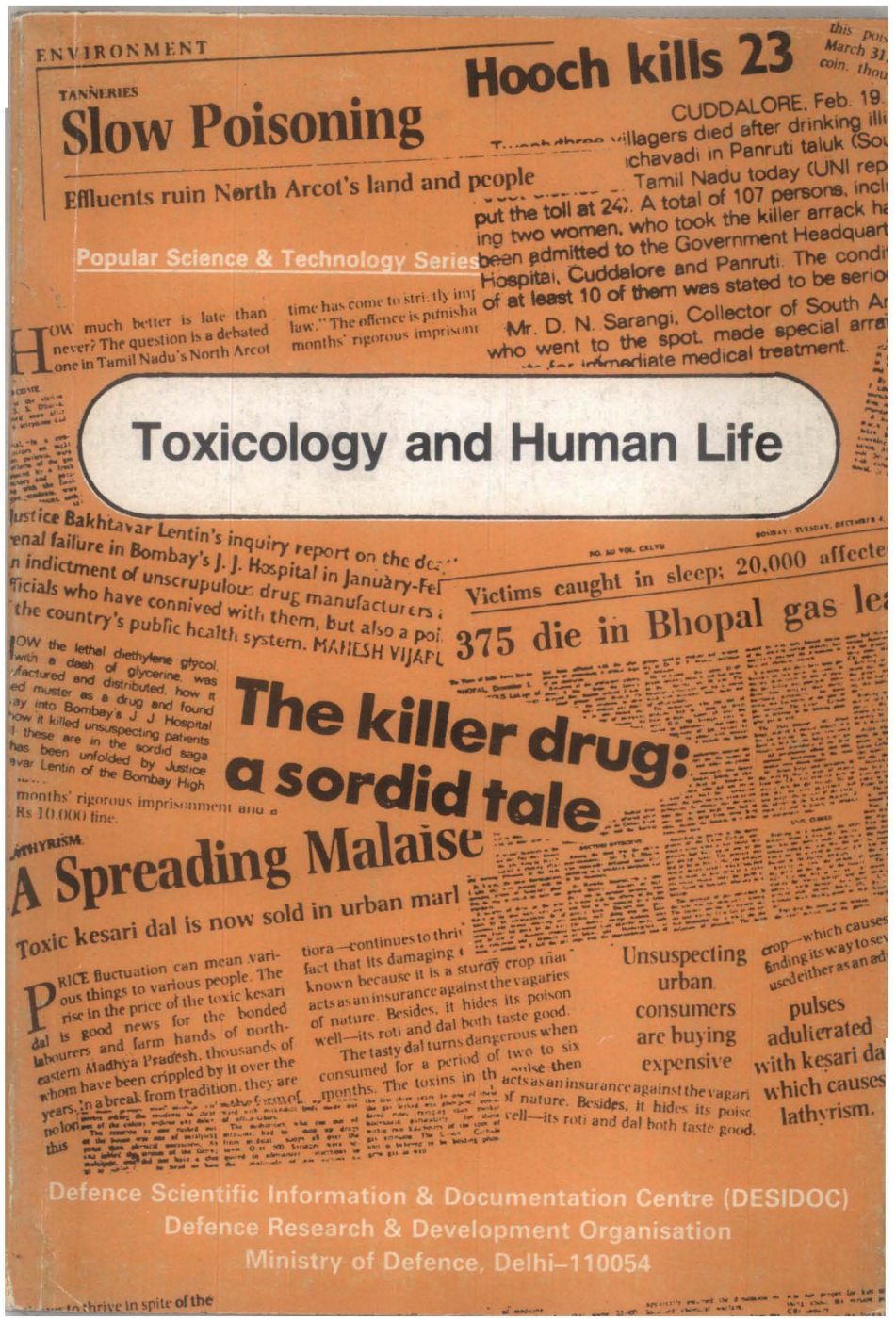
Popular Science & Technology (PST) series is being published by DESIDOC to promote the knowledge and understanding of the applications of science and technology in Defence among defence personnel, students, and general public. The contents covered in each of the titles are current to the year of publication.

**This title Toxicology and Human Life was published in the year 1989.**

**For subscription details please contact:**

Director
Defence Scientific Information & Documentation Centre (DESIDOC)
Ministry of Defence, DRDO
Metcalfe House, Delhi – 110054.
Tele: 011 – 2390 2527/29; Fax: 011 – 2381 9151

# Toxicology and Human Life



ENVIRONMENT

TANNERIES

## Slow Poisoning

Effluents ruin North Arcot's land and people

Popular Science & Technology Series

HOW much better is late than never? The question is a debated one in Tamil Nadu's North Arcot

time has come to strictly imp law." The offence is punisha months' rigorous imprisonm

## Hooch kills 23

CUDDALORE, Feb. 19.

villagers died after drinking illi ichavadi in Panruti taluk (Sou Tamil Nadu today (UNI rep put the toll at 24). A total of 107 persons, incl ing two women, who took the killer arrack ha been admitted to the Government Headquart Hospital, Cuddalore and Panruti. The condi of at least 10 of them was stated to be serio

Mr. D. N. Sarangi, Collector of South Ar who went to the spot, made special arra for immediate medical treatment.

this pos March 31 coin. thou

Justice Bakhtavar Lentin's inquiry report on the de renal failure in Bombay's J. J. Hospital in January-Fe an indictment of unscrupulous drug manufacturers officials who have connived with them, but also a poi the country's public health system. MAHESH VIJAPU

HOW the lethal diethylene glycol with a dash of glycerine was ufactured and distributed, how it ed muster as a drug and found ay into Bombay's J. J. Hospital how it killed unsuspecting patients these are in the sordid saga has been unfolded by Justice avar Lentin of the Bombay High

months' rigorous imprisonment and a Rs 10,000 fine.

## The killer drug: a sordid tale

Victims caught in sleep; 20,000 affecte

## 375 die in Bhopal gas le

LATHYRISM

## A Spreading Malaise

Toxic kesari dal is now sold in urban marl

PRICE fluctuation can mean various things to various people. The rise in the price of the toxic kesari dal is good news for the bonded labourers and farm hands of northeastern Madhya Pradesh, thousands of whom have been crippled by it over the years. In a break from tradition, they are no lon this

tiora—continues to thri fact that its damaging known because it is a sturdy crop that acts as an insurance against the vagaries of nature. Besides, it hides its poison well—its roti and dal both taste good.

The tasty dal turns dangerous when consumed for a period of two to six months. The toxins in th

Unsuspecting urban consumers are buying expensive pulses adulterated with kesari dal which causes lathyrism.

crop—which causes finding its way to sev used either as an ad

acts as an insurance against the vagari of nature. Besides, it hides its poise well—its roti and dal both taste good.

Defence Scientific Information & Documentation Centre (DESIDOC)
Defence Research & Development Organisation
Ministry of Defence, Delhi–110054

to thrive in spite of the

Popular Science & Technology (PST) series is published by DESIDOC to promote knowledge and understanding of the applications of science and technology in Defence among Defence personnel, students and the general public. Since the aim is to create awareness of current developments in frontier areas of science and technology among these groups, the presentation of material in the PST publications is lucid and generally in non-technical language. The text is supported by illustrations. Each issue of PST is devoted to a particular topic of current interest. PST is a half-yearly publication.


**Editorial Staff**

| | |
|---|---|
| Chief Editor | S S Murthy |
| Editor | Anuradha Ravi |
| Asstt Editor | S S Bagga |
| Production | Ashok Kumar<br>S B Gupta |
| Sales | G P Uniyal<br>R Kohli |
| Cover Design | Babu Lal Prasad |

Published by DESIDOC,1989

# Toxicology and Human Life

P.K. Ramachandran
Emeritus Scientist
Defence Research & Development Establishment
Gwalior

Defence Scientific Information & Documentation Centre
(DESIDOC)
Defence Research & Development Organisation
Ministry of Defence, Delhi-110 054.

# Foreword

DESIDOC is to be commended for its signal services towards popularisation of science in frontier areas by publishing books under its banner Popular Science and Technology (PST) series.

Although poisons have been known to man from time immemorial, during the last few decades, their implications have been widened beyond comprehension. The rapid growth of the chemical and pharmaceutical industry has unleashed the spectre of potential hazards not only to man, animal and plant kingdom but is also causing great concern about pollution of the land, air and water resources. Before harnessing the ever-increasing array of chemicals for the larger good of mankind, it is imperative to know all about the potentially hazardous chemicals and the steps to be taken to remove their adverse health effects. It is a matter for some consolation that, quickly on the heels of the phenomenal progress in several fields in science and technology, the new science of toxicology has arrived. Its ramifications extend beyond the confines of known emerging scientific frontiers particularly chemistry and pharmacology, pathology and pathophysiology, biochemical and metabolic detoxification, immunological responses, foetal losses, congenital malformations, teratogenicity, genotoxicity and the Damocles's sword of cancer not to speak of the cell of inexorable progression of systemic diseases of the respiratory, cutaneous and nervous systems.

Much enlightenment on these obscure or grey areas has been achieved with the application of refined and

up-to-date methods of investigative and analytical techniques of forensic sciences and experimental toxicology, on the one hand and the regulatory law enforcing agencies on the other.

Dr. P.K. Ramachandran has suceeded admirably in epitomizing the newer knowledge of toxicology in a free, lucid, elegant, easy and comprehensible manner, even by lay persons. He has covered succinctly the diverse subjects such as Historical landmarks in the growth of toxicology both during war and peace, growth of sophisticated methodology of clinical, forensic and experimental toxicology, assessment of body's defence mechanisms, problems associated with the treatment or therapeutics and management and up-to-date methods of chemical analyses of tissues and body fluids. Under the broad canopy of toxicology, due attention has been paid to some of the well-known areas of food and microbial toxicology, drug toxicity and the dangers of environmental pollution.

All in all, this small book with discreetly packed information, is bound to arouse curiosity and open up new vistas of ecological harmony. By harnessing toxicology especially from the analytical, promotive and protective aspects, is the price of eternal vigilance when the society cannot but choose the path of scientific progress of which Chemical Industry is an integral part.

(S. Sriramachari)
Addl. Director General (Retd.) ICMR
INSA Senior Scientist
Institute of Pathology, ICMR

New Delhi
February 1990

# Preface

'A little knowledge is a dangerous thing' says the old adage; it cannot be truer than in the case of toxicity of items in day-to-day use. And to add on to the already confused mind of an average educated housewife, sensation mongering lay media with the connivance of publicity seeking scientists would splash head lines "Petrol is carcinogenic" or "Diesel produces skin rashes" hardly ever reminding the reader that Petrol is not drunk by humans or diesel is not used for bathing atleast by the Indians. Toxicity is a dose-related phenomenon, pertinently meaningful only if th end-use of the substance is reckoned with.

It is hard to record many of these facts of science in popular language without loosing the precision of expression; however, an attempt has been made in this booklet to put facts straight, as also to give a general understanding of the involvement of the science of toxicology in all aspects of human life.

Many of the mass toxic episodes in the book have been occurrences abroad; it is not that such did not happen in India prior to Bhopal, but have been poorly recorded. Careless and ignorant handling of chemicals, as well as intentional adulteration have led to loss of a large number of human lives. Insecticides alone account for a large number of deaths, accidental, suicidal and homicidal, which possibly would increase in the years to come. But unlike in the developed world, all these incidents fade out of public memory very fast, till the next such incident occurs.

There are many aspects of toxicology which could not be dealt in here, as the impact on legal systems and enforcement legislations. May be that would have made unpalatable reading.

(P.K. Ramachandran)

Gwalior,
September, 1989

# Acknowledgement

Shri D.K. Aherwar who helped in transfering all my thoughts from the paper to the word processor has earned my sincere gratitude; he has done an excellent job in record time. Shri S.S. Rao, Scientist C helped me by reading the manuscript as also with suggestions and ideas. I record my sincere thanks to both these collegues of mine at this establishment.

## In This Issue


| | | Page |
|---|---|---|
| | **Foreword** | 1 |
| 1. | NIGHT VISION TECHNOLOGY | 9 |
| 2. | INFRARED OPTICS | 25 |
| 3. | PASSIVE IMAGE INTENSIFIERS | 31 |
| 4. | THERMAL IMAGING | 43 |
| 5. | NON-THERMAL NIGHT VISION | 51 |
| 6. | APPLICATIONS OF NIGHT VISION TECHNOLOGY | 57 |
| 7. | DEVELOPMENTS ON NVDs IN FOREIGN COUNTRIES | 65 |
| 8. | DEVELOPMENTS ON NVDs IN INDIA | 119 |

# 1. What is Toxicology?

Toxicology is not a study of poisons today, as many would say in one breath; and also quote in support Webster's dictionary. Science has left the lexicographer way behind.

Then what is it? Toxicology is the study of interaction of materials (drugs, chemicals, foods, polymers, pesticides, etc.) with a biological system and its responses. In simpler terms, it is the study of the changes in a living being, especially its different organs, on exposure to or administration of a chemical in different doses.

The word 'poison' has in fact lost its relevance today. The Poisons Act was legislated in 1918 and much has happened in science since then. Centuries ago, Paracelsus who can be called the father of toxicology, said, all substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy. An old Sanskrit adage says that even nectar (amrit or that which removes mrtyu or death) is a poison if consumed in excess. This has been proven more than correct in recent times. Response is related to the dose and the same chemical, which acts effectively as a drug in low doses, could be a poison in higher doses. Mercury and arsenic were two of the remarkable remedies for many illnesses in the early days of allopathy; today, no country will ever permit a new drug containing mercury or arsenic to be introduced without in-depth toxicological evaluation.

Apart from the normal responses, many chemicals could produce adverse reactions on widespread use. A very interesting case relates to hexachlorophene, widely used since 1945 as a mild bactericide in talcum powders and soaps. In the 1970'5, hundreds of children were affected in France and over 30 died after using a talcum powder containing five per cent hexachlorophene. In fact, the chemical had been cleared after necessary sub-acute study by administration to rats for 30 days. On a re-evaluation In of the toxicology of the drug, it was found to react with a white matter in the brain and spinal cord; it interfered with the mitochondrial metabolism leading to myelin (covering of nerves) degradation which resulted in spasms, convulsions and coma leading to death. The chemical was subsequently banned from cosmetic use in many countries.

The role of the toxicologist is clear from the above episode. His function is to study all desired and undesired effects of a chemical (a drug, an insecticide or any material) specially taking in view the end use. He has to lay down the safe dose as well as the toxic dose. If it is a drug for treating a disease, the safety margin or the gap between the safe dose and the toxic dose should be as large as possible, unless it is absolutely warranted as in the case of a terminal disease like cancer. Most of the time a toxicologist is active in collecting safety data or lack of toxicity.

Toxicology is essentially a predictive or speculative science, as humans cannot be used in experimenting with unknown chemicals. All toxicological studies start with rodents, that is, rats and mice. This mistaken on to larger animals like dogs and monkeys, when the situation demands. The results or the animal studies have to be extrapolated to humans

and this is the most difficult exercise, considering the wide difference in the organ systems of men and animals.

We can also define toxicology as the science that defines the safety of chemicals. This science is a melange of many disciplines with everybody claiming that his discipline only can make a toxicologist. The medical doctor, whom you ring up in a hurry, when a child eats up her mother's facial make-up, is a clinical toxicologist (not available in India). Here poisoning cases are mostly attended by anaesthesiologists, even in leading hospitals. Poisons and antidotes form a small part of the toxicologist's study. The medical pharmacologist, who does the clinical evaluation of drugs has a role as a toxicologist, if he is observing the adverse symptoms. The basic science of toxicology encompasses biochemistry, pharmacology, pathology, and, to a good measure, chemistry. The forensic toxicologist who goes into the cause of death played a dominant role in the earlier part of the evolution of toxicology. Statistical probability being an important ingredient of prediction, one cannot ignore the role of bio-statistics in toxicology

## 1.1 IMPORTANCE OF TOXICOLOGY

Since toxicology is the science laying down toxicity parameters of chemicals or materials; naturally toxicologists have to work closely in association with regulatory agencies for enforcing discipline in private industry. This evolution itself has a very interesting past.

The post-1950 years found an immense boom in the chemical industry with a variety of chemicals and materials being produced for human consumption like DDT, organophosphorus pesticides, many new drugs, polymers, etc. About. 500-1000 chemicals were being added to the market stock every year without much of a detailed investigation. In 1962, it took a lesser known lady, Rachael Carson, with her explosive writing Silent Spring to rudely awaken the U.S. public to the stark realities of indiscriminate use of most of these chemicals. The book was a sensation and marked the beginning of the environment movement in the U .S.A. Close on its heels happened the thalidomide. tragedy in Federal Republic of Germany, a few other European countries and England. Thalidomide was discovered as a safe sedative for pregnant women in 1954 by Chemie Gruenenthal in Germany and Distillers Company in U.K. It was marked under different names like Distaval, Contergan, etc. The large number (4000-5000) of deformed. Children born during a short period alerted the medical profession and toxicologists, who traced the deformities as due to thalidomide taken during the first seven weeks of pregnancy. Further toxicological investigations in animals proved thalidomide to be a teratogen or a chemical capable of producing malformations in foetus. The U.S. Food and Drug Administration had refused permission for import of the drug, not for teratogenicity, but for suspected production of nerve lesions.

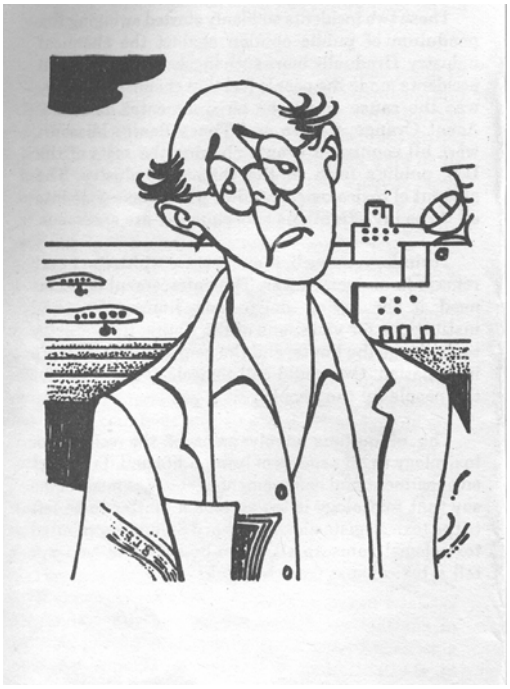
These two incidents suddenly started swinging the pendulum of public opinion against the chemical industry. Gradually more such incidents and chemical accidents made the people feel that chemical industry was the cause of all the environmental disasters. Agent Orange, Seveso and Times Beach, Missouri l were all connected events shaking the root s

of the U.S. public's faith in the chemical industry. The amount of toxicological studies which have gone into all these investigations subsequently are enormous.

Toxicology, as such, is one science which can never remain in an ivory tower. The enforcement agencies need it for laying down safe limits; the legal institutions for violations of the limits; the industry to challenge the limits; and the general public for more information. One could call toxicology a science 'for the people by the people'.

The ubiquitous involvement of the science of toxicology in all aspects of human life and its impact on enactment and enforcement of statutes made some say that toxicology is too serious a matter to be left to the toxicologists alone. Gerhard Zbinden, a reputed toxicologist sums up all, when he says that 'you can tell a toxicologist from his looks'!

# 2. History of Toxicology



Toxicology predates man and in many ways is a part of his history; and to be true to the lexicographer, the early days of toxicology began with an interest in poisons from plants and animals.

## 2.1 THE EARLY ERA

The early civilizations, the Hindus, the Egyptians and the Greeks were unduly concerned about poisons; mystifying poisons with divinity. Hemlock was the state poison of Greeks; aconite an arrow poison of the Chinese and peyote worshipped as a cult by South American Indians. Hippocrates was the first to introduce primitive principles of toxicology around 400 BC. He gave quite a few tips to prevent the absorption of toxins.

## 2.2 THE MIDDLE AGES

Down to the middle ages, the royal families appear to have had an obsession with poisons. The poisoner seemed to be an integral part of the scene as in many Shakespearean plays, as a political tool and as a custodian of a common social expedient. Poisoning was perfected as almost a sport with codes of unwritten rules of honour and fatalistic attitude of the selected victim. Out of this milieu rose up Catherine de Medici (1519-1589), the Italian queen of France. She was, so to say, the first experimental human toxicologist. Under the guise of treating the sick and the poor, Catherine tested toxic concoctions O on humans; carefully noting the rapidity of onset of actions,

effectiveness of the chemical, action on different parts of the body and complaints of the victims.

But amongst all stood out marl who had a farsighted wisdom and understanding of the science of medicine and toxicology-Philippus Aureolus Theophrastus Bombastus Van Honheim or simply Paracelsus (1490-1541). He challenged the existing Aristotelian tradition of a divine intelligence controlling the world. Paracelsus viewed the human body as a conglomeration of many existing elements.His foremost contribution toxicology was the Identification of toxicon, a chemical entity, and the interrelationship between chemical structure and toxicity. Paracelsus held the view that dose and response were related and can be experimentally proved. He also differentiated between the therapeutic (curative) and toxic effects, as also the specificity of action of chemicals. His Interests were very wide and covered the whole gamut of present-day toxicology. Even in the 20th century, many medical historians have not been able to gauge the long-lasting contributions of Paracelsus. As one author has commented, Paracelsus was the first to turn the magic of toxicology and medicine into a science.

## 2.3 TILL THE MIDDLE 20TH CENTURY

Matthieu Joseph Bonaventura Orfila (1787-1853), a cat Spanish physician occupying a place of respect in the court of Louis XVIII was the first chemist to identify toxicology as a new discipline and brought out a monograph titled *Study of "Poisons or General Toxicology* in 1813. Being a chemist, he introduced analytical techniques for enquiring into the cause of homicide and suicide. At that time, Marsh came out with his famous test for arsenic, which was one of the widely used agents for murder. With the advent of an analytical approach to autopsy, the hold of the occult as a cause of death gradually started dissipating. Orfile rose to become the Professor of jurisprudence at the University of Paris. He pioneered forensic toxicology.

Around this period, a few others like Francois Magendie (1783-1855), a leading physiologist, got fascinated with the study of mechanism of action of emetine and strychnine. His infectious interest spread to his students, amongst whom was the later celebrated physiologist, Claude Bernard (1813-1878).He used poisons as tools in analyzing physiological aspects of organic systems. Bernard's study of curare, the arrow poison recognized.as a ganglion blocking- agent; strychnine, the bitter alkaloid from Nux Vomica nuts; and carbon monoxide-haemoglobin association were landmarks in toxicological studies.

Coming to the twentieth century, Loris Lewin(1854-1929) contributed a text book of toxicology. Dr.Ramnath Chopra's fine book on indigenous drugs of India in 1933 is another notable study. Initially, there was a tendency to treat toxicology as a part of pharmacology; but with all the developments and catastrophies, toxicology did not take much time to catch public attention. Toxicity has now become a much misused household word, as a too politicised word

## 2.4 WAR AND TOXICOLOGY

Rapid progress in all applied sciences owe greatly to wars-Fermi's nuclear chain pile on a Chicago to university tennis court in 1942 within a span of in twelve years became a devastating weapon of PE destruction at Hiroshima and in another five years p~ a source of energy in the first nuclear power plant in the U .K. Did toxicology too get its share from the wars?

The use of chemicals in World War I from 1916-1918 forced scientists to study the toxicity of a variety of compounds and engage in synthesizing more toxic chemicals. While using any of these compounds, antidotes also had to be stocked, for a If possible retaliation in kind.

Toxicology of phosgene used by Germans in 1916 was studied well at that time but the mechanism of action was wrongly attributed to the hydrochloric acid produced in the system. when gas masks were introduced, compounds which could be taken In through the human skin called mask-busters like lewisite (named after Prof. Lewis, its discoverer), or 2 chlorovinyl Arsine, and sulphur and nitrogen mustards were introduced. An excellent piece of work on the mechanism of action of lewisite on human body by Carl Voegtlin and associates in 1924 led to the discovery of dimercaprol as an antidote-the well known British Anti Lewisite or BAL by RA Peters and group in 1945.

More interesting were the developments during World War II (1939-1945) especia11y in the area of organophosphorus chemistry and toxicology .Gerhard Schrader at Bayer, Germany and Prof.B.C. Saunders t at Cambridge were on a neck-to-neck race for the production of highly toxic phosphonates or nerve gases. When the war ended in 1945, Bayer was able to cash on their knowledge of basic toxicology by introducing a number of organophosphorus pesticides, starting with highly toxic tetra ethyl pyrophosphate, Parathion, Schradan and others and gradually reducing the mammalian toxicity by moving over to carbamate insecticides.

Development of antidotes to nerve gases has also been equally interesting. When the action of nerve gases was recognized as inhibition of cholinesterase enzyme, attempts to revive the inhibited enzyme by externally administered reactivator chemicals were made. A bit of deductive logic and more of serendipity led Wilson and Nachmanson in 1956 to synthesize and test the efficacy of pyridine-2-aldoxime hydrochlorides (2 PAM-Cl) in combination with atropine. Till today, this 2 PAM-Cl has held the field even though its high efficacy could not be understood fully

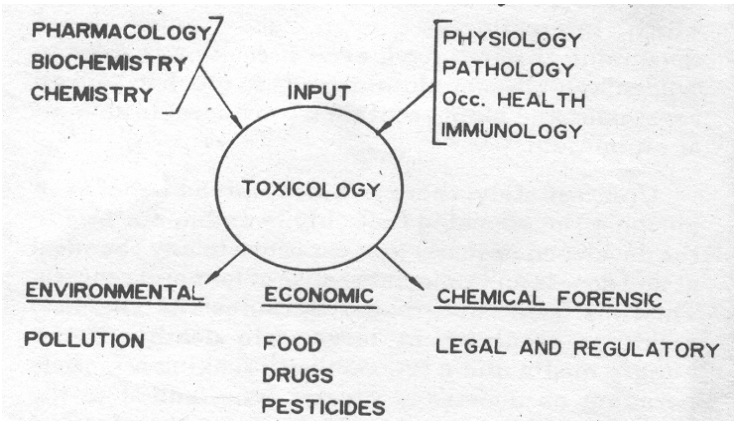
When Alfred Hoffmann described a new type of hallucinogenic compound, lysergic acid diethylamide (LSD), around 1943 this mind twister definitely caught public attention. Some wit in Pentagon thought that this would be the ideal compound to conquer without ki11ing but unfortunately, he did not cater to the diversity of human kind and mind. A variety of natural compounds were isolated and quite 11 a few were synthesized too in this field of psychochemicals. These types of compounds today have become social poisons an big business too! But no one can blame toxicology for it.

# 3. Role of Toxicology

Toxicology has its impact on human life, from the stage of conception in the womb to the last moments of life. There are chemicals which can harm the sperms as wen as the ova. Teratogenic chemicals malform the foetus. Neo-natal deaths can also happen from chemicals ingested by the mother. Disease of the o]d age, like Alzheimer's disease are being attributed to once-upon-a-time innocuous aluminium, which interesting]y enough, one cannot avoid consuming through food, even if cooking is done in 'golden' vessels-for aluminium is so much in soil; all vegetables and plants contain a considerable amount of aluminium.

Unfortunately, there is a widespread belief even amongst the educated (not only in India, but a]so in the developed nations) that exposure to any chemical at any dose is an immediate cause offer and concern. This has been exacerbated by industrial chemical accidents resulting in large-scale deaths. News- hungry media and a few publicity seeking scientists parading as doomsday oracles have added to the chaos. Interestingly, analytical instrumentation and techniques have improved so much, that we can identify quantities as small as 10-9 or 10-12 g. forgetting the stark reality that we no longer live in a pristine civilization, these scientific findings are turned into social and political problems almost driving the general public to mass hysteria.

When emotions yield place to logic and reason, science finds it difficult to establish the truth. This is where the most important role of a toxicologist comes in-the assessment of the 'chemical-risk- potential' by in *vivo* studies on appropriate test species or in vitro model systems, collecting. Quantitative and quantitative data and, utilizing the limited scientific data, predicting the probability of occurrence of a given adverse reaction In a normal human being by extrapolating from animal data. And world certainly cannot be improved by scientists who cry 'wolf' at every mouse in an attempt to hit the newspaper headlines. And it is this that a true toxicologist has to counter effectively.



What goes into the making of a toxicologist? As more on date, there is no university in India giving a formal course of instruction and degree in toxicology; so any one in the constituent disciplines can move over and function as a toxicologist by virtue of his experience. Advanced countries, of course, offer toxicology as a separate subject

Toxicology, being concerned more with poisons in the beginning, had a place with medical jurisprudence; the normal functioning of a biological system go to make physiology and abnormal functioning forms pathology. The effect of any chemical is studied pharmacologist and the fate of a chemical in the biological system by the biochemist. The analytical chemist with his instruments and ho techniques capable of detecting, identifying and quantitatively estimating very small quantities of chemicals and degradation products in biological fluids or environment plays a big role. Changes in the immune status form apart of the newly arising field of immuno-toxicology. A basic understanding of all the above areas would go to make a toxicologist. Above all, he should be a level-headed, practical thinker who should be designing his experiments and interpreting results based on the end-use of a particular chemical. For example, a tear gas cannot be toxicologically tested as a drug, because the hazards or risk from an occasional exposure to tear gas is different from that of a drug which has to be taken daily for curing an illness, So the regimen of testing in both cases have to be different. Similarly, an insecticide cannot be tested like a food-additive; the insecticide is meant to destroy insects and it will have a certain toxic effect on humans, while a food- additive which would be taken in very frequently has more chances of getting inside the biological system to produce long-term effects.

The study of toxicology could be taken up system-wise as well as agent-wise. Thus we have *systemic toxicology* involving toxicology of central nervous system, liver, kidney, respiratory system and predict eyes. The study would include defences of each of proper these systems against assault from foreign chemicals, responses and reactions as also injuries caused by excess.

An agent-wise study would take up drugs, pesticides, food additives, chemicals, heavy metals, polymers, radiation, chemical carcinogens, teratogens, etc. There is hardly much of again generalization which can be done and many of the agents have to be studied individually in different species before coming to any conclusion.

Application of toxicology for practical purposes can be broadly divided into three are as which are not rigid groups but flexible and overlapping.

- Clinical and forensic toxicology where medical science has a role;
- Industrial or economic toxicology which includes drugs, pesticides, foods, additives, containers (like cans, boxes),etc;
- Environmental toxicology incorporating pollution studies, residue analyses, industrial hygiene and occupational health.

For the sake of convenience, this book will deal with the subject in the above format.

There are many other divisions and names given by different authors. When standard procedures may not be able to predict the toxicity in man, then the predictions have to be based on chemical and physical of properties, molecular structure-biological activity relations, pharmacokinetics, etc. This is termed 'speculative toxicology'. There is an element of speculation in this study.

'Comparative toxicology' is the study of different species of animals to evaluate how close these are to human systems. Geographical or ethnic toxicology is again a recently evolving area, concerned with the puzzling phenomenon. That certain toxic drug reactions seem to be more frequent in particular countries or on one continent. It analyses various factors-climate, nutritional practices, genetic factors and other environmental influences-which might account for these differences in the responses of the human body, to a foreign chemical.

One could add on a long chapter on 'political toxicology' or as someone else named it, 'bio-politics'. Most of the difficulties a toxicologist encounters are not scientific or technical but political, psychological and sociological. The toxicological results can be distorted by any of the above forces to suit their purpose. A toxicologist, very philosophically pondered that 'only when avarice and ignorance are eliminated from the human kind, will toxicology become a dull study'.

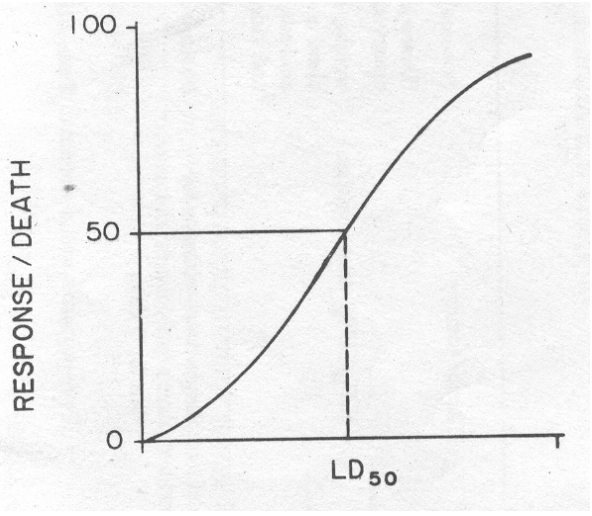
# 4. Index of Toxicity and Laboratory Determination

The potential toxicity of chemicals have to be evaluated on laboratory animals. These initial studies have to be designed in such away that maximum information for prediction of human toxicity would be available.

For any chemical there are three phases for production of a response: exposure phase, toxicokinetics (absorption, distribution, metabolism and excretion) and toxicodynamic phase (chemical reactor interaction)

A chemical can enter the human system in three ways-through nose (inhalation), mouth (ingestion) and skin. When it is a medicine, it can be given as an injection also, i.e., parenteral administration. Injections can be subcutaneous (just below the skin), intravenous (into the vein and directly to blood circulation) and intramuscular (into the muscles). In experimenting with sma1l animals, it is given intraperitoneal or into the visceral cavity or peritoneum. By each one of these routes, the effects produced are different. In normal practice, responses by different routes are elicited so that differences can give some clue to the metabolism of the chemical.

Three types of tests are carried out on laboratory animals acute or single dose effect; sub-acute or prolonged tests where single doses are given daily for 90 days; and chronic tests which involve administration over 90 days (up to one year). An outline of various animal toxicologic tests is given in Table 2.1.




The toxicity has to be measured quantitatively and this is doneby estimating the dose needed to kill 50 per cent of the animals in a group. This is called be Lethal dose50 and abbreviated as LD60. The route of dies administration, species and sex have to be specified. The dose-response curve is linear in the major portion of the graph. This value has to be statistically expressed, with a confidence limit of reproducibility fi for 90 or 95 tests per 100. Several methods are available for such calculations like Litchfield and Wilcoxon (1949) logarithmic probit graph paper method (1944), range finding procedure of Weil (1952), Finney's method (1964) and Dixon's up and down method (1964). The LD5o is expressed as milligrams per kilogram weight of the animal used; dose lesser the LD5o value numerically, higher the given toxicity.

Table 2.1 : Animal toxicologic tests

| *S No.* | *Tests* | *Acute toxicity* ($LD_{50}/LCT_{50}$ determination) | *Sub-acute toxicity* (Doses lower than $LD_{50}$ and observations on weight, animal morphology, blood chemistry, urine analysis, haemotology, liver and kidney functions) | *Chronic toxicity* (Very low doses and observations on state of health, weight physical examination, liver and kidney function tests) |
|---|---|---|---|---|
| 1. | Duration of administration | Single dose-observe for death in 24 hours and survivors for 7 days | 90 days | For about one year (90 days depending on animal's life span) |
| 2. | Species | Two-a rodent and a mammal | Two-rat and dog | Selected on the basis of pharmaco-dynamic studies |
| 3 | Route of administration | Two-one to be the intended route | Intended route of use | Intended route of use |
| 4 | Dose level | To be found | Three dose levels | Two dose levels |
| 5 | Autopsy | No | Yes, including histology of organs | Yes, including histology of organs |

In addition, there are some special tests: (8) Potentiation with other chemicals, (b) Effects on fertility, (c) Teratogenicity, (d) Mutagenicity/carcinogenicity.

If the chemical whose toxicity is to be determined is a gas, vapour, aerosol or dust, then the lethal dose has to be calculated in a slightly different way. Exposure can be in static or dynamic systems. The Sodium duration of time for which the animal is exposed to a particular concentration would become important. So, for inhalation exposure, the toxicity index is expressed as $LCT_{50}$ or Lethal Concentration Time for killing 50 per cent of the animals used. This is expressed in units of milligram per litre, multiplied by exposure time in minutes.

Table 2.2 gives the $LD_{50}$ values which can be used as a rough index of human toxicity of chemicals.

## Table 2.2: LDIO values

| S.No. | Oral LDso | Effect |
|---|---|---|
| 1 | > 15g/kg | Harmless |
| 2 | 5.0-15g/kg | Non-toxic |
| 3. | 0.5-5g/kg | Slightly toxic |
| 4 | 50-500 mg/kg | Moderately toxic |
| 5 | 5-50 mg/kg | Highly toxic |
| 6 | < 5mg/kg | Extremely toxic |

Most of the medicines (drugs) fall in category 4. Thus aspirin or largactil may appear 'more toxic' than a favourite brand of whiskey; the volume consumed and cumulative effect not being the criterion here.

For comparison and understanding, oral LD50 doses of a few "Yell known chemicals for humans are given in Table 2.3.

## Table 2.3: $LD_{50}$ levels 'of IOme common chemicals

| Name | $LD_{50}$ value |
|---|---|
| Sodium chloride (Common salt) | 12.357 g/kg |
| Copper sulphate (fungicide) | 272 mg/kg |
| Phenobarbitone(sedative) | 36 mg/kg |
| Parathion (insecticide) | 5.67 mg/kg |
| Sodium cyanide | 2.857 mg/kg |

Toxicity cannot be based on LD50 values alone, as these are dependent on age, sex, weight, genetic background of animals, conditions of breeding and a variety of other factors.

In certain cases, the time of death of animals would make a lot of difference in the calculated LD50-for example, in cyclophosphamide (an acute cancer drug) oral LD50 for rats is 720 mg/kg after two days; 235 mg/kg after five days and 94 mg/kg after 14 days. The toxicity increases if delayed deaths are considered, but this is mostly due to the secondary action of the drug.

### 4.1 Sub-chronic or Prolonged Toxicity Studies

Most of the chemicals, especially drug and food additives, are taken in repeated doses and it is essential to know what would happen on long-term use. Normally a certain fraction of LD50 is used for such studies, but there need not necessarily be any consistent correlation between LD50 and chronic toxicity. For example, dexamethasone which has LD50 (subcutaneous) in rats of 120 mg/kg, could not be tolerated in daily doses of more than 0.07 mg/kg.

All chronic toxicity tests are accompanied by clinical evaluation of the animals and observation of animal behaviour. Routine biochemical studies like sodium, potassium, urea and glucose in blood; pH protein, glucose, ketone, bacteria in urine; Bromosulphalein retention or thymol turbidity for liver function; SGOT, SGPT'; etc. are done to understand which of the organs gets impaired.Haematocrit, total RBC (Red Blood Cell count), total and differential WBC, thromobocyte count, etc. are also studied. All dead animals are subjected to autopsy studies as also tissue pathology.

Apart from the above general tests, special tests may have to be designed based on the results obtained. Tests for potentiation of action by drug combinations, so also antagonism, allergic reactions, hypersensitivity induction, etc. are some of these.

Combination of EPN (o-ethy1 o-para-nitropheny1 phenylphosphonothioate), a medium level toxic chemical, and malathion, a very tow toxicity compound to rats increases the toxicity of each of the chemical by 12 times. similarly, compounds like phenobarbitone and chlorinated hydrocarbons which induce mixed function oxidase enzymes in liver, which can metabolise the foreign chemicals, reduce the toxicity of many chemicals.

Teratogenicity testing is a direct offshoot of the Thalidomide tragedy. Teratology literally is a study of monsters, and is now called as the study of congenital malformations. Foetal malformations can be genetic or malnutritional as well; so the chemically induced malformations have to be distinctly established by testing. Teratologic studies are normally carried out in guinea pigs or rabbits. The known human teratogens are not that many in number; some very useful drugs for humans found teratogenic in animals are salicylates (pain relievers or analgesics), Adreno-corticoids, Hydantoins anticonvulsants), for vitamins A and D and insulin. In most of these to experiments, the chemicals were used in very large doses. If a chemical is found teratogenic in testing, then the dose is reduced till the 'no-effect' dose is obtained. This is compared with the effective dose or therapeutic dose for predicting the safety of the chemical.

### 4.2 Carcinogenicity Tests

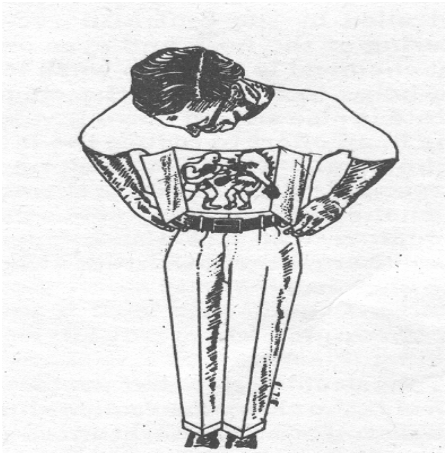
In the 1960's when the animal carcinogenicity tests gradually evolved, a large number of chemicals were found to be tumorogenic in rats. The familiar response was to pronounce the chemical as 'carcinogen' and ban production. Subsequently it has been realised that the testing procedures and prediction need to be re-evaluated because man cannot be treated as a 'mega-mouse' in the etiology of cancer and rodents are very susceptible to tumour growth by chemicals.

### 4.3 Mutagenicity Tests

A number of simpler tests that are less time- and animal-consuming have been developed for prediction of carcinogenicity. These tests carried out on bacterial test systems or animal cells help in cutting down time and indicting a chemical as capable of producing cancer. Further tests need be done only on 'positive' chemicals.

# 5. The War within the system

No living organism would accept a chemical without a fight and the toxicologist has necessarily to know how a biological system would face a particular chemical. An the tests mentioned in the earlier chapters record the happenings after a chemical gets over the body's defences and establishes itself in a system. But right from the moment of exposure to a chemical, a number of events occur which are the body's 'fight' with the 'aggressor' chemical.

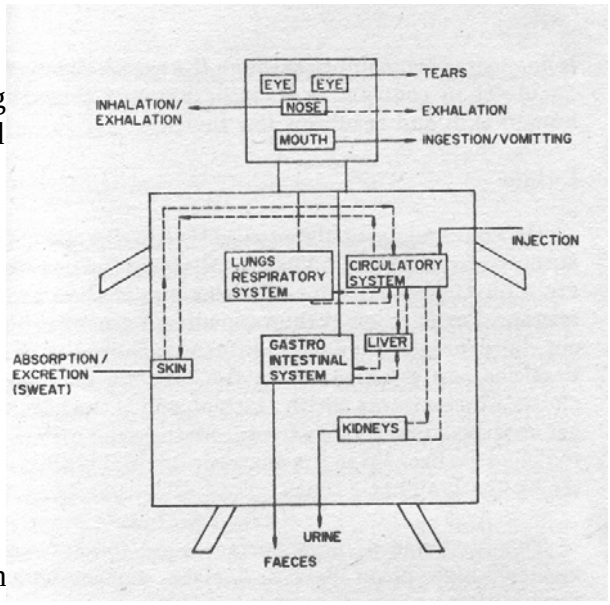


The route of entry into a human system can be through the nose, mouth or skin. After making the entry, the chemical has to get absorbed into the blood stream, get distributed to the tissues, metabolised, stored in the tissues or get excreted through urine or faeces. The three phases are (a) absorption, (b) distribution and metabolism and (c) excretion. The effects of a toxicant are dependent on these three phases and are manifested only when the chemical reaches a site of action, may be an enzyme, a receptor, etc. Caustic, corrosive and irritant chemicals of course show the effects right from the point of contact.

## 5.1 Peripheral Defences

Sneezing and coughing are two ways of getting rid of unwanted chemical from breathed-in air and these two are controlled by the central nervous system. Profuse watering of the nose and eyes on exposure to an irritant chemical is a way to wash it out of the body; it also helps to hydrolyse the chemical. The respiratory rate is also slowed down involuntarily at the beginning in an effort to reduce the intake of the offending chemical. Then comes nausea and vomitting, especially if the intake is through mouth, by which action the body tries to eject out the toxicant. Purging through rectum can also happen. Profuse sweating is another method, wherein sweat helps to wash out the chemical.

## 5.2 Absorption through Skin




The main physical barrier that protects the body wide from a chemical is the skin; an excellent lipoid (fatty) barricade consisting of horny keratinised epidermis, dermis and corium. The transport across the skin is mostly a passive process and fat/oil soluble chemicals is have an edge over water solubles in this process. If an ant or a mosquito bites one on the skin, immediately the body responds with a small swelling in which body fluids accumulate to wash out the toxin and prevent it from spreading. The skin also holds some hydrolytic enzymes which can detoxicate some of the chemicals. The permeability of skin varies widely between animals and man; the animals nearest to man in skin structure are pigs and guinea pigs. The effect of DDT as a contact poison in insects is due to its permeability through the exo-skeleton of absorb an insect in contact poison in insects is due to its permeability through the exo-skeleton of an insects in contrast to its poor passage through absorb human skin and resultant low toxicity.

## 5.3 Lungs

Gaseous and particulate toxicants can directly get absorbed from the lung. The cilia (hair) in the nostrils are a physical barrier for particles bigger than ten microns. Particles lesser than one micron are brought for out in exhalation or can diffuse within alveoli. Particles can get cleared by the mucous through glottis. The toxicants which reach alveoli of the lungs get absorbed directly into the blood; they can also be removed via bronchi to the gastro-intestinal tract, so too by the lymphatic route. The lung has a large surface area (50-100 sq. metres), high blood flow and close contact with external atmosphere. As such it is an important organ in detoxication.

## 5.4 Gastro-intestinal Tract

This is a major route for absorption of toxic chemicals gaining entry through food and water and is the most common route by which children are poisoned. Absorption of toxicants can take through the gastro-intestinal tract right from mouth to rectum; e.g. vasodilators like nitro-glycerine are given as sub-lingual capsules which are absorbed from the mouth. Enemas are administered rectally. The toxicants have to face a number of enzymes in their path, varied pH like high acidity in the stomach, the intestinal micro-organisms, etc. before getting absorbed. Fat soluble chemicals are more easily absorbed but water solubles are also absorbed from the intestines.

## 5.5 Distribution

The toxicant chemicals have to enter the blood stream for distribution throughout the body. This is the reason why medicines are injected intravenously for quick action. Chemicals can get stored in different tissues and get slowly released into the circulation. Carbon monoxide has a higher affinity for haemoglobin; lead gets stored in bones. Storage depots, in a way, prevent the toxic action, even though they prolong the life of the toxicant in the system. Protein-binding is one way of storage in the body and is a very important factor for toxicity evaluation.For example, antipyrine is not bound at all to plasma with proteins while seco-barbital is bound to the extent of organ 50 per cent and thyroxine to 99.9 per cent. In the management of premature infants, it was observed that penicillin-sulphonamide mixture treatment produced a large number of deaths. When this was investigated, it was found that penicillinsulphonamide mixture bound to plasma albumin displacing normally bound bilirubin which diffused into the brain producing severe brain damage consequent death. Switching over to tetracycline avoided this problem.

Liver and kidney have the capacity to eliminate a large number of toxicants from the blood. Similarly body fats store up toxicants like DDT, saving injury to the individual. The blood-brain barrier which prevents the entry of non-lipid soluble toxicants into the brain is an in-built safety system. This barrier is poorly developed at birth as evidenced by the high mortality from morphine and lead in newborns.

## 5.6 Excretion

All body secretions like tears, sweat and milk try to eliminate toxicants, but kidney and liver are the two major organs of detoxification and excretion.Kidney and liver function tests are of paramount importance to the toxicologist in assessing a chemical injury. The kidney eliminates the toxicants by passive glomerular filtration or active tubular diffusion into mixed the urine. High lipid soluble moieties are re-absorbed a pass and water soluble components are eliminated. Bases are eliminated more in an acidic urine and acidic compounds in a basic urine. Sodium bicarbonate administration to eliminate phenobarbitone from blood stream in poisoning cases is based on this finding-barbiturate being weak acid, alkalinity increases excretion

Organic acids, both anions and cations bound to proteins are eliminated by an active tubular secretory process. Various compounds compete with one another in this process. In the early days of penicillin, the half life of penicillin in the body was extended either by administration as a suspension in oil or by giving another acidic drug like probenecid as a competitively faster eliminated compound.

The liver is very critically situated for detoxication as the blood after absorption of toxicants from gastrointestinal tract passes through the liver. A number of bio-transformations take place in the liver and the metabolites can be excreted into the bile and on to the high the small intestines for faecal excretion. Diethyl stilbosterol (DES) is 130 times more toxic in bile duct ligated rats, showing that biliary secretion is the only

way of elimination of DES. Compounds which can induce liver enzymes like phenobarbitone increase the biliary metabolism of chemicals, so too are the drugs like spironolactone which can increase bile production and excretion

The lung has been found to have some drug oxidising enzymes like super oxide dismutase as also mixed function oxidases. It is no longer considered as a passive gas exchanger, but has an active role in detoxication process.

Unabsorbed material from oral intake is excreted through faeces. The best way of eliminating Paraquat, a herbicide which produces exclusive]y lung damage from accidental poisoning victims, is by binding it with an inert adsorbent powder and eliminating through rectum.

## 5.7 METABOLISM

It is obvious that many detoxification metabolisms have been developed in human beings during the evolutionary pathway. If humans were not exposed to environmental pollutants, these enzymes possibly could not have been developed, as there would not have been a need. Metabolism of the toxicant is one of the defence mechanisms of the body and includes (a) elimination of unchanged toxicant through urine, faeces and other excretions, (b) bio-transformation or modification of the structure mostly in the liver to make these more water soluble for excretion via kidney, (c) structure modification for detoxification alone and (d) host defence mechanism like immunity, tolerance, phagocytoses, etc.

All metabolic reactions are enzymatic and can be categorised into hydrolysis, oxidation and reduction in the first phase-occurring in the soluble mitochondrial or microsomal fractions of the liver. In the next phase, these bio-transformed molecules are conjugated with highly polar or ionic moiety to increase their water solubility. There can be more than one metabolite in many of these reactions and many other metabolic transformations as also alternate pathways in a biological system for an emergency; when all these pathways collapse by overburdening of the biological system by a toxicant, then only a man collapses.

# 6. Applied Areas of Toxicology

As has been said earlier, applications of toxicology have been broadly divided into (a) Chemical and Forensic Toxicology, (b) Economic Toxicology including food, drug and pesticide toxicology, and (c) Environmental Toxicology which includes pollution studies, industrial and occupational health.

## 6.1 CLINICAL AND FORENSIC TOXICOLOGY

### 6.1.1 Clinical Toxicology

A clinical toxicologist is concerned with humans-his, laboratory is the hospital and his experimental subjects are human beings, who do not give an option for experiments. A clinical toxicologist is concerned with the action of chemicals on human system and many a time he will have to attend on someone who has taken in a lot more drugs than one should normally have or a dose of pesticide to bring an end to one's life. He has also an important role in the controlled clinical testing of newly developed drugs.

A substantial portion of human illnesses are due to toxic substances: It accounts for about 1.6 per cent of an deaths in children under five years of age in the U.S. The main functions of a clinical toxicologist would be to attend on occupationally related and accidental poisoning, study of adverse effects of therapeutic agents in large-scale human trials, and attend on suicidal and homicidal cases involving toxic chemicals to bring back the victims to life by appropriate action.

Much information in clinical toxicology can come from epidemiology, i.e., by collecting information on victims of previous exposures. This is done in the U.S. through established poison centres, which handle poisoning cases, document these and provide information to other centres for their use. These are stored in computers and through a network can be retrieved by users.

Drug interactions within the human body where more than one drug is administered is a problem faced by the clinical toxicologist often. Barbiturate administration in epileptics increases the metabolism of diphenylhydantion (anti-convulsant), so that an increased dose becomes necessary. Barbiturates induce the liver enzymes which metabolise the hydantions. Severe haemorrhage (bursting of vessels) in a heart patient with thrombosis, discharged from hospital where he was stabilised on anticoagulants, used to be common. When the living barbiturate sedation is discontinued in the house, the metabolism of the anticoagulant is reduced, leading the to haemorrhage.

Another interesting instance is the protein binding with an elite corps of 1021 albumin molecules which is the most important defence mechanism of the biological system. There are other proteins and red blood cells to increase the binding. The bound part of the drug cannot immediately produce any action, but is also not metabolised fast. When two drugs compete for protein binding, many unexpected effects can happen as in the case of tolubutamide (oralantidiabetic drug) and sulphonamide (antibacterial). Both are bound to

proteins, but sulphonamide can competitively displace tolubutamide from protein-binding sites; if these two drugs are given together to a diabetic, it can result in hypoglycemia or reduction of blood sugar to dangerously low levels.

Old regimens of treatment are improved with better understanding of basic metabolism of the toxin, Cyanide intoxication used to be treated with nitrite for producing methemoglobinemia, which release the cyanide from cytochrome oxidase; subsequently sodium thiosulphate converts the circulating cyanide to harmless sodium thiocyanate. The recent advances have better compounds for production of methemoglobinemia like dimethyl aminophenone and dimethylpropiophenone; cobalt-EDTA and vitamin B12 have also been utilised usefully in therapy.

### 6.1.2 Forensic Toxicology

While a clinical toxicologist is concerned with a living human being and how to keep him alive if, intoxicated, a forensic toxicologist is concerned with the dead human, investigating the cause of death, utilising the same techniques as the clinical toxicologist. The forensic toxicologist can be called a medicolegal scientist.

About five per cent of all accidental deaths, and 25-30 per cent of all suicides involve poisons. In addition, for every case of suicide there will be 5-10 cases of attempted suicides, which are saved. by timely medical attention. A popular drug with suicidal maniacs is barbiturate as well as its derivatives normally used as hypnotics or sleep inducers in medical practice. Household insecticides are also a popular item.

Deaths from illicit liquor which many a time is adulterated with methyl alcohol and other substances are a common occurrence in our country. Adulterated drugs also do cause deaths as in the recent (1986) case N of glycerin adulterated with ethylene glycol which was the subject of Justice Lentin Commission in Bombay. Such occurrences are far less in the developed world and is reminiscent of a similar incident in the U.S. which occurred over 50 years ago in1937; 11 gallons and six pints of a sulphanilamide elixir was dispensed with 72 per cent ethylene glycol as solvent which led to the death of over 100 persons due to nephrotoxicity or toxicity of the kidneys. The mechanism of ethylene glycol toxicity was investigated and found to be due to the precipitation of calcium oxalate crystals in the kidney and stone formation. This episode led to a tighter control of food and drug laws and such occurrences are rare in those countries now mainly due to heavy public claims for compensation.

The trade in addiction forming drugs like cocaine, opium, morphine, heroin, marihuana, etc. is big time business in the world today and a large number of deaths also result from these drug abuses.

One of the main objectives of forensic toxicology is to collect data on human toxicology which could lead to recognition of particular chemical hazards and means of therapeutic handling of such cases.

Matthew J.B. Orfila (1787-1853) the Spaniard who was appointed as the First Professor of Medical Jurisprudence in Paris can be called the first forensic toxicologist. He created a sensation in Paris by identifying arsenic in the stomach contents of a victim of Madame Marie la Farge by Marsh's test in 1840 and getting a conviction. It should be remembered that it took another century to analyse the hair of Napolean Bonaparte for arsenic by neutron activation analysis and conclude that he could have been slowly poisoned with arsenic. It takes along time for these scientific evidences to get an acceptance in the courts of law. A medico-legal laboratory was established in New York city only in 1919 and the American Academy of Forensic Sciences was established as late as 1949.

The important samples for analysis by the forensic toxicologist are heart blood, catheterised urine and stomach contents. Kidney,liver, lung and bone tissue are also submitted to analysis in necessary cases. The improvements in instrumentation like liquid chromatography, gas-chromatography, mass spectrometry, etc. enable the analyst now to go into the minutest specs of chemicals.

### 6.1.3 Regulatory Toxicology

In the developed countries, a large number of legislations have come up to protect the humans from real and imaginary chemical onslaught especially since 1970. Federal Insecticide Fungicide and Rodenticide Act, Toxic Substances Control Act, Safe Drinking Water Act, Clean Air Act, etc. in the USA and the regulatory agencies Environment Protection Agency and Food and Drug Authority have been strengthened substantially. In India too, we have similar acts which are very weakly enforced; an environmental protection act was legislated in 1986 but no enforcing agency has been created.

All these enactments need functioning toxicologists to establish violations as also to amend and rectify technical aspects. The legal institutions have to handle cases of violations of the acts and have to take decisions on matters scientific and technical. But as many of these matters are transscience, a nonscientific judiciary with a strong common sense would be able to pronounce level-headed final judgments on these matters.

Regulatory laws are legislated always in all countries only after a crisis. It is interesting to note that one or two deaths per day from an event like a road accident is taken as a part of the risk to be faced; but if two people die by consumption of a particular medicine, that is headline news and a new regulatory restriction appears.

'When a regulatory law is passed, it always gives birth immediately to one or more government agencies or expansion of an existing agency. Then we get a new set of laws as an outgrowth of the parent act as a result of promulgation, by rule making, by practice, by interpretation and by administrative decisions to settle questions growing from the new regulation. Thus we get regulatory law not only by legislation but also by bureaucratic fiat.'

These were the words of wisdom from a U.S. Government publication in 1968 on 'Environmental have quality'.This is exactly what is happening 20 years after in our country too.

## 6.2 ECONOMIC TOXICOLOGY

The subjects classified under this head-food, drugs, pesticides, etc.-directly and indirectly are involved in the economics of the country, e.g., pure food contributes to good health and is of real value in the commercial export market.

### 6.2.1 Food Toxicology

The contamination of the food could be accidental, unintentional or intentional-adulteration for higher like a profits. In freshly cooked food many of the contaminants would be eliminated at the high temperature; an interesting example is the poisoning from eating uncooked raw cassava (tapioca) in Africa due to cyanogenic glycocides. In India, people eat cooked cassava and this problem does not exist. The possibilities of contamination are more in the processed foods. Food adulteration is an art in itself in our country and so not much can scientifically be said except about the common adulterants.

### 6.2.2 The Beer epidemic of 1900

Large scale poisonings used to occur in the early part of this century and an interesting episode is the beer epidemic in England In June 1900, a few people died, alleged, to be beer drinkers in the North of England, around Liverpool. More deaths ensued in the following months at Manchester, Salsford, Lancashire and neighbouring areas. Initially, it was attributed to alcoholism, peripheral neuritis or multiple neuritis. But when the numbers affected reached a few thousands, the Government appointed a commission with Lord Kelvin, the famous physicist, as chairman. Analysis of the beer showed 1.45-2.6 per cent arsenic. Deeper investigation proved that the beer was from the brewery of Bostock at Garston near Liverpool. Bostock used sulphuric acid from Nicholson in Leeds to mash up various starches to fermentable biomass. The sulpuric acid was made from pyrites imported from Spain, which contained copper and arsenic. Copper was extracted out, but arsenic was not carefully stripped off, thereby remaining as an impurity in the acid which passed on to the beer.

This was an excellent piece of toxicological investigation at the turn of the century; further it was observed that if the malt was cooked over coke kilns, the beer would

always be contaminated with arsenic.By the way,the permitted level of arsenic for liquids is 143 parts per million in U.K.

Poisonings are not limited to olden days. Cooking oil contaminated with Triorthocresol phosphate (TOCP) killed 300 people in Spain in 1981 and left another 20,000 with neurotoxic lesion. In 1971, 113 babies (out of 12,000 victims) died from dried milk powder contaminated with arsenic. In the 1960's, a large number of people died in Kerala by using sugar contaminated with an insecticide, Folidol.

The use of Kesari dal (which is a banned item) if consumed for extended periods of time produces lathyrism due to the chemicals oxalyl diamino propionic acid and B-amino propionitrile. The Minimata disease due to consumption of fish contaminated with methyl mercury dumped as ,industrial waste into the Minimata Bay of Japan took toll of a large number. Nearly 6000 people were affected and 500 fatally by consuming bread made from cereals contaminated with alkyl-mercury fungicides in Iraq in 1972. ltai-Itai disease another mysterious disease, again in Japan, which produced severe bone and joint pains like arthritis.This was traced to higher cadmium content in rice receiving effluent from a zinc-cadmium mine upstream.

### 6.2.3 The Major Contaminants in Food

The major contaminats of food are microbial organisms, environmental contaminants natural toxicants, pesticide residues and food additives. The microbial organisms are the major toxicants In the food system even in advanced countries, and according to one expert, the other hazards from , pesticide residues and food additives are less than a thousands in magnitude to the people; but enforcement agencies always give more priority to the pesticide residues and additives.

### 6.2.4 Microbial Intoxications

Quite a number of infectious diseases like typhoid, cholera, etc. are spread through food, but these are not categorised as microbial intoxications; these are infections. When a toxin produced by an organism in foods causes an illness, then only can it be called a microbial intoxication.

### 6.2.5 Viral Infections

Two food-borne virus diseases were identified in e England and Wales-acute gastro-enteritis (dysentery) due to small round virus (SRV) and acute hepatitis due to Hepatitis A virus. A large-scale outbreak of gastro-enteritis attributed to eating cockles did not show bacteria in the food, but SRVs were seen in the stools of the victims. The cockles were collected from sewage polluted waters of' Thames estuary and improperly cooked. Between 1977 and 1983, 269 cases were reported in the U.K. from consumption of shell fish, mussels and oysters. Over 400 cases of Hepatitis A were also reported amongst shell fish eaters. Food handlers suffering from viral infections can also infect others.

### 6.2.6 Bacterial Intoxications

Salmonellosis and botulism are two of the well documented food intoxications. A very recent salmonellosis infection was in a hospital in the U.K. affecting over 400 people, leaving 27 dead. The causative organism was identified to be *S.typhimurium* phage type 49 and infection through cold roast beef which was not properly refrigerated. The use of antibiotics in cattle feed in the Western countries has resulted in some resistant strains developing in animals and infecting humans through milk which is poorly pasteurised.

Botulism intoxication from clostridium botulism has become rarer in humans but more in cattle. The organism cannot survive heat and acidity and recent occurences in other countries have been mostly from home-canned vegetables and meat.

Chinese food which is very popular all over the world was responsible for a number of cases of *Bacillus cereus* intoxications in the U.K The cooked, kept over, reheated cereals were excellent medium for the. above bacteria. Staphylococcal infections and vibrio haemolyticus infections also were quite a number in the reporting.

### 6.2.7 Natural Toxicants

Mycotoxins: Ergot, the fungal infection of rye leading to ergotism probably is the oldest known mycotoxic disease. Ergotism was supposed to have thwarted the armies of Sparta and Athens in 1480 B.C. and also stopped the progress of Peter the Great's annexation of Turkey,because the staple food of the army was infected.Mycotoxicology as a discipline by itself started coming up in 1960's and got established firmly in the 1980's by the political adventurism of the U.S. in Kampuchea.

The Turkey x-disease killing thousands of turkeys in the U.K initiated toxicological studies on the causative factors, traced to fungus infected groundnut meals used as poultry feed, imported from India. The fungus was identified as *Aspergillus fiavus* and the toxin was named aflatoxin. Chemical structures and biochemistry have been fully studied. Significant changes are in the pathology of liver; proliferation of a bile duct, epithelium and appearance of ascites are characteristics of aflatoxicosis. Human fatalities by eating uncooked infected cassava have been reported.The aflatoxin BI levels in cattle feed has been restricted to 0.01 mg/kg to 0.05 mg/kg. Pressure, heat and moisture would degrade aflatoxin and as such,the hazard from cooked food is minimal. Aflatoxins have been indicted as carcinogens as well.

A few other toxicants like psoralenes found in vegetables, mainly peas, beans, etc. have been identified. While many of the natural toxicants occur at parts per million levels. along with other constituents in plants, fruits or fishes, the laboratory animal toxicology studies are done with far higher concentrations. If the results are extrapolated to humans, this can lead to very fallacious conclusions even if the effect of heat, moisture and other processes involved are not considered.

### 6.2.8 Environmental Contaminants

Metals which are toxic getting accumulated in animals, fishes or plants from environmental po11ution, pesticide and fertiliser residues on processed and unprocessed food materials are the more common contaminants. There can be contamination from packaging materials, like tin cans and plastic containers.

Lead is one of the metals indicted in poisoning from the hoary past of Roman days, from the pewter dishes used. Chronic lead poisoning occured in alcoholics by drinking alcohol distilled in lead vats.

Among the toxic metals which can get concentrated in the food chain is mercury from the methyl-mercury fungicides used, getting concentrated in fishes growing in polluted waters, as in the Minimata bay episode. The concentration can go up as high as 1 mg/kg of mercury .Birds also accumulate mercury from their feed.

Food additives form a negligible part of the hazard even though this has got the maximum attention from safety enforcement agencies. During the last 20 years,after continuous research, only three chemicals have been removed from the combined total of 300 permitted additives in the U.K.These were brominated vegetable oils used as weighting agents in soft drinks.which cause accumulation of lipidbound bromine in the tissues of animals and possibly humans.The red-dye Ponceau Mx and artificial sweetener, cyclamate also have been removed as possible carcinogens, even though the current status of cyclamates as a human carcinogen is not established.

Leaching out of toxic chemicals from packaging materials is another possibility which has been fully verified. Extraction of wax and coating from paper packaging, chemical reactions within cans leading to dissolving of tin and lead and leaching or migration of monomers, catalysts, plasticisers, etc. into the food products have been well studied and documented.


But the worst of all in our country is the toxicity from food adulterants which probably outstrips any other hazard. Potassium chromate is used to intensify the colour of turmeric and toxic seeds for adulterating pepper; animal fat mixed with ghee and mineral oils (like white oil) being added to cooking oils are common occurences in our country, like the recent Calcutta episode. When it affects a large number of people and a few die, then only it becomes headline news creating a temporary furore. Enforcement of legislations properly would need better paid and technically qualified staff for the purpose. The Lentin Commission report on Maharashtra Food and Drug Authority (1988) should be an eye-opener to all concerned.

### 6.2.9 Drug Toxicology

As mentioned in Chapter II, the evolution of toxicology began with the work on poisons used as drugs. As said by Paracelsus (which is worth repeating many times over), it is the dose which determines the toxicity and not the drug by itself.

In studying the toxicology of a potential drug,apart from studying the major action for which it is used as a drug, side-actions or side effects are also to be thoroughly studied. The chemical would become a drug, only If the side actions are not significant In comparison to the major desired effect. Morphine,which was being used as an analgesic earlier produces , respiratory depression as also addiction. So the use of morphine as an analgesic today is very low. Cortisone or penicillin can produce immunological reactions, but the good effects so much outweigh the adverse effects that these continue to be in use.

Another interesting instance is that of Practolol, a beta blocking agent for the control of cardiac arrhythmias (intermittent missing of heart beats), found valuable subsequently in high blood pressure cases as well. This was fully studied and licensed but when marketed, adverse reactions were reported such as skin rashes, impairment of secretions, corneal damage and sometimes loss of sight.

The margin of safety or the therapeutic index is the guiding factor in developing a chemical as a drug. Margin of safety is the difference between lethal dose LD50 and effective dose ED50; the wider the gap between the two values, better the chemical as a drug.

A large number of guidelines for toxicity testing of drugs, including many issued by drug licencing authorities, are available. Most of the potential drugs would show some toxicity like skin rashes, nausea, etc. in a heterogenous human population, as against animal studies in a homogenous group. What are the weights to be attached to the results of ominous but uncertain significance? Chloroquine, which is an essential antimalarial drug is mutagenic in Ames tests but we continue to use it, because there is no replacement and nothing untoward has happened in humans on large-scale use.

The major tragedies which created public alarm against drugs were the ones like Thalidomide tragedy detailed earlier, which virtually shook the western world. The subtilities of drug interactions appeared in another episode in Japan in the late sixties known as sub-acute myelo-optico-neuropathy or SMON. A large number of people were affected by abdominal pain and persistent diarrhoea followed by a kind of paralysis of the extremeties of the legs. In severe cases, blurred visions, blindness and sensory disturbances preceded death. Investigations traced SMON as due to intake of clioquinol which is iodo chloro-8 hydroxy quinoline, marketed as mexaform and enterovioform in India. This is an over-the-counter drug administered by housewives for common enteric infections. It was initially thought to be a neurotoxic response of clioquinol, but toxicological investigations proved this to be due to amine rich diet of Japanese, mainly fish, interacting with the clioquinol, which was found to be a mono-amino oxidase

inhibitor, causing disturbances of amine metabolism and accumulation. This is an excellent example of geographical toxicology where drugs and food habits are so closely connected; SMON may not In be produced in India where dietary intake of amines(fish products)are far less. Drugs like antipyrine and theophylline are metabolised at a faster rate by high protein-low carbyhydrate diet intake.

The biggest problem in drug development is the public demand that all the drugs should be totally safe-a zero risk drug is an Utopian concept. While the public accepts the risk faced in a heart or kidney transplant surgery and even death on the operation table, the small element of risk from a drug is magnified out of proportion. This has resulted in increasing the expenses on toxicological investigations and lengthened the time between first reporting of a possible drug and licensing for production. This lead time has increased from six years to 10 years approximately in the U.K and U.S.A. The number of man-days needed for the work and the cost also has gone up three or four times after adjusting for inflation.

### 6.2.10 Fallacies in Extrapolation from Animals to Humans

The unsatisfactoriness of predicting human effects from animal experiments has been well known for a long time especially in carcinogenicity testing of chemicals in rats and mice. Furosemide, a well tolerated and valuable diuretic in man, causes severe liver necrosis in mice because of a metabolite which is not formed to that extent in the humans. The nucleoside 6-azauridine is well tolerated in cancer chemotherapy of humans but it produces severe bone marrow depression in much lower doses in dogs.Intramuscular injection of iron sorbitol causes sarcomas at the site of injection in rats and rabbits but not in humans.

A few of the very difficult toxicological predictions on drug action are in case of newborns and infants,in very aged, and in malnutritioned people.

### 6.2.11 Newborns and infants

Quite a lot of information has been collected on drug toxicity on newborn and immature rats, and higher toxicity has been observed.The scientific basis for this difference has not been specifically established.

Antihistaminics, chloropheniramine maleate and diphenhydramine show high toxicity in very young rats due to rapid absorption from the site of injection.These drugs are poorly tolerated by young children too, but the reason is not known.

Two of the possible causes of higher toxicity of drugs in infants are (a) the larger volume of extracellular fluid in infants (almost thrice that of in adults), which increases drug retention and slows down excretion giving rise to higher blood levels and(b) the immaturity of drug metabolising enzymes.Chloramphenicol (chloromycetin) can cause cardiovascular collapse in infants due to the latter reason.The easy permeability across the blood-brain barrier has been given as another reason in the case of 2, 4 dinitrophenol

producing lens opacity on in in very young rabbits. Slower excretory mechanism can be yet another reason; nalidixic acid is retained in blood for 15 days in newborn calves, while its half life is 90 minutes in seven-month old calves.

### 6.2.12 Old age

Ageing of tissues, decrease in enzyme activities, degenerative deterioration and higher incidence of diseases are supposed to be the reasons for higher drug reactions in the aged. Clinical observations have shown that regulatory mechanisms of blood pressure are upset more easily in the aged people. Intravenously injected propanolol caused greater decrease in systolic blood pressure in old subjects than in young.

### 6.2.13 Malnutrition

The clearance of drugs from the blood is decreased in severe cases of malnutrition such as Kwashiorkar, marasmus and nutritional oedema. In malnutritioned children, clearance of drugs like Phenazone, chloroquine, para acetamol, sulphadiazine, etc. are considerably decreased. Renal clearance is also decreased in severe malnutrition.Drugs with low margin of safety have to be very cautiously administered to the malnutritioned.

### 6.2.14 An Unexpected Turn

The stiff regulatory practices in the western world have created a number of unexpected impacts. Many of the multinational drug companies are using the third world for surreptitious clinical testing of drugs because both, the awareness of the public and enforcement by the authorities, are comparatively poor in these countries. The number of new drugs has considerably reduced as also most of the research work is towards drugs for major diseases which will have a large volume sale. Drugs for rare diseases called 'orphan drugs' like triethylene tetramine for Wilson's disease or 1, 5 hydroxytryphophan for myoclonus are not viable for a commercial market.Many drug companies are turning their attention to profitable diagnostic kits.

### 6.2.15 Pesticides

Unlike drugs, pesticides would have a certain toxicity, without which they cannot kill the insects.These chemicals have now become inevitable due to their use in control of vector-borne diseases in public health practices, increasing agricultural productivity and in urban pest control. The negative aspects are indiscriminate use leading to environmental contamination as also entry into the food chain and direct exposure in production and spraying operations.

The large number of deaths and accidents from insecticides makes it necessary to study the toxicology elaborately. One of the earliest pesticide tragedies in India was the Folidol episode in the early sixties.Folidol (Parathion) being transported from Bombay to Cochin leaked from a container and soaked the sugar bags kept nearby. Sugar was unloaded at

Cochin port and distributed through retailers. Most of the deaths happened on drinking tea with the contaminated sugar.

Another was in Karnataka where a large number of agricu1tural workers spraying the fields with diazinon, got it in the face due to the shifting direction of winds. Helicopter spraying of plantation is now common, but we hardly know how many get affected.

Bhopal, of course, pygmied the rest of the accidents.The toxicology of the highly toxic product Methyl Isocyanate (MIC) was fairly unknown to the manufacturers themselves; or whatever known, was obviously concealed from the Indian subsidiary. The subsequent developments like the inclusion of MIC in the U.S. National Toxicology Programme for detailed study by the U.S. National Institute of Environmental Health studies confirms the absence of full knowledge on the toxicology of a chemical produced in large quantities and marketed as well.This escaped the attention of enforcement agencies because MIC was an intermediate and not the final product.

There are a variety of chemicals having insecticidal action but broadly these can be classified into: organochlorine group, organophosphorus group, and carbamates. Organochlorines held the field from1940-1960 and include the well known DDT, BHC,aldrin, dieldrin, heptachlor, etc. The discovery of the insecticidal action of DDT in 1939 by Paul Hemann Mueller led to the search of other compounds in the group. Methoxychlor is the only compound which has less acute toxicity than DDT.

DDT is one of those insecticides which has not produced a human fatality yet. All organochlorine insecticides including DDT are metabolised by the system very slowly, so that accumulation in biological systems have been reported. Birds, fishes, and even human milk have been reported to have a burden of DDT. In the environment also, the reports from subtropics showed it to be very persistent. But in the tropical climates like in India, the environmental degradation of DDT seems to be much faster.

The amazing fact that so far no one has been able to demonstrate the ill-effect of DDT accumulation in a human system.Indiscriminate use has lad to the contamination of food chain as also effect on non-target organism.The USA and some other countries have banned DDT, but for the developing world, DDT is much needed because of its low cost, human and effectiveness. Development of resistance is mainly due to indiscriminate use only.

Organophosphorus insecticides are a spin-off from the research on nerve gases during World War II in Germany. Tetraethyl pyrophosphate was the first to be produced as a substitute for nicotine which was in short supply. These compounds are of higher toxicity than organochlorines generally but are not persistent, being very easily metabolised in the biological system as well as in the environment.

These groups of insecticides are called anticholinesterases because they inhibit the cholinesterase enzyme which hydrolyse acetylcholine, the chemical transmitter at the neuromuscular junctions. The mammalian toxicity of these insecticides are enough to kill humans in large doses.The highest number of medico-legal cases are death or other effects due to organophosphorous insecticides.

### 6.2.16 Delayed neurotoxicity

A few years ago in Egypt a number of buffaloes developed paralysis of the hind limbs initially followed by death on drinking water from a pond. Very painstaking toxicological sleuthing by Prof. Abou-donia revealed that the paralysis was due to drinking of water contaminated by an insecticide, leptophos, which was being sold in India as Phosvel. Subsequent investigations found that quite a few organophosphorus insecticides do produce delayed neurotoxicity.

It was later found that the 1930 episode known by the name of 'ginger-jake paralysis' was also a delayed neurotoxic syndrome due to taking tri orthocresol phosphate (TOCP). Myelin degeneration of nerves in the extremities occurred, leading to paralysis. This is being attributed to neurotoxic esterase inhibition, but it is not established very clearly. However, conventional treatments do not cure this paralysis.

### 6.2.17 Carbamate Insecticides

These are further improvements on organophosphorus compounds in that the mechanism of toxicity of these compounds are exactly like the OPs, but the inhibition of cholinesterase is reversible. As such, there are better chances of recovery in cases of human poisoning. Some of the very wen known household insecticides like Baygon, carbaryl (sevin),etc. belong to this group.

## 6.3 ENVIRONMENTAL TOXICOLOGY

A healthy human being has to be in harmony with his environment an the twenty-four hours a day. A working man is in his work environment eight hours a day and the rest of the time in his home and natural environment. The individual's health status vis-a-vis his work environment, termed occupational health and the efforts to keep the work place clean, called 'industrial hygiene', both go hand in hand. Occupational health can be defined as the science and art devoted to the recognition, evaluation and control of those environmental factors or stresses arising in or from the work place which may cause sickness, impaired health and well-being or significant discomfort and inefficiency among workers.

If the same definition is extended to include the environment outside work place and illness or death among citizens of a community, then it is called environmental health. Occupational health relates to work place pollution while environmental health relates to environmental pollution; the methodology for both studies is the same.

The stresses at workplace can be caused by noise, heat, chemicals, etc. while in the general environment, it can be the pollution of air, water and food. In India, while we hear quite some controversies over environmental pollution, much less is heard about work-place pollution. The duties of a toxicologist. is very many in both these studies-detecting the type, nature, extent and source of pollution, studying the effects on humans as also ways and means of reducing this pollution, are all part of a toxicologist's functions.

### 6.3.1 Occupational Toxicology

In all occupations, there exists some hazard; there can never be a zero-risk occupation. Noise, explosive hazards, electrical hazards, etc. also exist but an occupational toxicologist would be more concerned with exposure to toxic materials at the work place. Scientists have been aware of this hazard for a few centuries but it did not take root as a discipline, till enforcement legislations were introduced in the developed nations.

Ramazzini, a Florentine physician in the 1700s wrote about illnesses seen in workers, but he could not name the causative agent. In 1775, Percival Potts had reported cancer of the scrotum in chimney sweeps, due to excessive exposure of that part on their job. Lung cancer in two workers in the asbestos industry was reported in 1935; but it took 30 years more to establish the direct connection between asbestos and lung cancer, because the deaths have to be distinctly more than in the general community. An association of the suspected causative agents with death or illness, then specificity and statistical significance have to be established before indicting a chemical as an occupational hazard. Occupational Safety and Health Administration (OSHA) of the USA has named twenty-one chemicals as of consequence in the industry and enforced compulsory medical surveillance of workers in these industries. These include asbestos, bischloromethyl ether,nitrosodimethylamine, vinyl chloride, inorganic arsenic, lead, coke-oven emissions, cotton dust and acrylonitrile.

Exposure limits to hazardous chemicals in the industry are laid down in the U.S. by the American Conference of Governmental Industrial Hygienists (AGCIH). These are called Threshold Limit Values or TLVs and are worked out on the best available information from industrial experience, experimental human and animal studies. The basic philosophy in laying down TLV is that a concentration does exist for all substances at which no injurious effect should result, no matter how often the exposure is repeated. The first set of TLVs in the U.S. was framed in 1941 beginning with coal dust exposure. It is very important to remember that these values are based on the conditions existing in the U.S. such as the general health and nutritional status of the individual.

In India, the Factory Act of 1948 is the guiding legislation. Section 14 of the act talks about effective measures to be undertaken to prevent the accumulation of dust and fumes in work places and section 36 on precautions to be taken. Section 89 identifies 22 notifiable occupational diseases under the act. Most of it is more ignored than heeded by the Indian industry .

An authority on occupational health in India has recorded the following-' Functioning of such an elaborate system of safeguards for controlling health hazards all these years should normally have provided with enough documented statistics/evidence on the pattern of changes in the hazard levels taking place and the resultant health impairment. Unfortunately no such information is available either from the health records maintained in the factories or from the records of the enforcing agencies'. Legislations are left to slumber inside the statute books, unless rudely awakened by incidents like Bhopal.

Information on the 22 notifiable diseases is also scarce. It appears that 7 per cent of the workers in asbestos industry suffer from asbestosis but none of the studies probed the incidence of carcinoma of the lungs or mesothelioma. In a study conducted in 1979 in an alkaloid extracting unit using benzene on a large scale, it was observed that all over the work place, the concentration of benzene was more than the permissible limit, going as high as 90 times the limit of 30 $mg/m^3$. Most of this was due to improper ventilation. In 71 per cent of workers, urinary phenols, an indicator for benzene exposure was more than 50 mg/litre, 44.8 per cent of the workers were in a state of benzene intoxication and 14.6 per cent had haematological abnormalities too. Benzene is not used as a solvent in most of the developed countries.

In a study of workers using lead, such as printing presses, lead-acid battery industry, etc., 9.1 per cent of the workers were diagnosed as lead poisoning cases. Similar are the pattern in industries using carbon disulphide, mercury and nitro-amino compounds. No evaluation has been done for many hazardous substances like vinyl chloride monomer and organophosphorus insecticides.

Large-scale unemployment in India forces individuals to hold on to their jobs under all adverse working conditions. A person has to live with the risk of falling ill due to work exposure. The industrialist is concerned only about deaths at work because these will be subjected to police enquiries; a clean work place and presence of occupational diseases do not interest him; as workers, mostly in a malnutritioned state, can never establish evidences to show that their illness arose from their occupation. Enlightened workers' unions only can take up these causes, but these unions get diverted on to party politics rather than science-based facts. To enforce a very clean work place would also not be very meaningful, as in sixteen hours in the outside environment, the worker would be exposed to the worst type of water, air and food pollution. A proper balance has to be struck and blind application of the U.S. and U.K. standards to Indian industry is not a practical solution.

### 6.3.2 Toxicology in Environmental Pollution

While exposure to toxicants at the work place will affect adults, when a whole community is exposed to the harmful effects of a chemical, this will affect a group including newborns to near-grave individuals, both sick and healthy. It would surprise many to know that in 1849, John Snow had a lot of difficulty in convincing others that the cholera epidemic of London arose from water pollution. He based his finding on the variation of mortality in different districts of London during the 1849 epidemic.

A variety of environmental pollutants are talked about now, beginning with carbon dioxide, oxides of nitrogen, oxides of sulphur, particulate matter, etc., all arising out of industrial activities. Communities near to industrial facilities are exposed to low doses round the clock. Toxicological studies of such chronic low level exposure is very difficult and one has to depend mostly on epidemiology for the purpose; and this needs well-maintained health data files of the group of exposed people.

'Acid rain' due to sulphur oxides from Germany (FRG) being wafted on to Sweden and endowing rain water with high acidity which comes down to destory Swedish forests is a matter of international dialogues. To establish that the Swedish acid rain has its origins from the factory smoke of FRG needs the help of meteorologists and others. Similarly, to establish that the ozone layer in the upper atmosphere is depleted by the halohydrocarbons used in the aerosol industry, multinational collaborative studies were required. Even then, further confirmation of the ill-effects on humans from an excess of ultraviolet radiation due to depletion of ozone will take a long while.

The more definable pollution toxicology problems are on the pesticide residues getting into the food chain, excessive use of fertilisers getting leached into the drinking water sources, dumping of industrial wastes and effluents which again can pollute ground water resources, etc. In most of these cases, industrial activities are marked out as the culprit in the advanced countries and public affair litigations are also initiated. The environmental protection agency (EPA) in the U.S. is saddled with all the responsibilities of laying down standards, identifying violations and ensuring prosecution.

The major sources of environmental pollution causing human distress in our country are poor quality drinking water and adulterated food-stuff. Human faecal pollution of drinking water sources far exceeds contamination from industrial pollutants. Particulate pollution of air is far more, especially in our capital and surroundings due to the geographical location near the Thar desert. It will be a folly to fix the air and water standards laid down by U.S. EPA for India, as it will not be possible to keep up to the standards.

Toxicological problems of environmental pollution have not been handled in a comprehensive way and serious studies in some industrial areas show that the rise in pollution is seasonal. being mostly in summer months. The tropical rains clean our atmosphere very well. Many of the industrial pollution problems are localised and limited to small areas. Environmental Protection Act has been legislated in 1986 but the infrastructure and wherewithals for strict enforcement are totally lacking.

# 7. Toxicology in Political Conflicts

One would really wonder what toxicology has got to do with political conflicts, but two episodes prove how the distant connections can kick up immeasurable amount of polemics as also toxicological and epidemiological research. Enormous scientific labour and financial investments involved in these have brought out some useful scientific information-but disproportionate in all ways.

One of these episodes was an aftermath of the U.S.-Vietnam war and the other from the U.S.- U.S.S.R. cold war. The interesting turns and twists of these episodes read more like fiction than anything else.

## 7.1 The TCDD Affair or the Agent Orange

Agent Orange is the code name for a defoliant used for about eight years in massive quantities by the U.S.forces in the Vietnam war during 1962-1970, for the denial of food operation to Vietcong by destroying the standing crops. The chemical 'Orange' consisted of equal parts of 2,4,5 trichlorophenoxy acetic acid (2,4,5T) and 2,4 dichlorophenoxyacetic acid (2,4 D). Both the compounds are innocuous to humans, but contain a trace impurity, tetrachloro p-dibenzodioxin or TCDD, which has earned a certain notoriety. TCDD is formed from trichlorophenol on heating and up to 2 ppm were permitted by the manufacturers. But as the demand far exceeded supply, quality control could have been relaxed temporarily.

Hospitals at Saigon had reported in the early 1970's congenital deformities in newborn children which were attributed to TCDD. A number of factories all around the world producing chlorophenols had been reporting since 1949, mainly chloracne as an occupational disease on accidental exposure to TCDD.As research and controversies were going on, an incident in the U.S. further aggravated the issue.

In 1971, a stable owner sprayed used furnace oil in the paddocks to contain dust at Times beach,Missouri; a resort and.entertainment area. Birds, cats and dogs started dying In the area. 43 of the 85 exposed horses also died during the year.Investigations showed that 16,000 gallons of trichlorophenol waste residue was mixed with the waste oil. Concentration of TCDD in the oil was nearly 300 ppm and in the soil 30 ppm. Ultimately,in 1982, the EPA purchased the whole beach to avoid human casualities.

The next incident was an explosion in 1976 in ICMESA factory in Seveso, Italy producing 2,4,5 T. The neighbouring community of 37,000 were exposed to different concentrations of TCDD. The whole township was evacuated, decontaminated and then only were people allowed to go back. About 184 cases of chloracne were reported; but no birth defects of children born to exposed pregnant women or deaths were reported.

## 7.2 Toxicology of TCDD

TCDD is an ubiquitous chemical. When scientists honed the techniques and started looking for it, they could find it all over the earth. It has a half life of ten years in sub-tropical soil; both sunlight and microorganisms degrade it. Guinea pig is the most sensitive species with oral LD50 of 0.6 µg/kg body weight; hamsters are the least sensitive with oral LD50 of 1157 µg/kg. This has been explained as due to faster metabolism in hamsters.

TCDD produces a variety of reproductive effects in rats like cleft palate, kidney abnormalities, foetal deaths, etc. It is also a proven carcinogen in rats and mice. Human acute symptoms were limited to chloracne, digestive disorders, effects on essential enzyme systems and a few psychiatric disorders, which could be an indirect effect. Soft tissue sarcomas (a type o cancer) reported in wood workers of Sweden as due to TCDD could not be confirmed by studies in Finland and Washington state, USA.

## 7.3 After-tale

The U.S. veterans who fought in the Vietnam were quite agitated by the results of animal studies and in 1979, filed a class action law suit against the chemical companies that had manufactured Agent Orange; and these companies in turn sued the US federal government for negligent misuse of the herbicide.These law suits led to a number of epidemiological studies by different agencies.Veterans Administration, Communicable Diseases Centre and U.S. Air Force, all had independent studies conducted for the purpose of evaluating human injury due to Agent Orange handling. All of these studies concluded that TCDD levels in serum of people who handled Agent Orange was higher than those who did not; but this was in no way related to any health injury .The Air Force study,'Operation Ranch Hand', is to go on till 2002 and the NIOSH study to cover 85 years of occupational exposure to TCDD. A fund of 200 million U.S. dollars has been created for compensation payments. Whatever be the outcome, TCDD will take the cake for being the most widely studied man-made compound at a cost of billions of dollars. No doubt, an unpopular programme in an unpopular war has given the science of toxicology, a big boost.

## 7.4 THE YELLOW RAIN

On 13 September 1981, the then U.S. Secretary of State, Alexander M. Haig made a dramatic announcement at a Berlin press conference that the Soviet Union was using lethal chemical weapons in Laos, Kampuchea and Afghanistan and the U.S.Government had physical evidence of the use of three potent mycotoxins or fungal metabolites, not common to the region.

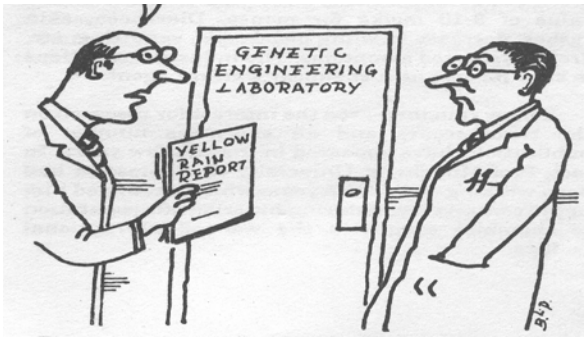
The toxins being referred to are called tricothecenes and are produced by fungus, Fusarium. These at times contaminate cereal grains and are reported to cause skin lesions, vomitting, diarrhoea and gastro-intestinal bleeding. These were identified as tricothecenes by Prof. Marocha at the University of Minnesota. As these toxins were supposed to have come down as yellow droplets, which later on settled on trees and soil, this was given the name yellow rain'. The major supporters for corroborating yellow rain attack were amongst the refugees from Laos on the non-scientific aspects and Chemical Research and Development Centre of the U.S. Army on the scientific evidences. But many ill the U..S. academic world did not take these evidences at the face value. One among them, Prof. Mathew Meselson of Harvard University decided to take on the cross in search of truth.

In January 1982, the British Chemical Defence Establishment at Porton downs found that yellow rain samples contained a large amount of pollen; this observation was confirmed by the Canadian Agriculture Laboratory in Ottawa. The U.S. military scientists could not explain the presence of pollen in a chemical warfare agent.

Prof. Meselson convened a conference of experts from all relevant fields like Botany, Mycology, Chemistry, Medicine, etc. at Harvard in 1983 at which the identification of the yellow rain pollen as belonging to a plant species in South-East Asia was brought out. This led Prof. Meselson to take up scanning electron microscopic studies of pollens from the South-East Asian species as also examination of the excreta of honey bees from India. He observed the lack of proteins in the yellow rain pollen as well as honey bee excreted pollens, while presence of proteins was fully established in fresh pollen. All this led to the conclusion that yellow rain was nothing but honeybee faeces.

To get conclusive proof of this, Prof. Meselson with a bee-expert, Dr. Seeley, went to Thailand in 1984.They observed copious showers of yellow rain from the bee nests, collected and analysed it-in fact, the members of the team were caught in a yellow rain shower !

One would ask: 'what about the toxicological content of this controversy?'Enormous interest was created in tricothecene chemistry and toxicology because of the indictment of mycotoxin as a chemical warfare agent, even though baselessly.



## 7.5 WHAT ARE TRICOTHECENES

These are products of the metabolism of a species of fungi called Fusarium. These were being studied in different countries because they used to contaminate animal feed as also infest plants. T-2 toxin was identified in 1971 as the cause of death to dairy cattle. Reports of human poisoning also existed.

The three compounds mentioned in the above episode are T-2 toxin, nivalenol and HT-2 toxin. These are of comparable toxicity with intra-peritoneal $LD_{50}$ value of 5-10 mg/kg for mouse. Diarrhoea, skin rashes, decrease in white blood cells, vomitting, etc. are the reported symptoms. The induction of actions is slow for use as a chemical warfare agent.

Yellow rain increased the interest for research on the tricothecenes and an enormous number of publications have appeared in the last few years. In fact, Prof. Mirocha at University of Minnesotta had been working on it for 15 years which entangled him in the controversy, reducing his scientific reputation to shambles caught in the web of international politics.