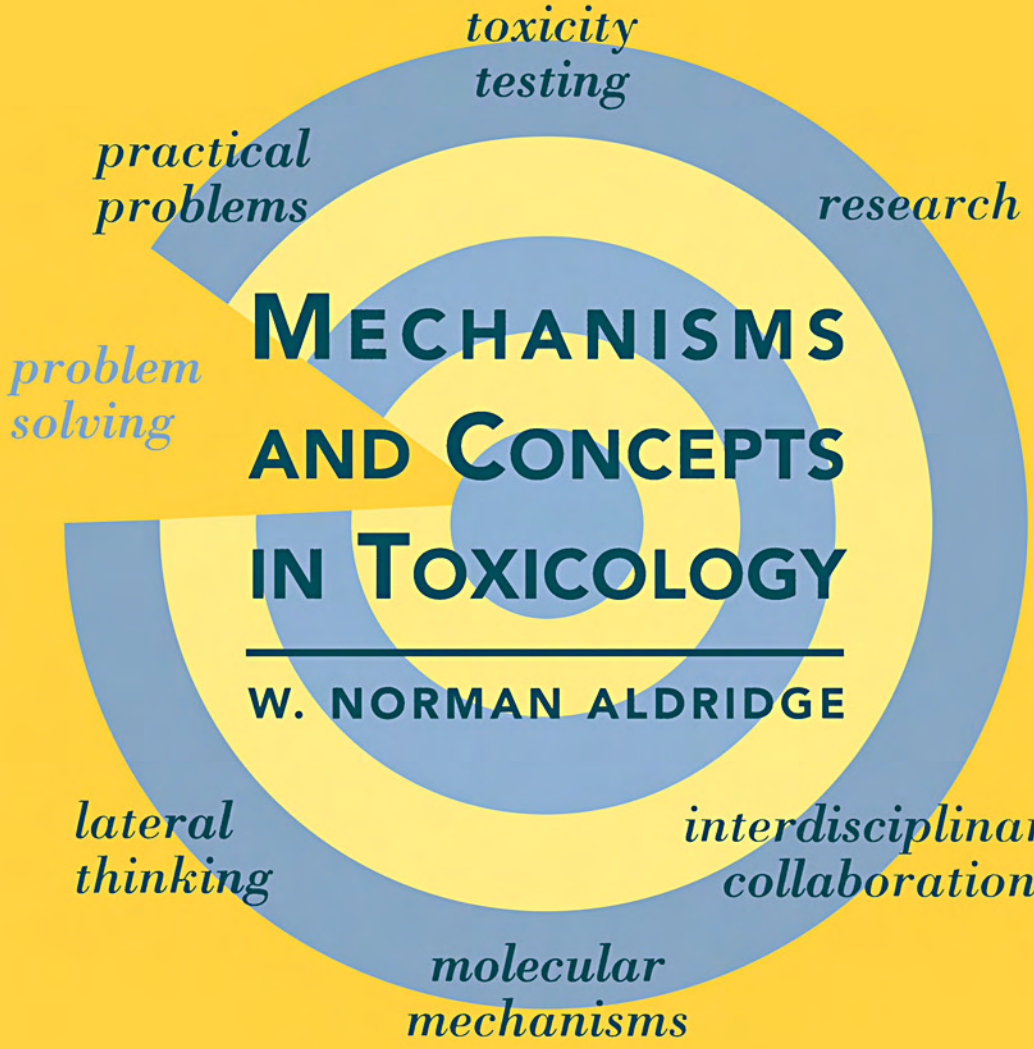

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Mechanisms and Concepts in Toxicology

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Preface

Toxicology is the science of poisoning by chemicals, natural or man-made, small or large molecular weight. Historical studies have often been in essence descriptive but now we can define the early reactions and adverse consequences of the interaction of chemicals with biological systems in chemical terms. This chemical approach also requires a redefinition of some of the basic toxicological terms.

Biology is rapidly absorbing information from molecular biology and the control of biological processes is being defined in molecular terms. Research in toxicology aims to define the specific interactions of an intoxicant with molecular control points in living organisms. In biochemistry and physiology the control of biological systems by hormone and transmitters, and in pharmacology their modification by drugs etc. is now being described in terms of specific interactions with macromolecules (receptors). Although toxicity may be initiated by interactions with these same receptors there are other macromolecular components of cells (targets) whose physiological, biochemical and/or structural function is as yet unknown in detail but whose modification by a chemical causes toxicity.

Many advances in biology have resulted from the experimental and controlled manipulation of biological systems. Claude Bernard's famous quotation made in 1875 is explicit about the value to basic science of the understanding of the consequences of the perturbation of biological systems by intoxicants:

> Poisons can be employed as means for the destruction of life or as agents for the treatment of the sick but in addition there is a third of particular interest to the physiologist. For him the poison becomes an instrument which dissociates and analyses the most delicate phenomena of living structures and by attending carefully to their mechanism in causing death he can learn indirectly much about the physiological processes of life….

Research in toxicology thus serves a dual purpose—it defines the factors determining the degree of interaction with the primary target and also illuminates the molecular aspects of the biological processes involved.

The focus of this book is research and not test procedures. Test procedures are to provide data on specific questions. In research, described by Medawar (1986) as 'that restless endeavour to make sense of things', experimental procedures are continually modified so that the results may prove or disprove a hypothesis. Mechanisms and concepts are illustrated as well as the research route by which they were established. Occasionally unproven mechanisms are presented which, because of our state of knowledge, stimulate working hypotheses leading to new research. The concepts of the 'biochemical lesion' and 'lethal synthesis' developed by Peters (1963) are milestones in toxicological research. Research on mechanisms in toxicology leads not only to greater understanding of the biology involved but also to the means to design substances with

selectivity among species, for example pesticides with large differences between their toxicity to the pest and to humans.

Society now demands that reassurance be given prior to the introduction of a chemical that that chemical will not cause illness in those unavoidably exposed. Exposure can occur during medical treatment, at work or in the general environment. Governments have responded by increasing the extent of toxicity testing both *in vitro* and *in vivo*. However, the interpretation of the results of these tests in terms of the risk to humans may be difficult and often depends on other background information. Rational decisions on how a toxic chemical may be used safely, development of objective biological monitoring procedures, design of safer drugs and chemicals, development of quicker and more precise predictive tests (*in vitro* and using animals) for particular types of toxicity require more understanding of mechanisms in toxicity. A better definition of the factors which influence chemico-biological interactions and a greater knowledge of dose-response and structure-activity relationships enhance the intellectual climate necessary for the rational prediction of the hazards and risks of exposure.

Toxicologists, in the future, will have to utilise an increasing amount of information arising from expanding knowledge of the molecular complexity of biological phenomena. Experience gained in teaching postgraduate students with primary degrees in medicine, pathology, physiology, biochemistry, chemistry or physics (and more mature students wishing to gain a general view of principles and approaches) has shown that a conceptual framework helps in the assimilation of information from diverse disciplines. This book provides such a framework for interactions of any chemical with any biological system.

Although this book presents a chemist's view of mechanistic toxicology, other disciplines play an important role. Contributions from many disciplines are brought together and focused on the importance of understanding the molecular science of chemical-induced perturbations in complex biological systems for both practical and basic toxicology.

Norman Aldridge

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The subject of toxicology is so wide and needs the expertise of so many disciplines that it is obvious that the author, before writing this book, has received help during his career from many colleagues including the staff of the Medical Research Council Toxicology Unit, UK, and many others from home and abroad who have taken part in discussions whenever we met.

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1
Scope of Toxicology

1.1
Toxicology and Mechanisms of Toxicity

Toxic substances have always been used by many species of animal including humans for defensive and offensive purposes. Textbooks of pharmacology are full of examples of such agents used by man, e.g. arrowhead poisons such as digitalis from *Strophanthus* and curare from *Strychnos* and *Chondrodendron*. Although there are many examples of man using natural toxins for his own purposes there are probably many more of one animal species using toxins to control another, to deter an attack by another, to immobilise a food source either for immediate or later consumption. Pharmacology has a rather positive image being concerned with the treatment of disease by natural products, structural modifications of natural products and by synthetic drugs designed for treatment of a particular disease. In contrast, toxicology is sometimes regarded in a negative way. This is a misconception, for in its wider context natural toxins have a respectable position in evolutionary development which has allowed the equilibrium between species to be attained and maintained. Man-made chemicals also have provided and will continue to provide protection by the control of disease vectors (in many species) and also enhanced comfort, convenience and security in modern society. Toxicology and pharmacology are complementary and the thought processes involved in mechanisms of toxicity are similar to those in pharmacology.

Novel therapeutic agents follow from new basic knowledge of disease processes and are now positively designed to interfere with a particular receptor. Even though it may be possible to design a chemical with a high affinity for a particular receptor it will not always result in an acceptable therapeutic agent. During the development of new chemical structures (drugs), undesirable and novel side-effects may appear in tests on experimental animals. Sometimes, even when a potential drug passes all safety checks, side-effects may appear during clinical trials or even after the drug's release for general use. Such side-effects highlight areas of biological activity about which little is known and new biological research is required.

The meaning of mechanistic and mechanisms when applied to toxicology is not always clear. It is often limited as if it were only research which established the primary interaction of a chemical with a macromolecule as the initiating event for the disease. This is an unhelpful and unnecessarily narrow view. Studies in the area of mechanisms are those whose purpose and intention is to provide a holistic view of the process of toxicity; there are many levels of biological complexity which, when combined, allow a reasonable hypothesis of each mechanism of toxicity to be stated.

In a historical context, mechanistic toxicology could not begin before a certain knowledge in basic biology. Research in the seventeenth and eighteenth centuries, largely on plants by Hooke and vøn

Leeuwenhoek, led to the view that tissues consisted of many small entities, i.e. cells. The importance of the nucleus as a feature of all cells was promoted by Schwann, Purkinje, Brown and Schleiden followed by Virchow's clear statements:

1 All living organisms are composed of nucleated cells.
2 Cells are the functional units of life.
3 Cells arise only from pre-existing cells by dividing.

The foundation on which scientific toxicology developed probably derives from the work of Orfila who lived in times of great advancement in chemistry, physiology and pathology. In the early nineteenth century, Orfila was the first to treat toxicology as a separate scientific subject and introduced chemical analysis as an essential component. His first textbook on general toxicology laid the foundation of experimental and particularly forensic toxicology. During the same period Magendie was making many discoveries which became the basis for the experimental analysis of the site of action of poisons (for an extensive discussion of the contributions of Orfila and Magendie see Holmstedt & Liljestrand, 1963). Later in the nineteenth century it became clear that toxic substances could be useful as tools for the dissection of living tissues and thus to learn about the way they are organised in a functional sense (see Preface; Bernard, 1875).

Following such work and the ability to identify by histopathological techniques the tissue and cells attacked by a toxic substance, advances in many relevant sciences took place, e.g. methods for the separation of the active principles of plants, morphological detail of the structural arrangement of cells, the relationship between organ and cell function and the biochemical reactions occurring in them. The development of the electron microscope has led to understanding of intracellular organisation, e.g. subcellular organelles and the existence of other compartments. Methods for the separation by fractionation of tissues and of cells in a functioning state are now being devised so that reactions can be studied *in vitro* under controlled conditions separate from the controlling influences the whole organism. Analytical methods of great finesse are now available for the separation and identification of small and large molecules.

These advances in the biological sciences signalled that there was a reasonable expectation that mechanisms of toxicity should be able to be identified at the cell level and often down to changes in reactions occurring within cells and sometimes to a specific interaction with a macromolecule. The latter is an early event in contrast to changes in morphology which indeed may be a rather late consequence of the primary chemical event. The concept of a 'biochemical lesion' was first proposed by Rudolf Peters as a result of research on the effects of vitamin B (thiamine) deficiency (Gavrilescu & Peters, 1931) and was later used following research on the toxicity of vesicants such as the arsenical, lewisite (Peters *et al.,* 1945) and on the intoxicant fluoroacetate (Peters, 1963); in the latter the concept of 'lethal synthesis' was also introduced. Lethal synthesis is when the toxicity of a chemical is changed and increased by biological conversion of one chemical to another.

Thus understanding in toxicology depends on information derived from many other disciplines, i.e. toxicology is an interdisciplinary subject (Figure 1.1). This is not exceptional for biological disciplines e.g. pharmacology, physiology, microbiology and so on. As in other areas of biology, in addition to the importance of the acquisition of new information, mechanisms of toxicity provide the intellectual climate for the solution of practical problems in toxicology and the subject moves from a descriptive to a predictive science.

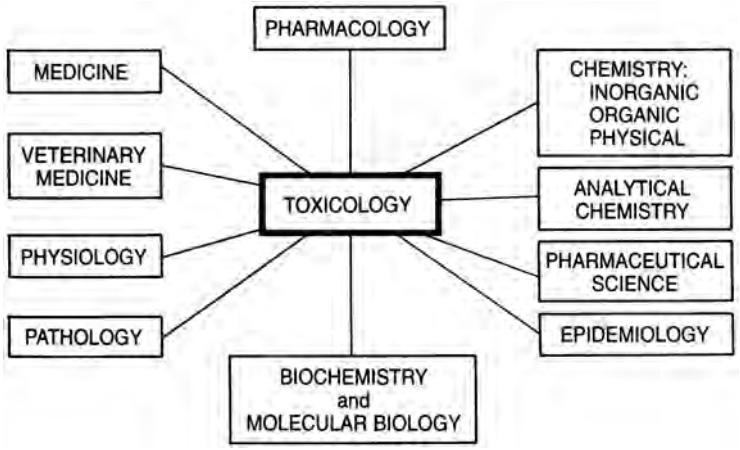

Figure 1.1 Areas of scientific study relevant to toxicology. These relationships imply that crucial steps catalytic for progress in mechanisms of toxicity may arise from research, not only in departments or institutes of toxicology but also in many other areas

1.2
Definition of Toxicology and Toxicity

1 Toxicity by chemicals is changes from the normal in either the structure or function of living organisms or both.
2 Chemicals causing toxicity are intoxicants or poisons.
3 Toxicology is the science of poisons.

These definitions are wide and open-ended with respect to organisms, responses and toxic chemicals.

1.2.1
Organisms

The aim of most studies is to provide safety for human beings. In the past half century there has been a radical change in public attitudes about exposure to chemicals. Prior to 1940 few chemicals were synthesised in large amounts and exposure often occurred during the mining of metals and their subsequent purification and fabrication. If illness resulted, then following medical advice conditions were improved. For a variety of reasons attitudes have changed; these include the advance of chemistry, the increased longevity due to treatment of infectious diseases and better nutrition, and an awareness through education of the possibilities of toxicity. It is now generally accepted that before (not after) a chemical (pesticide, drug, etc), is used, industry and governmental authorities take responsibility for ensuring that sufficient information about potential hazard is available to protect the public. This has resulted in a huge increase in the use of experimental animals for toxicity testing and for research.

Veterinary practice has always been faced with toxicological problems. Some are accidental as in the feeding to livestock of feed supplement containing polybrominated biphenyls instead of magnesium oxide (Dunckel, 1975). Other examples are the fatal exposure of animals in the Seveso episode (Holmstedt, 1980), drinking water contaminated by toxic cyanobacteria (Carmichael *et al.,* 1985) and consumption of

feed on which organisms have grown and produced toxic metabolites as in the initial poisoning of turkeys with aflatoxin (Stoloff, 1977). Toxic plants in fields and pastures have always been a cause of poisoning in animals, e.g. consumption of ragwort containing the toxic Senecio alkaloids (WHO, 1988; Mattocks, 1986).

While the egocentric attitudes of humans have provided the main driving force for the evaluation of the toxicity of new chemicals in recent years there has been a growing view that the ecological balance in nature should be preserved. This view may be challenged and debated *in extenso;* it has resulted in greater attention being given to effects of chemicals on species other than the usual experimental and farm animals. For example, fish toxicity is now considered necessary before the introduction of most pesticides and the toxicity of effluent resulting from their manufacture is more carefully evaluated. The range of organisms which can be studied from a toxicological point of view is potentially very large. In many cases where exposure to chemicals is postulated to be the cause of the change in the population of particular organisms, proof that this is so often necessitates much more information and research (see Chapter 13).

The increase in the use of animals for experimental purposes has resulted in demands from some members of the public for the introduction of *in vitro* techniques to replace them or at least to diminish their numbers; they might utilise a pure enzyme, isolated animal tissues and in some cases model bacterial systems (see Chapter 10).

As a consequence of all the above demands, the range of species of organism and biological systems to be considered in a toxicological context is now very large and is increasing. This expanding range is mainly being driven by questions of a practical nature but, in another context, researchers in biological science have always been prepared to widen their studies to other species in order to solve particular academic problems (e.g. when an animal model is not available within the usual range of experimental animals; see Chapter 10).

1.2.2 Responses

Current perception is that the range of responses is almost infinite; any attempt to produce a useful list for the huge range of potentially affected organisms would fail. Sometimes the site of toxicity is decided by the route of exposure, e.g. through the skin, by inhalation or by absorption from the gastro-intestinal tract, when the toxicity evinced may be to the skin, to the lung, etc. However, all responses are not of equal practical importance. In most cases tissue damage (e.g. loss of cells) can be repaired by mitosis from remaining cells. Thus ingestion of the commonly consumed chemical ethyl alcohol no doubt from time to time causes the death of some hepatocytes but provokes few long-term consequences due to efficient repair processes. However, neuronal loss is permanent and although this may not be clinically significant owing to a large reserve capacity, a permanent change has taken place. Damage to long axons in the central (spinal cord) and peripheral (sciatic nerves) has different long-term consequences; peripheral nerves can be repaired and reach the end organ while axons of central nerves do not reconnect. Secondary consequences in the whole organism, such as scar tissue formation or long lasting inflammatory processes, clinical signs or symptoms which after a short exposure take a long time to appear or which require a long exposure, continue to present challenges to our current understanding in biology and toxicology (see Chapter 8).

Some chemicals cause toxicity following a reversible interaction with a tissue component, whereas others interact to yield a covalently bonded and modified tissue component. Biological consequences of such interactions can be very different but much remains to be explored, particularly in relation to chronic and long lasting toxicity (see Chapter 7).

Clinical responses are obviously emphasised but one of the most important tasks in toxicology is to identify early interactions which result in changes which are part of a chain of events leading to toxicity (a

biological cascade). The identification of early changes may provide the basis for rational biomonitoring of human populations. The definition of such early changes, their dose-response relationships and the identification of longer-term consequences require a rather profound understanding of the biology involved. Sometimes current basic biology is inadequate for the task; thus the nature of a large human poisoning, known as toxic oil syndrome which in 1981 involved thousands of victims (Sections 8.10 and 12.5), is not yet fully understood at a biological level and the aetiological agent has not been identified.

1.2.3 Toxic Chemicals

Traditionally, material which causes toxicity has been divided into toxic chemicals and toxins. This division is arbitrary and is often based on molecular weight; sometimes it is based on whether toxicity is caused by a synthetic chemical or a natural product. Such a division is almost impossible to sustain when, for instance, an effluent is released into a river and then reacts with a natural constituent to produce a toxic entity. *Botulinum* toxin is not usually considered to be within the province of toxicology but plant lectins sometimes are. With few exceptions the toxic chemicals used in this book as examples of particular mechanisms have a molecular weight of less than 1,000; no distinction is made between those chemicals synthesised in chemical industry, mined, produced naturally by organisms in the environment or resulting from the release of synthesised chemicals or by-products into the environment.

There is a popular misconception that chemicals synthesised by chemical industry are dangerous and natural chemicals are not. This is untrue, for some of the most toxic agents occur in the natural world. Table 1.1 lists examples of both natural and synthesised chemicals. All have a molecular weight of less than 1,000. A wide range of toxicity is shown for both natural and synthetic chemicals, and extremely toxic chemicals occur in each group, e.g. with a toxicity of less than 0.1 μmol/kg. This selection of chemicals causes many types of toxicity by different mechanisms and illustrate a central fact in toxicology: chemicals are selective and specific in the toxicity they cause. In one example a natural product has been structurally modified to give a synthetic chemical with enhanced toxicity and properties. Natural pyrethrins derived from the Pyrethrum plant is an effective knock-down pesticide but is very unstable to light. Many pyrethroid

Table 1.1 Examples of natural and synthetic toxic chemicals

Toxic chemical (source)	Molecular weight	Tissue affected	Ref.
NATURAL TOXICANTS			
Aflatoxin B_1 (*Aspergillus flavus:* growth on nuts)	312	Liver necrosis and carcinogenesis	1
α-Amanitin (Mushroom: *amanita phalloides*)	902	Gastro-intestinal tract/liver necrosis	2
Anatoxin-a(S) (organophosphorus produced by cyanobacteria)	252	Nervous system: (anticholinesterase)	28, 29
Fluoroacetate: Na (Plant: *dichapetalum cymosum*)	100	Heart/skeletal muscle/ nervous system	3, 4
Monocrotaline *(Crotalaria spectabilis)*	325	Liver/lung	5, 6

Toxic chemical (source)	Molecular weight	Tissue affected	Ref.
Ochratoxin A (*Aspergillus ochraceus:* growth on nuts/seeds)	403	Kidney	7, 8
Pyrethrin I (Plant: *pyrethrum cinariaefolium*)	328	Nervous system: (nerve conduction)	9
Tetrodotoxin (Puffer fish)	319	Nervous system (nerve conduction)	10, 11
SYNTHETIC TOXICANTS			
Deltamethrin (pyrethroid insecticide)	505	Nervous system (nerve conduction)	12, 13
Ethylene glycol monomethyl ether (solvent)	76	Testis	14
Hexachloro-1,3-butadiene (intermediate)	267	Kidney	15
2, 5-Hexane dione (bioactivated product from hexane)	114	Nervous system (long axons)	16, 17
Methyl isocyanate (intermediate in synthesis of carbamates)	57	Lung/eye	18,19
Methylmercuric dicyandiamide (fungicide)	298	Nervous system (cerebellum)	20, 21
MTTP: 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine (impurity in pethidene derivative)	173	Nervous system (substantia nigra)	22, 23
Paraquat (bipyridinium herbicide)	186	Lung	24, 25
Pentachlorophenol (fungicide)	266	General metabolic poison	26
Soman: pinacolyl methylphosphonofluoridate (nerve gas)	182	Nervous system (anticholinesterase)	27

References: (1) Stoloff, 1977; (2) Weiland & Faulstich, 1978; (3) Peters, 1963; (4) Hayes, 1982; (5) McLean, 1970; (6) Mattocks, 1986; (7) Purchase & Theron, 1968; (8) Chu, 1974; (9) Elliot, 1971; (10) Kao, 1966; (11) Narahashi, 1990; (12) Elliott, 1979; (13) Verschoyle & Aldridge, 1980; (14) Thomas & Thomas, 1984; (15) Hook *et al.,* 1983; (16) Divincenzo *et al.,* 1980; (17) Cavanagh, 1982a; (18) Nemery *et al.,* 1985; (19) Ferguson & Alarie, 1991; (20) Tsubaki & Irukayama, 1977; (21) Aldridge, 1987; (22) Jenner & Marsden, 1987; (23) Langston *et al.,* 1984; (24) Conning *et al.,* 1969; (25) Smith & Nemery, 1986; (26) Gaines (1969); (27) Black & Upshall, 1988; (28) Cook *et al.,* 1989; (29) Hyde & Carmichael, 1991.

structures have now been synthesised with high biological activity and stability to light (Elliott, 1979; Hassall, 1990).

1.3
Origin and Types of Exposure

Exposure and toxicity occurs in different circumstances:

1 Medical and veterinary practice.
2 At work (occupational).
3 In the general environment (environmental).
4 Accidental or intentional (including forensic).

Other subdivisions have been used: environmental toxicity for incidental or occupational hazards; economic toxicology for intentional administration to living organisms, e.g. therapeutic agents for human and veterinary use, chemicals used as food additives or cosmetics and chemicals used by humans selectively to eliminate another species; and forensic toxicology including both intentional and accidental exposure.

1.3.1
Medical and Veterinary Practice

Modern-day therapeutics are often designed from knowledge of the way particular physiological functions are controlled and are thus intended to be specific in their action. Many are intended to rectify defects in normal control or to modify normal physiology and thus are also often prescribed for use for many years, e.g. treatment of hypertension or the contraceptive pill. Extensive animal studies are carried out so as to discard undesirable side-effects but, in practice, others sometimes emerge after the pre-marketing clinical trials or when the drug has been released for general use and the side-effect is recognised during post-marketing monitoring. Thus, experience indicates that drug side-effects continue to be a problem, as stated by Fingl and Woodbury (1966):

> Drug toxicity is as old as drug therapy and clinicians have long warned of drug-induced diseases. However with the introduction into therapeutic practice of drugs of greater and broader efficacy, the problem of drug toxicity has increased, and it is now considered the most critical aspect of modern therapeutics. Not only is a greater variety of drug toxicity being uncovered, but the average incidence of adverse effects of medication is increasing, and unexpected toxic effects occur relatively frequently. There is an urgent need for the development of methods in animals that accurately predict the potential harmful effects of drugs in man.

Although improvements have been made since 1966, problems remain. Side-effects which are novel forms of toxicity quite different from their therapeutic action may appear. Also, control of the dose necessary for the drug's therapeutic action is vital; this is the province of the pharmacologist and the clinician.

1.3.2
Exposure at Work

Exposure at work takes place during the development period, production on a pilot plant scale, full-scale production, formulation and use. In recent years there has been an irresistible centralising tendency in industry so that fewer places synthesise a particular product or an intermediate for a future manufacture. This is beneficial for working conditions but the product then has to be transported in large quantities over

great distances with a potentiality for accidents. In practice, the environments of formulation plants are often less controlled and exposure may be greater. It is difficult to generalise about the extent of the danger of exposure at work; however, during the dissemination of pesticides for agricultural pest control or vector control for public health purposes in developing countries, long hours of work under poor conditions, problems of diluting the concentrates and of disposal of empty containers often lead to high exposure.

1.3.3
Environmental Exposure

A major problem in environmental exposure is to reassure the public that long and perhaps life-time exposure to low residues of compounds in food and water, e.g. pesticides, food additives, fluoride in drinking water, will not cause undesirable effects. Neglecting the obvious problem of proving a negative, the potential for such a consequence can more confidently be predicted if a sound basis for mechanism of action is known and the scientific basis of threshold is established. From the results of basic research, dose- and biomonitoring techniques may be devised to follow up those people who are, during their work, exposed to a relatively higher concentration for a long time—such studies can be very useful for risk assessment.

1.3.4
Accidental and Intentional Exposure

As mentioned above there is always a potential hazard in the transport of the final product or the chemically active intermediates used in its synthesis. The use of a chemical for purposes for which it was not intended, failing to follow the recommended procedure for its use, the re-use of containers and the storage of a chemical in unsuitable and often unlabelled bottles has led to many fatalities by accidental poisoning. Experience shows that when a toxic chemical becomes available it will be used in suicide attempts, and even in the best regulated circumstances accidents will happen. Although such accidents are regretted when they happen they provide a source of information about the susceptibility of humans to poisoning by particular chemicals. Systems need to be available to collect and utilise this potential information (Aldridge & Connors, 1985). Expertise is required, as an immediate need, to care for those exposed and to contain the accident. However, others trained in epidemiology, analytical chemistry and experimental toxicology are needed at an early stage to ensure the conditions for the collection of relevant information and samples for analysis.

1.4
Discussion

The purpose of this chapter has been to outline the potential scope of toxicology both in the natural and industrialised environments. It is not intended to suggest demarcation disputes about what is and what is not toxicology. Perhaps more than other scientists, those who are engaged in studies on toxicity rely on information from many other scientific areas. Those engaged in research on mechanisms of toxicity are engaged in basic research in the biological sciences. While it is possible to limit research to projects with a predictable practical benefit, it is equally valid to choose an unsolved problem in mechanisms of toxicity which will throw light on some area of biology.