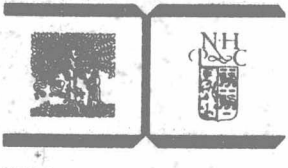
The Principles and Methods in Modern Toxicology

C. L. Galli, S. D. Murphy and R. Paoletti
Editors

THE PRINCIPLES AND METHODS IN MODERN TOXICOLOGY

Proceedings of the International Course on The Principles and Methods in Modern Toxicology held in Belgirate, Italy, 22-26 October, 1979

Editors:
C.L. Galli
S.D. Murphy
and
R. Paoletti



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PREFACE

The proceedings of the International Course on "Principles and Methods in Modern Toxicology" organized by the Fondazione Giovanni Lorenzini and by the Institute of Pharmacology and Pharmacognosy, University of Milan, held at Belgirate on October 22-26, 1979, are now presented to the international scientific community.

This Course is the first organized by the Fondazione Giovanni Lorenzini in order to discuss the most up-to-date developments in the field of the environmental toxicology. This Course is therefore general in nature and it will be followed by more focused activities devoted to methods for more specific problems in the area of modern quantitative toxicology. The distinguished group of international experts who have participated in this meeting has been asked to collaborate in planning the future series; they believe that such enterprise is timely. There is an evident need for common methodology, legislation and training for toxicologists in the industrialized countries.

The Fondazione Giovanni Lorenzini is proud to have collaborated with this scientific event in its tradition of interest in continuing education and pioneering activities.

The review and the experimental papers collected in this monography represent the results of a collaborative effort and they are offered to the scientific communities for evaluation as contributions to an emergent science.

We are particularly grateful to the lecturers, to the editors of this volume and to the distinguished audience that has stimulated a most fruitful discussion, and we hope that this monograph may be of use to active toxicologists and scientists training in many countries.

Prof. Rodolfo Paoletti
President, Fondazione
Giovanni Lorenzini

SYMPOSIA OF THE GIOVANNI LORENZINI FOUNDATION Volume 6

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The Principles and Methods in Modern Toxicology
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TOXICITY TESTING: SCOPE AND DESIGN

R. TRUHAUT

Toxicological Research Center, René Descartes University, Paris (France)

GENERAL OVERVIEW

It is a pleasure and an honour to have this opportunity to speak to you all this morning and to talk about one of my favorite topics.

Since the end of the last century, society has entered a new era, the chemical era. This era has been characterized by an extraordinary expansion of industrial development and by a consequent increase in the use of chemical agents in most varied applications. This spectacular progress in the chemical sciences and technology has undoubtedly brought about a great economic and social benefit and, therefore, an indisputable improvement in the living standards of many world populations.

However, the hazards which might result from people's exposure to a considerable and ever increasing number of chemicals in modern life should not be overlooked. Certain products may be hazardous to human health and, therefore, present important problems for toxicologists, hygienists, physicians, engineers, and technologists who are concerned with the protection of public health.

Toxicology focuses on the detection of toxic risks to humans. This is essential for establishing preventive measures since one can only prevent risks which are known and have been identified. The term 'toxic' is derived from the Greek word 'toxon' that means bow which recalls that, unfortunately, humans have always been concerned with finding ways to kill. That may well have been the reason why toxicology or the science of poisons first developed along the lines of legal or forensic medicine.

In the second stage of development of toxicology, interest shifted to drugs and pharmacotherapy; emphasis was given to establishing relationships between effective (medically beneficial) and toxic (side action) effects and the corresponding doses responsible for these effects.

In the course of the chemical era already mentioned the field of toxicology has grown considerably. It includes the study of agents to which workers in industry and agriculture may be occupationally exposed (occupational toxicology), components of air pollution, aqueous effluents of industry, automobile exhaust (environmental toxicology), pesticides used to combat parasites and agricultural

pests, intentional and non-intentional food chemicals (food toxicology), multiple chemical agents, material in household ingredients, cosmetics, packaging materials, etc.

Toxicity testing is necessary to establish risks of these chemicals to human population. Experimental toxicology, thus, is concerned with the design of tests which would enable the determination of the potential of a substance to cause injury and with the development of enough data to warrant conclusions that levels of exposure should be so low in relation to harmful doses that there would be a practical certainty that no harm can result. Such information can usually be obtained by studies in animal models; since emphasis is generally on the detection of subtle long-range effects deriving from chronic low level exposure, suitable designed chronic or lifetime studies are the basis for most decisions regarding safety of chemicals.

THE DESIGN OF TOXICITY TESTING

The problem of designing animal experiments includes two sources of uncertainties: a) the uncertainty whether the animals chosen for the toxicity testing are appropriate models from which to extrapolate the results to humans, and b) the uncertainty whether effects that may occur only in very low incidence in the population can be detected with the number of experimental subjects that are practical in laboratory investigations. Since the goal of toxicity testing is to insure the least possibility to harm to humans, the experimental studies should be designed to detect any and all toxic effects. There is no ideal animal available which has the high susceptibility to every possible adverse effect and in which the induced adverse effects are comparable to those observed in humans; thus the inherent limitations of animal studies and the consequent difficulties are evident. These difficulties represent scientific challenges of great practical significance and require that the maximum creative competence be harnessed to solve these problems.

For practical purposes, several national and international groups concerned with toxicity testing have made specific recommendations in this regard.

Essential to the success of toxicity testing are proper experimental design and proper interpretation of results. Essential also is the maintenance of good laboratory management and practice so as to prevent or minimize contamination of air, food, water and equipment, to minimize the incidence of intercurrent disease, and to assure adequate records of, and the preservation of important experimental material.

In designing the studies, basic minimum requirements should include observa-

tions on growth, food intake, clinical examination, hematology, blood chemistry, urinalysis, gross pathology and histopathology. Additional observations or tests should be included either as a direct result of observations made during interim sacrifices or as a result of prior knowledge based on structural similarities to compounds studies previously or on earlier screening studies, either acute or subacute.

It is clear that toxicity testing should be done under the guidance of qualified scientists who, by training and experience, are competent to respond to unforeseen toxicological manifestations noted during the course of the study by initiating reasonable additional experimental procedures or modifications of established protocols. An earlier decision to follow a certain protocol cannot in any way obviate the requirement for data to answer new questions raised by the experimental results of the original protocol when these questions are pertinent at the time of the final evaluation for safe use of the chemical in question. For a better understanding of the problem, it is necessary, at this point, to introduce some general notions which are very well established in human toxicology. It is believed that the application of these concepts to the specific field of experimental toxicology would be useful. These concepts are: 1) the various forms of toxicity directly affecting certain living organisms; 2) the adverse effects indirectly affecting humans, caused by direct biological (possibly toxic) effects on other organisms living in the biosphere; 3) the effect of various factors on manifestation of toxicity; 4) the importance of establishing qualitative and, particularly, quantitative dose-effect relationships so that toxicity thresholds and, therefore, allowable limits can be established.

VARIOUS FORMS OF TOXICITY

1. Acute or subacute toxicity

The first form of toxicity that will be considered is acute or subacute toxicity, i.e., toxicity resulting immediately or after a short delay from the absorption of an adequately large single dose or of several rapidly successive doses of a chemical. In humans, for instance, this occurs following the ingestion of many products, for some, by penetration through the skin and, in the case of gases or vapours, such as carbon monoxide, chlorine, or hydrogen cyanide, following inhalation.

The manifestations of this form of toxicity are spectacular since they may even be expressed in sudden death. That is the reason why the belief that poisons are substances which kill violently is so widespread. Experimentally, for the matter, the estimation of the acute toxicity of a given substance is

currently carried out in the laboratory by determining the lethal dose and particularly the lethal dose 50, i.e., the dose which produces death in 50% of the treated animals. This dose may vary widely, depending on the species of experimental animals as well as on diverse factors, particularly the route of the administration. In the case of gases and vapors, when the lethal concentrations is determined, the time of exposure is always specified.

2. Long-term toxicity from absorption of repeated small doses

It cannot be emphasized enough that toxic effects do not only result from absorption of relatively high doses in a short time. Quite often, they also result form the repetitive absorption of even minute doses, or of doses too low to cause acute toxic effects. This repetitive administration leads to intoxications which are much more insidious because they generally appear without any signal. This, then, is a matter of long-term toxicity resulting in the phenomena of cumulation of doses and cumulation of effects. Cumulation of doses occurs particularly in the case of so-called cumulative poisons, e.g., elemental derivatives of arsenic, fluorine, heavy metals (lead, mercury, cadmium, etc.) and halogenated aromatic compounds such as polychlorobiphenyls or inseticides of the DDT-type. These poisons are retained in living organisms due to their physical nature (much greater solubility in lipids than in aqueous liquids; adsorption, etc.), due to their chemical nature (fixation to certain cellular components), or due to their harmful effects on excretory organs which hamper their elimination (heavy metals). The absorption of small doses of such cumulative products which, if normally eliminated, would have no discernible consequences, brings about disorders at the level of the receptors when, after certain time, toxic concentration thresholds have been reached. Cumulation of effects is exemplified by substances with an inherent carcinogenic action. From results obtained in rat studies with paradimethylaminoazobenzene (butter yellow), a hepatoma-producing azo-dye stuff, Druckrey and Kupfmuller in 1949 forwarded the *a priori* paradoxical concept of addition of effects from each individual dose over the entire lifetime of the experimental animal, whatever the rates of elimination and metabolic degradation. There would not only be a cumulation of doses but a total summation of absolutely irreversible effects. Thus, carcinogenic substances would occupy a separate place among agents of long-term toxicity, because in their case, it would not be possible to establish threshold doses, since, due to persistence of the effect after elimination of the product, no dose, however small, would be without danger if the dose would be repeated and if a sufficiently long period would permit the manifestation of their activity. More recently, however, various considerations made one wonder whether the concept of absolute irreversibility of effects is not an exaggerated

presentation of the facts. Certain observations in the field of molecular biology, for instance, make one admit the possibility of a repair of lesions at the level of nuclear macromolecules, lesions which precede the development of malignant proliferation.This involves very important problems which are currently being discussed with great interest on an international level and which prompted research on dose- effect relationships of carcinogenic agents, both physical, like X-rays or radiations emitted by radioactive elements, and chemical.

3.Long-term effects resulting from absorption of a single dose

It should be emphasized that in addition to immediate acute or subacute toxic effects and more or less long-term toxic effects resulting from the repeated absorption of small doses, there are also more or less long-term effects which may result from a single dose or from a single exposure. During the last few years, various examples have been given of products capable of causing serious effects in humans and laboratory animals after a more or less prolonged latency period during which the products themselves had already disappeared from the organism. It is in this way, for instance, that the herbicide Paraquat, derived from bipyridinium, produces, several weeks after ingestion of a certain dose and having caused only a minor gastrointestinal disorder,a proliferation of fibroblasts at the level of the pulmonary epithelium which may become fatal by inhibition of oxigen diffusion. Another example is the delayed neurotoxic action of certain organophosphorus compounds, expressed as axon degeneration in the central nervous system, with demyelination leading to paralysis. These are the so-called 'hit and run poisons'. Research is currently being carried out to discover the biochemical lesions which are at fault.

In certain cases, the effects of a single dose may manifest themselves after a very long time. This has been shown, in experiments with laboratory animals, — to be the case with carcinogens like nitrosamines and related substances (nitrosamides). Thus, the administration to a pregnant rat half-way in the gestational period, of N-nitroso-N-methylurea, at a dose which does not cause any apparent toxic effect in that animal, produces cerebral cancers in the offspring when they reach adulthood (transplacental carcinogenesis).

4. Special forms of toxicity: teratogenic and mutagenic effects

The hint at an attack in utero directs attention toward effects on functions of reproduction and, particularly embryotoxic effects. This leads to the examination of teratogenic effects which represent a particular type of embryotoxicity.

Mention should also be made of mutagenic effects, i.e. the production of mutations giving rise to substances with genotoxic properties. Their identification, at least in higher mammals, is difficult, and, currently, work is very actively being carried out in specialized laboratories in an attempt to establish an adequate experimental method. Other very specific effects also deserve attention, for instance, immunosuppressive effects, behavioral effects, sensitizing effects, and many other, perhaps more subtle effects. It is believed that, by simply mentioning these various categories of effects, attention may be drawn to the multiple and multidisciplinary methodological approaches that come into play in the testing for possible toxicity of chemical agents.

CONCLUSION

This paper tried to put into perspective the general principles which should guide the scope and the design of toxicity testing.

We are, however, fully aware that because of the multiplicity and of the complexity of the problem, the approaches taken are far from being ideal in every case. Consequently, we understand the necessity to keep an open mind to new develoments permitting to correct and to improve the toxicological methodologies. Further research in this typically pluridisciplinary field must be encouraged and supported. Nevertheless looking at the situation as it presented itself some 20 years ago, we feel that the toxicological approaches so far followed were very useful in giving, on as much as possible scientific basis, guidance to the regulatory authorities to cope with the difficult task of protecting public health from the impact of the chemical era.

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CHEMICAL SAFETY EVALUATIONS AND TOXICOLOGICAL DECISIONS

G.VETTORAZZI
Scientist, World Health Organization, Geneva, Switzerland, and
Professor of Experimental Toxicology, University of Milan, Italy

INTRODUCTION

Safety evaluations as they relate to chemical substances may mean different things to different people. For example, to the drug toxicologist safety evaluation is the process by which therapeutic effects are related to the lethal effect by establishing a margin of safety or 'therapeutic index'. This practice has been a common procedure in pharmacology with regard to drug development for many years; it should be, however, noticed that the same practice has become more complex since the drug toxicologist today wishes to develop drugs that have not only a high therapeutic index but also a high index regarding all undesirable side actions and all undesirable effects of the drug.

To the occupational toxicologist safety evaluation indicates the process of identifying and interpreting data on uptake/response relationship with the aim of establishing permissible levels of occupational exposure. Similarly, to the environmental(including food) toxicologist safety evaluation, as currently practised, represents the determination, for a given compound, of the dose that is without detectable effect in the experimental animal of choice and then, by application of 'safety factors' to that dose, the estimation of the amount that can safely be consumed by man.

At first sight carrying out a safety evaluation on a chemical it may appear a relatively simple process whether the toxic material is a drug, industrial chemical, environmental contaminant or a

natural product. In reality, safety evaluations are anything but simple, particularly when concepts such as risk estimation and so= cially acceptable risk are brought to bear.

There seemsto be general consensus that a sound safety evalua= tion should be based on sound toxicological considerations and decisions. These decisions, by their very nature, belong in the do= main of technical and scientific decisions rather than in that of political and administrative ones, and they should, therefore, be based solely on technical and scientific knowledge. In turn, poli= tical and administrative decisions as to whether the use of a substance presents a socially acceptable risk are based on the toxicological decisions that have been taken after exhaustive scru= tiny of scientifica facts. It follows that the availability of scientific data when a toxicological decision is taken is crucial to the whole process of safety evaluation of a chemical. The qua= lity of the decision will reflect the quality and the quantity of the data on which it has been based.

Safety evaluations and toxicological decisions are commonly car= ried out by individuals operating in 'expert committees' and very rarely by one individual or by computer; in a matter which requires broad representation of many disciplines and vast partecipation of solid experience, society feels better protected when it relies upon collective judgements rather than upon whims of individuals. At present, there are many of these 'expert committees' at the national, regional and international level. In addition to carrying out safety evaluations on a great variety of chemical substances, these groups have also been operational in fostering the development of requirements for testing procedures, which, over the past several decades, have become more complex and elaborate. In the 1940's, for example,it was not uncommon to call a study of thirty days' duration a chronic toxicity study. Total testing of safety of food chemicals and drugs was commonly conducted in a few rats,