5. TOXICITY TESTING AND HUMAN HEALTH

R. Kroes

5.1 INTRODUCTION

"Toxicology is the scientific discipline involving the study of actual or potential danger presented by the harmful effects of substances in living organisms and ecosystems, of the relationship of such harmful effects to exposure and of the mechanism of action, diagnosis, prevention and treatment of intoxications" [1]. Paracelsus’ saying: “Dosis sola facit venenum” (it is the dose which makes the poison) is well-known and depicts a property inherent to almost every chemical: at a certain dose effects are inevitable. Whereas toxicity testing in the past was carried out using non-standardized methods, today many standardized test systems are available for the investigation of specific aspects of toxicity in experimental animal and in vitro systems. In addition, controlled studies in humans are increasingly used. Indeed, over the past two or three decades toxicity has emerged as a mature science, in which routine toxicity testing has evolved a protocol-driven, “cook-book” approach, which may occasionally make one forget that toxicity testing should also be an “art”.

The science of human toxicology includes both the producing and gathering of toxicity data in biological systems, and the subsequent evaluation and interpretation of these data, with the aim of predicting possible risk, or lack of risk, to humans. The toxicity testing of environmental chemicals initially focused on determining safe levels of human exposure to toxic chemicals. The testing has now expanded from simple acute and subacute tests to well-balanced consideration of data on acute, subacute and chronic toxicity, specific toxicity such as carcinogenicity, mutagenicity, reproductive toxicity and, more recently, immunotoxicity, neurotoxicity, dermal toxicity and other organ tests.

In addition to these toxicity tests, data on the mechanisms of action at the tissue, cellular, subcellular and receptor levels, as well as toxicokinetic data, greatly facilitate the interpretation of toxicity data and assessment of the potential hazard to humans. “Protocol toxicology” and “receptor toxicology” are essential to provide the optimal context for risk prediction [2,3]. Toxicology is also becoming increasingly complex. It takes a considerable amount of effort to determine the toxicity of just one agent, let alone the numerous variety of agents currently available. The large number of chemicals involved requires rules to be able to set priorities for toxicity testing.

5.2 GENERAL ASPECTS OF TOXICITY

Toxicity is the capacity of a chemical to cause injury to a living organism. This, of course, depends on the quantity of substance administered or absorbed. Ideally, administration of the substance to a model (animal) system should be comparable to human exposure, e.g. by ingestion, inhalation, topical application or injection. Adverse effects should always be described in relation to the quantity of substance administered and, ideally, a dose-response relationship should be established for the observed adverse effect(s). The type and severity of the effect and the time needed to produce such an effect are also essential elements.

In theory small doses of a toxic agent can be tolerated due to the presence of systems for physiological homeostasis or compensation, such as metabolic detoxification, cellular adaptation and repair. Thus, below a certain minimum dosage these compensatory mechanisms can counteract the effects of a substance and ensure the normal functioning of an organism without significant injury [4]. Therefore, a toxicological threshold can be defined as the dose or exposure concentration below which an adverse effect to the organism will not occur (Figure 5.1).

Figure 5.1. The impairment-disability curve. Impairment increases: (1) with aging, (2) as a result of illness and (3) due to excessive environmental stress. From Hatch [4]. With permission.
Above a given chemical-specific threshold the compensatory ability of the organism becomes saturated, leading to the loss of physiological homeostasis and impairment of function. This impairment may be reversible or irreversible and ultimately fatal. Since toxicity may occur in several target organs, different thresholds may apply for different endpoints, some of them being of more concern to human health than others. Other factors may also influence toxicity such as sex, age, genetic predisposition, nutrition, pregnancy, health status and combined exposure. Although an individual threshold dose for a specific mammal can be experimentally established, we are mainly interested in a population threshold which indicates the response or lack of response of all individuals within numerical limits of each group. This takes into account the natural variation in sensitivity existing within the population. When the body’s compensatory processes are overwhelmed at a given dose of a substance, the toxicity threshold is exceeded and pathological effects become apparent. When this pathological phenomenon occurs at a site distant from the site of entry of a substance, which is often the case, this is called systemic toxicity, in contrast to topical toxicity. Systemic toxicity thus requires absorption and distribution of the substance.

In addition to overwhelming the compensatory processes in the body (called deterministic toxicity, which, in principle, is a reversible response), non-deterministic toxic effects can occur due to insidious processes that are irreversible or poorly reversible at low doses or during the early stages of causation [5]. Examples of this include biological effects, such as irreversible changes in genetic material, and some chronic cumulative effects.

In principle, for changes in DNA leading to hereditary mutagenic diseases and cancer, there is no threshold, since they are stochastic processes, while for other chronic cumulative effects a threshold is likely since these effects are not necessarily stochastic. Toxicological endpoints involving alteration of the genetic material are further characterized by the fact that their frequency rather than their severity, increases with dose.

In toxicity testing, the aim is to identify and describe properly the adverse effects which a substance may produce and establish a dose-response relationship. In addition, information on the mechanism of action greatly contributes to the quality of a safety evaluation. Establishing a no observed adverse effect level (NOAEL) is another important aim of toxicity testing, since it provides the basis for the interspecies extrapolation which has to be performed to predict safety for humans. The NOAEL can be defined as the highest concentration or dose of a substance, determined by experiment or observation, which causes no detectable adverse alteration in morphology, functional capacity, growth, development or life span of the target organism under defined conditions of exposure [1].

The correlation between exposure and the incidence or severity of effects (in quantity or percentage) is usually called the dose-response relationship. This relationship is fundamental to toxicology. Understanding the relationship between effect and dose is the basis of safety evaluation, assuming that the effect is the result of the substance administered. In toxicology, we can observe a quantal response (mortality or number of animals affected) and a graded or continuous response (weight, enzyme activity, etc.). When a large number of doses is used in an experiment with a large number of animals, the result can be represented by a sigmoid dose-response curve (Figure 5.2). In this curve the dose has been given on a log basis. The curve can be transformed to a linear curve by a probit or logit transformation. Both methods are based on the assumption that dose-response exhibits a gaussian distribution, known as a normal frequency distribution. A normal frequency distribution shows the variation among animals in susceptibility to a substance, ranging from relatively resistant animals to hypersensitive animals, the majority being normally sensitive.

![Figure 5.2. Sigmoid dose-response curve and the probit transformation to a linear curve.](image)