# HEALTH BASED STANDARDS: TOXICOLOGY

**Luc Hens**
*Human Ecology Department, Free University of Brussels (VUB), Belgium.*

**Keywords:** Toxicology, environmental toxicology, dose-effect relationship, No Observed (adverse) Effect Level (NOEL), Acceptable Daily Intake (ADI), drinking water guidelines, chemical hygiene, toxicokinetics, toxicodynamics.

## Contents





## Summary

This contribution unravels the link between guideline establishment and toxicology. The results from laboratory experiments, in general animal experiments, provide data which are useful to determine guideline values.

To handle these data, the relevant basic concepts in toxicology are overviewed. They entail the action mechanisms of pollutants in the body. In this respect exposure (dermal, inhalation, ingestion) is important for understanding how the substance is taken up, transported, eventually transformed and eliminated (toxico-kinetic phase). Toxico-dynamics studies the effects and the action mechanisms of toxic substances.

This background information provides an understanding of the importance of fundamental criteria in toxicology and their relevance to standard setting: acute versus chronic exposure, reversible versus irreversible effects, locally versus systemic acting substances, stochastic versus non stochastic effects. Moreover the data provide a basis for classifying toxic agents according to their mechanism of action, the symptoms they cause, or their target tissue.

Quantification of toxic effects uses dose-response relationships. The derived curves may determine $LC_{50}$, $EC_{50}$, NOEL, NOAEL and LOAEL-levels. The NOAEL level is the scientific interpretation of the "safe" dose and provides the basis for guideline establishment. Experiments on which this value is based are subject to high quality standards, to assure the NOAEL is established with confidence.

Adjusting the NOAEL for body weight and scientific uncertainty allows for the calculation of the acceptable daily intake (ADI) which extrapolates the animal toxicity data to humans. The ADI is the daily dose considered to be without risk to humans for a lifetime intake. The ADI is the toxicological key factor to establish guidelines. An example on how to calculate guidelines for drinking water illustrates this point.

Reviewing the theoretical background, the scientific uncertainty and the practical difficulties in the establishment of guidelines using toxicological data, it is clear that standards are a necessary but partial instrument to protect human health from the effects of pollution. A more restrictive and precautionary attitude towards the introduction of chemicals into the environment might result in important health benefit. The application of the "chemical hygiene" concept might help in this respect.

## 1. Introduction

Environmental standards aimed at protecting human health are based upon guideline values which are considered within their context of prevailing exposure levels and environmental, social, economic and cultural conditions. Guidelines provide a basis for protecting human health from the adverse effects of pollution and for eliminating, or minimizing contaminants that are known or likely to be hazardous to human health and wellbeing.

Guidelines represent the best scientific judgment at a particular moment. This judgment is based upon the combined results of epidemiological and toxicological research. The relationship between epidemiology and guideline establishment is the subject of *Health-Based Standards: Epidemiology*. This contribution unravels the link between toxicology and guideline establishment.

This article discusses the aspects of research on animals and on in vitro systems which judge the toxicity of a substance for humans. The mechanisms of poisoning are described both in their qualitative and quantitative aspects. The paper also addresses how toxicity in animal experiments is determined and how results can be extrapolated to humans. The use of these data in establishing guidelines for drinking water is provided as an example.

## 2. Definition: toxicology and environmental toxicology

Toxicology deals with the interaction between a substance and an organism. The word relates to the Greek "toxicon" which means poison. Toxicity refers to the capacity of a substance to harm living organisms. Toxicology covers both the routes of exposure (air, water, soil, food) and the mechanisms of the effects. It provides a scientific basis for managing risks from toxicants. Toxicological risk assessment aims at combining the

bulk of toxicological knowledge on a particular substance in one figure, which provides an indication for its toxicity.

Toxicology was by origin a descriptive scientific discipline which provided very useful information e.g. on the use of medical drugs. As such it was related to pharmacology, physiology and pathology. Gradually however toxicology moved towards more understanding and more fundamental insight in the basic mechanisms underlying the toxicological effects. The more toxicology moved into this direction, the more fundamental disciplines such as biochemistry, cellular biology and genetics became important. The resulting picture is thus a very interdisciplinary area of science.

Toxicology is characterized by a wide variety of societal applications. In addition to the link to pharmaceutical and medical aspects of poisoning (clinical toxicology), toxicology is applied in research on chemicals in food, in industry, in criminal acts and also in the environment. Environmental toxicology analyses effects of exposure to contaminated air, water and soil. Environmental contaminants in food are also of clear importance to assess exposure. Therefore food toxicology is closely related to environmental toxicology. The research on and the prevention of poisoning in industrial conditions (industrial toxicology) also provides information which is relevant for evaluating environmental situations.

The starting hypothesis in toxicology is that each substance might act as a toxicant. The occurrence and the intensity of health aspects is a matter of dosage. The dose concept is of core importance in toxicology.

## 3. Qualitative aspects of the action of toxic substances in the organism

A substance in an organism acts in different phases. These phases can be globally subdivided into an exposure phase, a toxico-kinetic phase, which studies the fate of the pollutant during the transport, and a toxico-dynamic phase in which the interaction with the target organ is analyzed.

### 3.1. Exposure

Exposure to a substance can happen by three main ways: inhalation (through the lungs), oral (through mouth, stomach, intestine system) and dermal (through the skin) intake. A substance will often be taken in through a combination of these ways. In estimating the total exposure to a pollutant it is important to take into account the combined effect of these three exposure entrances. Exposure to contaminants in food and drinking water occurs through oral intake. Children are subject to a particular aspect of exposure to contaminated soil. Young children (1 to 4 years) usually put objects covered by dust into their mouths. Occasionally they might even eat soil. This habit is called pica, from the Latin name *Pica pica*, the magpie, a bird which collects bright objects with its bill. The total amount of soil ingested is estimated to be a maximum of 200 mg per day. Digestion as a process can also be subdivided into a number of steps. In each of these steps contaminants can be taken up by the body. The small intestine, which is characterized by an enormous surface of exchange (approximately 200 $m^2$) by the presence of villi and microvilli, is an important area of uptake. The cells covering the

intestine border are in close contact with the blood, where the substances are traced back. From the uptake area, the substances are transported to the liver and further distributed towards the tissues. Fat soluble substances can bypass the liver in the lymphatic system and get straight to the systemic circulation.

The amount of pollutant given orally will only be partially assimilated. The non-assimilated proportion will be excreted. The fraction which will be absorbed strongly varies from pollutant to pollutant.

In risk analysis it is important to estimate the amounts of intake and uptake. The food basket, however, differs substantially among countries, populations and individuals and changes over time. Only local investigations can provide insight into the variety and amount of food which is consumed. For water it is accepted that two liters a day are consumed.

Air pollutants are primarily taken in by inhalation. In the lungs an exchange between air and blood will take place. The lung alveoli are the most important in this process. With an estimated amount of 300 million per lung, they represent an exchange area of about 50 to 100 $m^2$.

Before the air reaches the alveoli, it has to pass a complex branching system of trachea, bronchi and bronchioli. These tubes are covered by cilia at their inner side which keep a carpet of mucous in continuous movement (the 'muco-ciliary escalator'), directed towards the mouth. This mucous layer is important in pollutant studies for two reasons:

a) It captures larger particles (> 5μm) and other substances in the air and removes them through the gastro-intestinal system. In this way it provides a cleaning mechanism of the lung for harmful substances. This does not mean that no pollutants at all can reach the alveoli, but an important part of them will be cleaned up using this system. The smaller 'ultrafine' particles do get down to the alveoli where they can be engulfed by scavenging macrophages that patrol the alveolar surface. However if the macrophage system is overwhelmed by the number of particles presenting, they will be assimilated into the blood stream or lymphatics.
b) Some pollutants such as $NO_x$, act on the effectivity of this system. The cilia are moved by small circular muscles which are found in the area of implantation at the wall of the airtubes. $NO_x$ inhibit the contraction of these muscles and in this way impair the cleaning capacity, which results in a higher intrinsic probability for harmful substances like carcinogens to interact with the lung tissue.

The amounts of air which are inhaled might vary according to age, sex and working conditions. The difference between rest and heavy work is a factor of 3. Nevertheless one bases risk calculation figures on 20 $m^3$ per day for adults and 10 $m^3$ per day for children.

Uptake through the skin is particularly important for substances in the air or in bathing water. Although the skin has a structure providing effective protection against many

agents, some substances can penetrate through the skin and reach the blood vessels which are underneath it. Moreover damage to the skin can facilitate the uptake of toxics.

Substances assimilated into the body by inhalation or dermal routes are potentially more toxic weight for weight, as the liver, the major site of detoxification, is bypassed initially.

### 3.2 Toxico-kinetic phase

Toxico-kinetics studies the fate of toxic substances in the organism. This includes the uptake, transport, transformation and elimination.

Substances have been effectively taken up at the moment they are present in the blood. From this moment on a substance can be subject to different processes.

Transport distributes a substance throughout the body. This distribution can be rather homogenous, as is the case for benzene. Substances might, however, have an affinity for particular tissues, such as PCBs and dioxins which accumulate in fatty tissues or the insecticide paraquat which accumulates in the lungs.

Substances which are subject to transport before reaching their target tissue are called systemic acting agents. Substances acting at the site of their intake are called locally acting agents.

Transformation processes like the ones in the liver can either detoxify a product or bioactivate it. Nitrite and amino-residues in the stomach can be bioactivated to carcinogenic nitrosamines. The half-lifetime is a parameter which is used to characterize the transformation speed. It indicates the period which is necessary to reduce the concentration of a substance in the body by 50%.

Elimination makes sure that a substance is removed from the body. This can be done by excreting the substance in the same chemical condition as it has been taken in, or after metabolization. In the excretion process the kidneys play an important role. Pollutants like cadmium primary affect the kidney tissue and the excretion process.

The liver will detoxify an important number of substances which are excreted into the intestinal system through the bile bladder. Other routes of elimination include sweat, lungs, milk and salivary glands. Breastfeeding is known as a major route of release of dioxins and persistent halogenated pesticides from the body of a lactating woman.

An important instrument in toxico-kinetics is the use of mathematical models. They quantify intake, absorption, distribution, biotransformation and excretion processes. The basic data used in these models are the result of experiments in which the concentration of the substance in a set of tissues (blood, urine, etc.) is followed over time.

### 3.3 Toxico-dynamic phase

Toxico-dynamics studies the effects and the action mechanisms of toxic substances.

The toxic action of a substance begins with the interaction between a substance and a molecule in the receptor organism. Such an interaction can be realized with molecules which are widely present in the body (generalized) or with molecules which are characteristic for one tissue (specific).

Changes in the receptor can either inhibit or stimulate a particular function. Carbon monoxide (CO) can bind to hemoglobin and inhibit the uptake of oxygen by the blood. The nature of the specificity of the interaction between a substance and its receptor allows a first classification of toxic substances which is presented in Table 1.

The different levels concern cholinesterase inhibitors, oxidative phosphorilating agents, inducers of methemoglobinemia, inhibitors of cytochromeoxydase, porfyrinogenic agents, alkylating agents and inductors of metabolic enzymes. For each of these groups, the Table provides examples of agents which are known pollutants.

| **Mechanism of action** | **Agent** |
|---|---|
| Chlorinesterase inhibitor | Organic phosphoresters, carbamates |
| Oxydative phosphorilating agents | Dinitro-ortho-cresol (DNOC), organotin compounds, parachloro-phenol (PCP) |
| Agents inducing methemoglobinemia | Nitrite, chlorates, chlorodiamine, aromatic nitro compounds |
| Inhibitors of cytochromooxydase | Cyanide (HCN), hydrogen sulfide ($H_2S$) |
| Porfyrinogenic compounds | Dioxin (TCDD), polybromobiphenyls (PBB), hexachloro-benzene (HCB) |
| Alkylating agents | Methylbromide, ethylmethane-sulfonate |
| Agents inducing metabolic enzymes | Polychlorobiphenyls (PCBs) |

Source: Alink (1989).

Table 1. Classification of toxic substances according to their molecular action mechanism

If many receptors are changed by the action of toxic products, damage to a cell or a cellular function might occur. Damage to a large number of cells might result in functional alterations of an organ, which may result in disease. In conclusion, toxic processes might influence different levels of organization of the organism: molecules, cell organelles, cells, tissues, organs and the organism itself.

The effects generated may be reversible or irreversible. Changing the uptake of oxygen by hemoglobin is a process which is perfectly reversible at low concentrations. Mutations and cancer induction are widely considered irreversible effects.

The effects caused by toxic agents are often specific for a particular organ. Organs specifically targeted by toxic compounds are called target organs. Effects in target organs are the result of the specific, non-homogeneous distribution of a receptor

molecule over the body. Agents can be classified according to their target organs. Substances can be:

(a) **neurotoxic**: if they damage the neurological system and/or its functions. Damage to cells belonging to the central nervous system is irreversible. Examples of neurotoxic compounds include: lead, methylmercury, and pesticides such as parathion and aldicarb.
(b) **immunotoxic**: the immune system provides a defense mechanism against substances external to the body and pathogens. Suppression of the immunologic system results in an increased susceptibility for infections. Examples of immunotoxic agents include: PCBs, dioxins, lead, sulfur dioxide, nitrogen dioxide. A particular case is provided by allergens, which cause the immunologic system to over-react.
(c) **hepatotoxic**: the liver is the main site of biotransformation. The metabolic liver processes provide the body with essential molecules and detoxify a number of agents. Changing the function of the liver changes these fundamental processes. Examples of hepatotoxic agents include carbon tetrachloride, ethanol, and nitrosamines.
(d) **nephrotoxic**: the kidneys play a crucial role in the excretion of compounds through urine. Alterations of the kidney function might result in changed excretion patterns and accumulation of toxics in the body or release of substances such as proteins which are necessary for the functioning of the organism. Examples of nephrotoxic agents include mercury and cadmium.
(e) **reproduction toxicants**: these are substances which impair fertility. In males this can happen by changing both the quality and/or quantity of sperm. In females endometriosis and functional changes of the gonads are an important cause of sub- or infertility. Examples of reproductive toxic agents include PCBs, heavy metals, polycyclic aromatic hydrocarbons and different xenoestrogens.
(f) **teratogenic**: these substances impair the normal development of the embryo or the fetus. In many cases this is due to a perturbation on the developmental mechanism of the embryo or fetus rather than to direct toxicity. They result in spontaneous abortion, embryonal death, stillbirths or neonates with congenital malformations such as spina bifida, dislocation of the hip or cleft lips. Examples of teratogens include PCBs and heavy metals such as methylmercury.
(g) **Feto-toxicity** refers to systemic effects, e.g. growth retardation in the fetus. Cigarette smoking provides a well-known example.
(h) **pneumotoxic**: these substances can influence both the tubule part (trachea, bronchi, bronchioli) and/or the respiratory part (alveoli) of the lung function. Examples of pneumotoxic agents include: ozone, nitrogen dioxide, sulfur dioxide, and ammonia.
(i) other effects include dermatologic, hematologic and cardiovascular impacts.

Not all compounds involve a specific target organ. Many agents interfere with different organs. Lead does not only affect the central nervous system and the learning capacity; it equally impacts intestines, kidneys and bone narrow.

For guideline establishment it is however important to know which organ is functionally impaired at the lowest dose. For lead, this is the nervous system. For benzene, the bone narrow producing white blood stem cells is the system which seems to be targeted first.

The toxic action of an agent is made clear by the disease it causes. This disease can appear after acute or chronic exposure to an agent. Although some pollutants like the chemical mixtures characterized by high ozone concentration might provoke acute effects, most pollutants act by chronic or semi-chronic exposure. Diseases can either appear immediately after exposure (immediate toxicity) or after some delay (delayed toxicity). Disease is another important criterion to characterize toxic agents. A selection of pollutants in relation to the disease they cause is shown in Table 2.

| **Type of toxic agent** | **Symptom(s)** | **Agents** |
|---|---|---|
| Irritants | irritation of mucous surfaces of e.g. eyes and lungs | Chlorine ($Cl_2$), ammonia ($NH_3$), nitrogen dioxide ($NO_2$), sulfur dioxide ($SO_2$), formaldehyde, accrolein (synthetic dey) |
| Asphyxiants | shortage of $O_2$ resulting from interaction with $O_2$ uptake, transport or consumption | Carbon monoxide (CO), nitrogen dioxide ($NO_2$), cyanide (HCN), hydrogen sulfide ($H_2S$) |
| Anaesthetics and narcotics | calming, pain killing, sleep promoting reactions | Methane ($CH_4$), methanol ($CH_3OH$), trichloroethylene, toluene |
| Carcinogens | cancer | Polycyclic aromatic hydrocarbons (PAH), asbestos, benzene, arsenic, 2,3,7,8 TCDD (dioxin) |
| Teratogens | congenital malformations | Xenoestrogens |

Source: after Alink (1989)

Table 2. Classification of toxic substances according to the symptoms they cause

The description of the process of toxicology as a combination of exposure, toxico-kinetic and toxico-dynamic aspects results in a set of key concepts for toxicological action which are recalled in Table 3.

| |
|---|
| Acute – chronic effect |
| Reversible – irreversible effect |
| Local – systemic action |
| Immediate – delayed effect |
| Stochastic – non-stochastic effect |

Table 3. Key concepts in the description of toxicological effects.

-
-
-



**Bibliography**

Alink G.M. (1989) Toxicologische basisprincipes. *Medische Milieukunde* (eds A.R.J. Stumpel and R. van den Doel), Utrecht, the Netherlands: Bohn, Scheltema, Holkema (in Dutch). [A textbook chapter explaining the basis of toxicology applied to standard setting]

Felter S.P., Dourson M.L., and Patterson J. (1998) Assessing risks to human health from chemicals in the environment. *Handbook of Environmental Risk Assessment and Management* (ed. P. Calow) 9-23. Oxford, UK: Blackwell Science Ltd. [A textbook chapter explaining the risk assessment approach to environmental chemicals]

Van Larebeke N. (2000) Health effects of a household waste incinerator near Wilrijk, Belgium. *Health Impacts of waste management policies* (eds P. Nicolopoulou-Stamati, L. Hens, C.V. Howard) 239-250. Dordrecht, the Netherlands: Kluwer Academic Publishers (In Press). [In this chapter of the proceedings of a conference on the relationship between waste management policies and health, the author advocates that a more precautious attitude towards the introduction of new chemicals in the environment is indicated based upon the precautionary principle].

Van Leeuwen C.J. and Hermens J.L.M. (eds) (1995) *Risk assessment of chemicals*. Dordrecht, the Netherlands: Kluwer Academic Publishers. [A textbook providing an introduction to the assessment of chemicals in the environment based upon the risk assessment methodology]

Vitousek P.M., Mooney H.A., Lubchencoo J., and Melillo J.M. (1997) Human domination of earth's ecosystems. *Science* **277**, 494-499. [An overview of human impacts on the global ecosystem, including comments on the uncontrolled spreading of chemicals in the environment]

WHO - World Health Organization (1996) *Guidelines for Drinking Water Quality, 2nd ed. Vol. 2, Health criteria and other supporting information*. Geneva. [A classical document showing how standards can be used to define the quality of drinking water]

**Biographical Sketch**

**Professor Luc Hens** obtained his Licentiate in Biology from the Free University of Brussels (VUB) in 1974, Aggregation of Higher Secondary School Teaching from the VUB in 1975, and PhD from the Faculty of Science of the VUB in 1981.

Professor Hens is a member of several professional societies and recipient of a number of honours and awards, including the prestigious award of the Belgian Royal Academy of Sciences and Arts which he was awarded in 1984. Currently he is the Head of the Department of Human Ecology at the VUB.

He has been responsible for organising and/or participating in several international research and postgraduate teaching programmes in many countries including Bolivia, Bulgaria, Brazil, Brussels, the Czech Republic, Ghana, Hungary, Turkey, the Ukraine and Vietnam.

To date the publications of Professor Hens number about 200 including twenty-six books. He is also the co-editor of the journals *Environment, Development and Sustainability* and *Environmental Pollution*. His teaching and research interests include environmental management, sustainable development, human ecology, and related issues.