HUMAN HEALTH AND ENVIRONMENTAL RISK ASSESSMENT OF CHEMICALS

Arielle GARD-FLOC'H and **François E. FLOC'H**
Limonest, France.

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Contents

1. Introduction

Toxicology is defined as "the science of poisons" and is based on experimental models used to determine potential hazards for humans by establishing toxic endpoints, which are the doses below which no toxic effect would be observed. Ecotoxicology, also based on experimental models, is used to determine the potential hazards of a substance to the environment, which includes humans. These models for hazard identification are extrapolated for human exposure to estimate risk. This risk estimate, or risk assessment, is also based on experimental models used to compare human or environmental exposure, either measured or calculated, to the determined endpoints of experimental models and to estimate a potential risk with uncertainty (or safety) factors (UF or SF).

2. Hazard Assessment

2.1 Development of Toxicology

In Paleolithic times humans learned through experience to distinguish between food and poisons in their environment. They were familiar with the hazards of nature and took risks in hunting in order to get meat. Since the development of advanced technology, it is more difficult to appreciate hazards and risk either from newly exploited natural sources or new substances developed by industry. This was particularly the case for drugs, which have to be tested for their toxicity as well as their therapeutic action.

Toxicology developed together with pharmacology, and as usual in the sciences, experimental models were developed and rules were established to enable comparison between assays (experimental study on animals—species and even special strains—or cells from different organs or tissues) done in different laboratories. This type of regulation was adopted first at the state level, as in the USA CFR (Code of Federal Register) at the beginning of the 1980s, then at the regional level as in the Chemicals in Europe Directive 67/548/CEE, and finally at the global level of OECD (Organization for Economic Co-operation and Development) guidelines under the Good Laboratory Practice (GLP). The latter ensured that standard rules and methods could be applied so that any country could accept the results.

Unlike the standards used in pharmacology, the basis for comparison is mg/kg or mg/m^3. However, a more appropriate basis would be Mole/kg (see glossary: mole) or $Mole/m^3$, which are used when measuring parts per million (ppm) (because it is based on molecular weight). Experimental models were developed for acute, sub-acute and chronic toxicity that generally use rats, except when another animal model is recognized as more sensitive to the chemical in question.

2.2 Acute Toxicity Endpoints

The following methods are used to measure acute toxicity endpoints for substances:

- LD50 and LC50 measure chemical toxicity for ingestion, inhalation or dermal exposure. LD50 or LC50 means the dose or concentration leading to the death of 50% of animals within 15 days of observation after a single exposure (OECD

guideline). Clinical signs are also noted to indicate the non-lethal effects. The high dose is set at more than 2000 mg/kg orally and dermally, and at 20,000 mg/m^3 for inhalation (for comparison, the limit for inert dust human exposure is 10 mg/m^3 due simply to visibility). If no death occurs at 2000 mg/kg the substance is declared not harmful; otherwise next steps are to repeat the procedure at 200 then 50 mg/kg. Inhalation exposure is not addressed here, but refer to IPCS (1995), which is International Program on Chemicals Safety under World Health Organisation) for more information. Even with well-defined strains of carefully bred animals, there are differences in their reactions to chemical exposure.

- Corrosion irritation to the skin and eyes is measured using rabbits, and alternative methods are being explored to avoid animal suffering. The requested test dose is 0.5g or 0.5 ml per 6 cm^2 on the sensitive skin of a rabbit and 0. 1 g (gram) or 0.1 ml into the eye.

2.3 Sub-Acute Toxicity Endpoints

After a substance's toxicity is studied in the case of acute exposure, the deleterious effects of repeated exposure after 28, 90 or 120 days of administration are studied. This must begin with a high toxic dose (maximal tolerated without death, though many major toxic effects result), a medium dose with limited toxic effects and a low dose that must define, if possible, a NOEL (No Observed Effect Level) or a NOAEL (No Observed Adverse Effects Level). Results must also indicate target organs for toxicity.
At this stage, because there are many parameters and a variety of doses are tested; the uncertainty of the NOEL estimation still has to be tested. The simple statistical significance of a test is too often applied to determine a substance's toxic effect without considering other parameter factors involved in the test (such as differences between animals and strains, peculiarities related to the time of the study, possible abnormal randomization…). Consideration of normal variation in comparison to historical controls (all data obtained with the strain in this laboratory, and possibly within all laboratories using it) in the study laboratory and consideration of the strain of animals used must be the basis for incorporating the significance of biological variation into risk assessment.

2.4 Chronic and Bioassay Endpoints

Chronic and bioassay toxicity endpoints are determined by the effects on animals that are exposed to a substance for one year or throughout their lives. Generally, rats and mice are used, and they can be of special strains that are prone to develop some types of tumors. High, medium and low doses are still used to find the NOEL. Comparisons are always made with a control group, and historical controls are sometimes used as well (from past tests with the same animal strain in the same laboratory—they can differ from another laboratory and from the global variation of the strain). The animal subjects selected for the trial are the most sensitive to the substances being tested, so in some cases the results are not relevant to humans. For example, Aspirin* (acetyl salicylic acid) would not reach the market if the laboratory test results of modern regulations were followed, but it is sold as an over-the-counter (OTC) drug and is clearly used safely.

A new way of conducting the repeated and long-term studies is to define a Benchmark Dose (BD) rather than a NOEL. A BD is a full statistical analysis of all endpoints (not only "toxic"), and it refers to a 1% or 5% toxic effect according to the model and threshold decided upon.

These points generally form an S-shaped curve that resembles a pH curve, and the BD determines the level at which no adverse affects occur. This will normally lead to a threshold dose at which toxic effects begin to occur. Despite its advantages, this approach does not take the beneficial effects of a drug into account (because it is only based on detrimental effect, and a variation from a normal parameter distribution is supposed detrimental, even lowering cholesterol level in blood).

Furthermore the uncertainty factors incorporated into the models, particularly by the choice of the most sensitive animal model, are not taken into account in the risk assessment. (See *Case Study: Dioxins and Dioxin-Like Compounds*).

2.5 Carcinogens

As a rule, a distinction is made between potential and probable carcinogens, as well as between carcinogens for which there is a threshold at which effects occur (non-mutagenic) and carcinogens for which there is no threshold at which effects occur (mutagenic or genotoxic). For any "non-threshold" or mutagenic substance, the probability of the effects occurring is defined in comparison to the natural occurrence of the effects.

Mainstream thought uses the threshold level of a substance rather than assuming that one molecule is sufficient to induce a mutation. Many compounds are mutagenic because they cause the formation of an epoxide (internal oxygen bond into a molecule, easily prone to open for linkage), which then links to and mutates DNA. However, it is more likely that such epoxide will in fact link *in vivo (whole body)* with proteins before DNA is affected. This is why it takes many more than a single molecule of a substance to reach the nucleus and the DNA of an exposed subject.

3. Ecotoxicology

The environmental hazard level is currently appraised on the basis of a set of ecotoxicology tests performed on organisms that are selected for their relevance in terms of their environmental stability. These organisms reside in several trophic levels in aquatic or terrestrial compartments. Because of the diversity and complexity of ecosystems, numerous parameters can be chosen to determine the effects of toxics.

However, these parameters, which can be global or can encompass particular ecosystems or species, are still poorly understood. Moreover, an acceptable level of effect on the chosen parameter(s) must be defined. The main difficulty in appraising the environmental hazard level lies in making an accurate approximation of the sensitivity of the whole environment while using cost effective tests.

The aquatic environment is the most studied area of the environment because it is far

easier to set up standard water quality and to measure concentrations in a laboratory than it is to do so in soils or sediments. Hazard via the air is appraised mostly by the inhalation tests in mammalian toxicology and to a lesser extent by studies in plants, though these are not standardized tests. The aquatic toxicity-testing scheme is referred to hereafter because its results are the most accurately extrapolated to the global environmental effects.

3.1 Aquatic Acute Tests

The evaluation of the effects of chemicals in the environment has to be simple, despite the high complexity of biota (living organisms in the environment). These biota are food webs composed of predator / prey related species, from primary biomass producers as photosynthetic organisms, to herbivores, to top predators.

Each food web prey or predator level is called a trophic level. The appraisal of the acute effects of toxic exposure in the aquatic environment is based on testing in three key trophic levels:

- Primary producers: A standard test in freshwater algae is to measure the inhibition of biomass production in a given time (72 hours in the European Union (E.U.) standard test) or to measure the growth rate reduction of an exponentially growing culture, expressed as EC50. This latter measure is the agreed standard for OECD member states, and although it is commonly used as an acute test in assessments because of its short duration, it should be noted that it is in fact a chronic test because it is applied to several generations of algae cells.
- Primary consumers: Invertebrates, often represented by Cladoceran crustacea of the *Daphnia* species, and mainly by *Daphnia magna* in the E.U., undergo a standard test that is based on mobility inhibition.
- Secondary consumers: A standard test based on the lethality of exposure to chemical substance concentrations gives the LC50 of a substance, mainly in 96 hours. Fish, which are predators of primary consumers, are often used for this test. (Different fish species are used in EU and USA. This is accepted under OECD guidelines: http://www.oecd.org/home.)

3.2 Aquatic Chronic Tests

The same principle of three trophic levels is applied in chronic tests:

- The above mentioned test in freshwater algae is designed to obtain a NOEC (No Observable Effect Concentration).
- A *Daphnia* reproduction test giving a NOEC or EC based on production and survival in 21 days.
- Early life stage tests on fry production; growth or survival in fish also produces a NOEC or an EC_{50}.

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Biographical Sketches

Arielle GARD-FLOC'H has a PhD in Biochemistry and an engineer diploma in chemistry from ENS Paris. She is currently Product Steward in a Chemical Company. She was previously expert ecotoxicologist in the same Group. She was involved in the last ten years in many chemical risk assessments using either direct assessment or tools like Simple Treat and EUSES, and developed a safety assessment screen for R&D and product stewardship (GARD-FLOC'H, A. and FLOC'H, F. Eco-Toxicological approach in R&D, a key issue in Development. To be published in Roots of Chemical Development Vol. 8, Editors, Elsevier). She was previously Head of environmental research Laboratories, working in those fields for 10 years and establishing Good Laboratory Practices. Se has published several papers such as on saturation behavor of the manganese-containing superoxide dismutase from *Paracoccus denitrificans*, BBRC 113 (1), 114-120 (1983).

She is an Expert for the following Competent Authorities:

Ministry of Environment: Member of the "Ecotoxicity Evaluation Commission.

European Commission: CEFIC observer on the Working Group "Classification of Substances: Environmental Effects" and "Technical Progress Committee" (European Chemical Bureau, Ispra).

OECD: National expert for aquatic toxicology, biodegradation, and bioaccumulation for the OECD guidelines programme

Francois FLOC'H D.Sc. is currently senior expert toxicologist in a Chemical Group. He has been involved over the last ten years in many chemical risk assessments using either direct assessment or tools like EASE and EUSES, and he has developed a safety assessment screen for R&D and product stewardship (GARD-FLOC'H, A. and FLOC'H, F. Eco-Toxicological approach in R&D), a key issue in Development, to be published in Roots of Chemical Development Vol 8 Editors, Elsevier). He was previously Head of the Anti-Allergic Research and Immunology Research Programme of pharmaceutical companies, working in those fields for 20 years. He has published several papers such as WERNER, G. H. and FLOC'H, F. (Editors) *The Pharmacology of Immunoregulation*, Academic Press, London, New York, 549 p (1978) and subchronic toxicity, mutagenicity and allergenicity studies of a cultured dextrose food product. Food and Chem. Toxicol, 41 (2003), 689-694. He has a PhD in Cell Biology, a degree in agronomy and a Pasteur Institute Diploma in Immunology.

As part of his current professional role in Product Stewardship, he is a Regulatory Toxicologist and a senior member of various standardisation or regulatory methods elaboration groups:

- Member of MESA group (Méthodes d'Etude en Sécurité Alimentaire) du CSHPF (Conseil Supérieur d'Hygiène Publique de France.
- French Expert for OECD, relative to carcinogenicity, immunotoxicity and risk assessment.
- Member of an expert group on Emergency Preparedness for the Ministry of Environment.