

SCIENCE BASED TOXICOLOGY

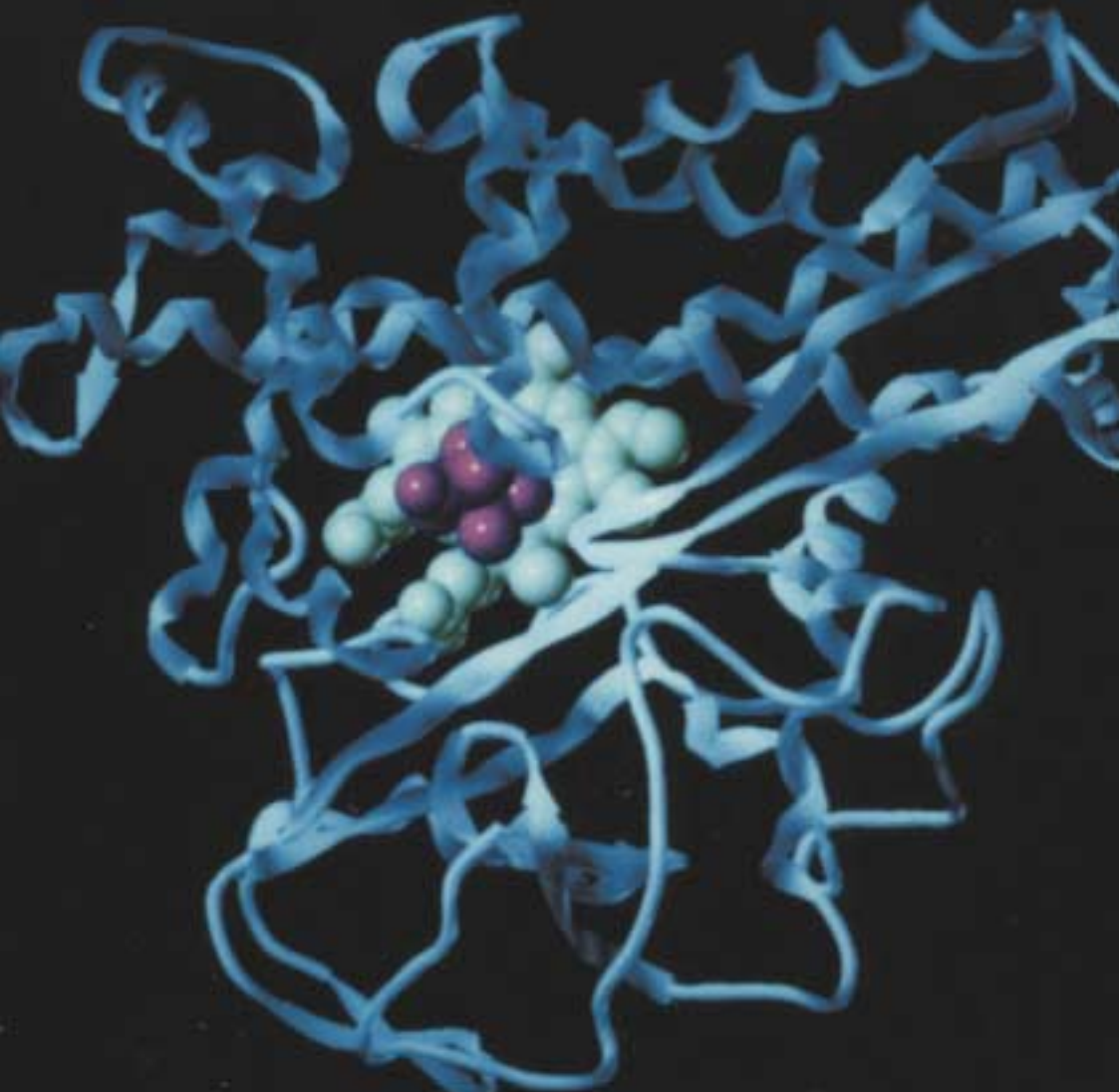
A New Strategy for Toxic Risk
Assessment in the 21st Century

By Claude Reiss PhD



Science Based Toxicology (SBT) represents a novel approach to a growing public health and environmental problem. The European Union is currently proposing a mass chemical safety testing programme, based on the use of millions of animals. This document is in part a response to that programme - which is known as REACH (Registration, Evaluation and Authorisation of Chemicals). It is also intended as a more comprehensive critique of traditional safety testing protocols. As such, it is of interest not only to scientists and regulatory authorities, but also to all persons concerned about public health and environmental pollution.

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Toxicology is the science of living organisms (biology) in contact with a toxic substance (the xenobiotic)

Over the past half century, biology has made unprecedented leaps, moving away from empiricism, and instead, towards exact science. Toxicology can benefit from the concepts, methods and tools developed in modern biology and thereby achieve the status of an almost exact science.

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While this document is intended primarily for scientists and regulatory authorities, it will be of interest to all persons concerned about public health and environmental pollution.

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INTRODUCTION

EU authorities are right in wanting to assess the 100,000 man-made chemicals in use today for their adverse effects on our health and our environment. However, the prospect that this assessment will involve a very large number of animals and that it will not be completed for several decades, at least, is cause for serious concern.

Those most concerned are consumer organisations, and private charities whose aim is to promote human health. Some of these groups have challenged the EU approach in its current format (REACH) because it relies heavily on animal-based research. These groups, supported by independent scientists, contend that the toxic effects of chemicals vary from species to species and that any safety testing must be species-specific. In other words, testing chemicals on rats or guinea pigs will not provide data relevant to human beings.

Whilst significant progress has been made over the past century by the petrochemical, agrochemical and pharmaceutical industries, the methods for assessing the risks of chemical products have remained almost unchanged and still depend largely on animal models. It is therefore timely, before starting the risk assessment of these 100,000 chemicals, to examine carefully whether recent advances in modern biology could of themselves provide the answers to human risk, thereby improving consumer safety and eliminating animal tests.

To this end, we will examine the following six topics:

- 1) Human health statistics gathered over the past few decades: do these statistics point towards negative health trends, e.g. an increase in the incidence of major diseases? If so, what proportion can be ascribed to insufficient disease prevention and to man-made chemicals?
- 2) Are the testing procedures currently in use satisfactory for reliable prevention of major diseases?
- 3) Are there better methods for assessing toxic risks? Can such methods be applied without delay?
- 4) Science-based toxic risk assessment (SBT).
- 5) Merits of SBT over animal model-based procedures.
- 6) Merits and benefits of SBT for interested parties.

MORBIDITY AND MORTALITY STATISTICS IN THE EU: ALARMING TRENDS



Despite the fact that life expectancy in EU countries is high, this benefit is offset by high morbidity rates. Several million EU citizens suffer debilitating neurodegenerative conditions (Alzheimer's and Parkinson's disease, multiple sclerosis (MS), autism, etc.) Although, for most of these diseases, the increase in the number of cases correlates roughly with the increase in life expectancy, a rapid increase of neurodegenerative conditions (MS in particular) has been observed among people aged between 20 and 40 years, and even in children (autism). The steepest rise in morbidity and mortality has, however, been seen in relation to cancer. In France, for instance, since 1990 the leading cause of death for people aged 35 to 65 years has been cancer. The proportion of deaths due to all cancers except lung cancer, among people of age 40 to 45 years, has increased six-fold between 1950 and 1980, and 300,000 new cancers are diagnosed annually, with a significant increase in the number of cancer cases likely to be linked to hormones. In 1970, one woman in 13 was affected by breast cancer. Today, it is one in seven.

It is generally agreed that 5-10% of all cancers are linked to genetic defects, and this figure has remained fairly constant. Hence exogenous (outside) factors, especially lifestyle (smoking, alcohol, dietary excess, etc.) and cancer-promoting (carcinogenic) products present in our food and in our environment are responsible for nine out of every ten cancers. Since lifestyle has steadily improved over recent decades, it is likely that, at present, environmental carcinogens are the main culprits responsible for causing the 1.7 million new cancers diagnosed in EU countries annually. This is clear evidence that either these products have not been tested for their carcinogenic potential, or else have been tested by methods which failed to detect this danger.

To assess the efficacy of these methods, let us examine how they perform in an area where they are applied most stringently: the assessment of prescription drug toxicity. Despite the fact that many years of research are invested in drug development and testing, adverse drug reactions (side effects) rank as the fourth leading cause of death in the EU, claiming 20,000 lives annually in France alone (and a total of 120,000 lives in the EU).

It is thus obvious that the current testing methods are failing to protect public health. As required by law, toxicity testing in general, and for prescription drugs in particular, must be performed on animals, i.e. "models" which are believed to display biological reactions similar to those of humans. It is therefore worthwhile analysing the relevance of the "animal model" concept in relation to human health.



IS RESORTING TO ANIMAL MODELS FOR HUMAN HEALTH ISSUES BASED ON RATIONAL PRINCIPLES?

There is remarkably simple, yet clear, proof that no animal species can substitute as a reliable biological model for another species. A species is defined in terms of its reproductive isolation, meaning that members of different species cannot interbreed. This is because a given species has its own unique genetic make-up (from number, organisation and structure of chromosomes, through to regulation and control of gene expression). Modern biology has clearly demonstrated that the genetic make-up of an individual determines the precise biological activities of its cells, tissues, and organs. Hence, individuals from different species have different genetic make-ups and therefore display different biological activities, even if some appear similar. The claim that members of a given species can substitute as reliable biological models for other species is therefore untenable.

In particular, the assumption that results obtained from some mammalian species are valid for humans is unfounded and seriously compromises human health. Consider, for instance, the chimpanzee, our closest relative (in evolutionary terms). If exposed to the human immunodeficiency virus (HIV), the chimpanzee does not become ill (in humans it causes AIDS); if injected with the hepatitis B virus, one out of ten or so chimpanzees might develop a mild form of hepatitis and will recover quickly (in humans, the virus causes chronic hepatitis and sometimes liver cancer); and when injected with the Ebola virus, the chimpanzee dies of hemorrhagic fever, as do humans. In other words, the best animal model we know behaves in opposite, different and identical fashion to humans. Nobody can forecast the result, which can only be arrived at after observing the test in both species. Testing animal models is, therefore, at best, useless, and often dangerous to humans (the French blood scandal occurred because “experts”, noting that the chimpanzee showed no response, approved HIV-contaminated blood products going onto the market).

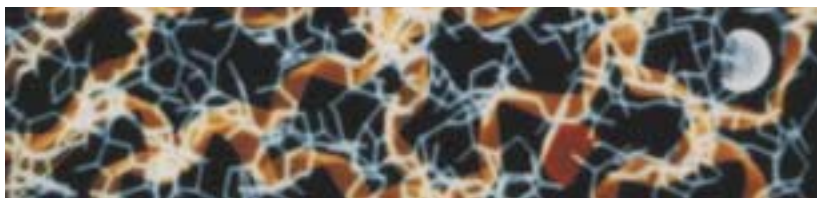
A conservative estimate of the number of deaths in France alone resulting from this flawed testing methodology of prescription drugs and carcinogenic products using animal models, ranges from 100,000 to 120,000 a year. Assuming that similar rates per capita are valid for other EU nations, some 600,000 to 750,000 citizens in total will die prematurely within the EU every year.

WHAT IS THE BASIS OF VALID TOXIC RISK ASSESSMENT FOR HUMANS?

Resorting to animals for assessing toxic risk in humans goes back almost to medieval times, as it was then the only way to gain a vague indication of risk. Modern science provides us with far more reliable means.

Toxicology is the science of living organisms (biology) in contact with a toxic substance (the xenobiotic). Over the past half century, biology has made unprecedented leaps, moving away from empiricism, and instead, towards exact science. Toxicology can benefit from the concepts, methods and tools developed in modern biology and thereby achieve the status of an almost exact science.

In addition, the cell is where life starts. It is therefore not surprising that the answers to practically all problems in biology must first be sought at the cellular level. Human diseases almost invariably have a cellular origin, whether the cause is endo- or exogenous. This holds true for cancer, neurological diseases and cardiovascular conditions, to cite the most frequent and life-threatening ailments in EU countries. It follows that harm done to the cell by a toxic substance is the first step to disease.



The stage is then set for Science Based Toxicology (SBT), as opposed to the traditional toxicity assessment using animal models. SBT has its roots in modern molecular and cellular biology. The study of what happens when human cells come into contact with toxic substances will thus be the first step required for reliable assessment in humans.

Modern biology has also made impressive strides into the study of integrated systems, at the tissue, organ and system levels. Non-invasive methods (various imaging techniques, organ function biochemistry, etc.) are available, allowing us to conduct molecular and cellular human risk assessment of substances to which consumers are extensively exposed (prescription drugs, food additives, pesticides etc.).

SCIENCE-BASED TOXICOLOGY

Our aim here is not to describe the SBT program in all its technical detail but, rather, to sketch the main outlines of molecular and cellular SBT, based on 15 years of experience with these methods and techniques. Indeed, not only have some of the scientists who contributed to this report been actively developing methods and tools for SBT, but they have also organised international workshops that succeeded in bringing together world-class specialists in molecular and cellular toxicology (see footnote - p12).

The response to a toxic event may be acute and systemic, or it may be delayed. The latter outcome may be due to an accumulation of minor damages, which manage finally to overcome cellular defence and repair mechanisms (a major cause of liver and kidney diseases); or a long induction period might typically result in the development of cancer - given that it takes on average five to ten years between the onset of proliferation of a cell and the detection of the resulting tumour. Hence, the necessity to assess both the short and long-term toxic responses.

Cellular studies are best performed on primary cultures, but established cell lines allow for preliminary investigations. An interesting complement to the methods described below is *in silico* evaluation of the toxic effects of a molecule, derived from its chemical structure (structure-activity relationship). This is a concept that has become increasingly reliable in forecasting the adverse biological activities of a particular molecule, even before it has been synthesized.



Metabolism Of The Xenobiotic

In order to enter the cell, the xenobiotic has to cross lipid or aqueous barriers and may need to be metabolised accordingly. This can be done by activating the expression of various cellular genes, which may involve specific metabolizing enzymes (mono oxygenases, including members of the P450 family, acetyltransferases, epoxide hydrolases, glutathio-s-transferases, methyltransferases, sulfotransferases, udp-glycosyltransferases etc.). Cell entry can also be accomplished through nuclear transcription factors, like the PRX receptor activated by the majority of drugs, and involved in many adverse drug reactions, xenobiotic transporters (metallothioneines, P-glycoprotein family, etc.). The resulting metabolites need to be carefully identified, since some happen to be highly toxic, even though the unmetabolized xenobiotic is not. Since the primary targets of xenobiotics are the liver and the kidney, cells from these organs should be tested first. Methods include: in vitro testing of the enzymatic activity of the genes involved, DNA chips (kits commercially available) to allow for the monitoring of the expression of many of these genes; and identification of metabolites by mass spectrometry.

Intracellular Toxicity Assessment

Once the xenobiotic or its metabolite has entered the cell, the effect on the latter and its fate must be monitored. In response to even mild aggression, the cell will mobilise a series of genes, either to protect itself, or to have the damage repaired.

Many members of the families of genes involved (stress genes and various repair enzyme genes) have already been identified and can be recruited as “reporters”, which inform the toxicologist of the target, the extent of the damage and the ability of the cell to overcome the damage. By standard genetic engineering techniques, the control element (promoter) of the stress or repair gene is fused to a DNA sequence coding for a coloured, fluorescent, or luminescent protein. This “reporter” is introduced into the cell. As an example, assume that the control element has been borrowed from a gene responsible for the repair of a DNA strand break, fused to the coding sequence of luciferase (a protein used by the firefly to produce its light). If the cell harbouring this “reporter” is exposed to a substance that lights up the cell, it can be concluded that the substance has entered the cell, has damaged the genetic material and is therefore potentially mutagenic. The fate of the cell, exposed to various doses of the substance, tells us about its ability to survive and how it will cope with the substance in the long run. Presently, reporter gene-loaded cells are commercially available, allowing

researchers to track the xenobiotics responsible for inducing the stress (including oxidative stress), various kinds of DNA damage, membrane damage, etc.

The disadvantage with reporters is the necessity to guess the gene targeted by the xenobiotic. This problem is overcome with commercially available DNA chips, carrying hundreds or thousands of gene elements known to be involved in toxic response (*Toxicogenomics*). A DNA chip is a checkerboard of squares of micrometer size carrying a short fragment of a gene. Using standard biochemical techniques, the expression of each of these genes can be individually visualised. The DNA chip allows the observation of the gene's behaviour (transcription) in the cell's nucleus as well as its interaction with all genes present on the chip, indicating whether the genes are stimulated, repressed or unaffected by the xenobiotic.

DNA chips are the ultimate tools for monitoring the first phase of gene expression (transcription). In order fully to observe the xenobiotic activity on gene expression, the second phase of expression (translation), during which the gene product is actually synthesised, must also be monitored. This can be done with the tools of *Toxicoproteomics* (e.g. 2D gel or capillary electrophoresis, protein chips, mass spectroscopy, and many other new methods currently under development). Toxicoproteomics accounts for the xenobiotic-induced protein modifications and other modifications of proteolytic processes, aggregation, etc., which have been identified recently as representing important stages in many debilitating diseases (neurodegenerative disorders, dementia, diabetes type 2 etc.).

In summary, using techniques based on molecular toxicity, we can obtain a clear view of the mechanism by which a particular substance or product is harmful, at what doses the cell can resist damage and most importantly, the long-term effect on the cell. The experiment takes a few days on average, can be performed in large parallel screening set-ups (various cell types or doses for instance), is relatively inexpensive, easy to standardize, and requires tiny amounts of the xenobiotic (important in drug testing). The results are quantitative (large range of linear dose-response), reproducible and, most significantly, valid for the species that provided the cells. These points represent clear scientific and economic advantages, even though advanced technical skills are required for most of these methods.

SCIENTIFIC ASSESSMENT OF ORGAN, TISSUE AND SYSTEMIC TOXICITY

We estimate that the assessment of the toxic risk by molecular and cellular approaches can be extrapolated to the organ, tissue and systemic level with about a 90% confidence level. The remaining level of uncertainty can and must be further reduced, especially for prescription drugs and products to which consumers are exposed over long periods of time, or at high doses (food additives, pesticides).

In special cases, the substance in question can be tested in perfused tissues or in organ slices. This allows one to monitor the response of the cells integrated in their normal environment. Due to problems of supply and rapid degradation of the slices, these tests are difficult to carry out routinely. It is much easier to rely on non-invasive methods, which allow one to monitor human patients, under strict clinical test conditions (with informed consent), using micro-dosing techniques on the tissue or functioning organ in situ. Of particular value are imaging techniques (MRI, PET scan, etc.), which allow one to identify the organ targeted by the xenobiotic, as well as the metabolism and elimination of the latter. Valuable supplementary information on the function of particular organs can be obtained by standard biochemical and other laboratory tests.



MERITS OF SBT OVER TRADITIONAL (ANIMAL-BASED RESEARCH) TOXICITY ASSESSMENT

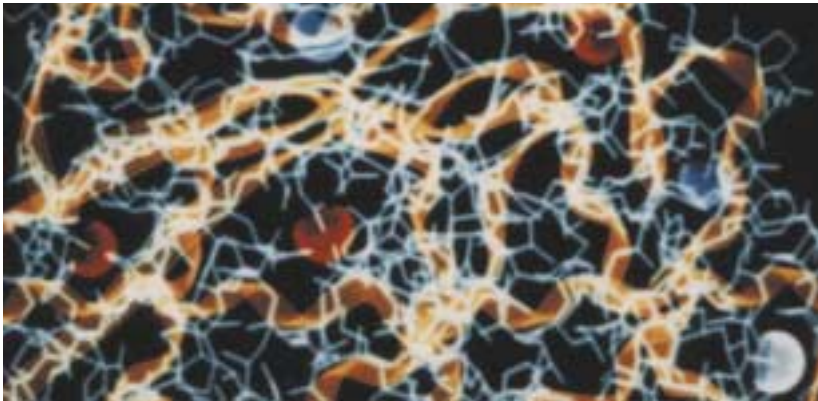
As shown earlier in this report, the biological reactions of individuals of a given species are unique. Occasionally, individuals from different species may display similar gross responses when exposed to the same toxic substance, but one should never be misled by these chance phenomena. First and foremost, the mechanism through

which a substance produces a particular pathological reaction can be rather different in different species. 60% of prescription drugs in use are metabolised in humans by the same member of the P450 family (which can lead to synergistic drug activation). But several different members of the p450 family are involved in primates, dogs and rodents. Additionally, long-term effects in humans cannot be assessed in species with a shorter life expectancy. In mice, spontaneous cancer development occurs at the age of ten months, whereas in humans it usually starts after the age of 40 years, and the mechanism of cancer initiation is known to be very different in each species. The susceptibility to cancer in different strains of mice can vary a hundredfold. Some strains tolerate oestrogen doses many times higher than others, with no apparent ill-effects. Even if the gross response in two different species looks alike in the short term, the underlying mechanism that determines the long-term outcome is likely very different and can therefore lead to vastly different results. In addition, it would be useless to perform SBT for a given species on cells or cell cultures belonging to a different species.



THE BENEFITS OF SBT FOR INTERESTED PARTIES

The most obvious benefit would be consumer safety. SBT allows one to understand the mechanism by which a substance produces its adverse effects, which in turn helps to predict its long-term effects. By identifying cancer-promoting substances, cancer prevention could be significantly enhanced. As a result, cancer morbidity figures could be halved within the next three to five years. By the same token, reliable assessment of prescription drug toxicity could save tens of thousands of lives each year in the EU. Neurotoxic substances (80% of insecticides are neurotoxic) would be identified and removed from the market, thereby reducing the risk of damage to the neuronal development of children (according to the FDA, this could be the case for rotenone). Detection and removal of endocrine proliferators would prevent both abnormal development of sex organs and most hormone-dependent cancers (breast, ovary, prostate).



Improved consumer safety would result in the immediate **alleviation of socio-economic costs** resulting from those preventable diseases, whose rates are presently soaring in EU countries.

THE BENEFITS OF SBT ASSESSMENTS FOR INDUSTRY

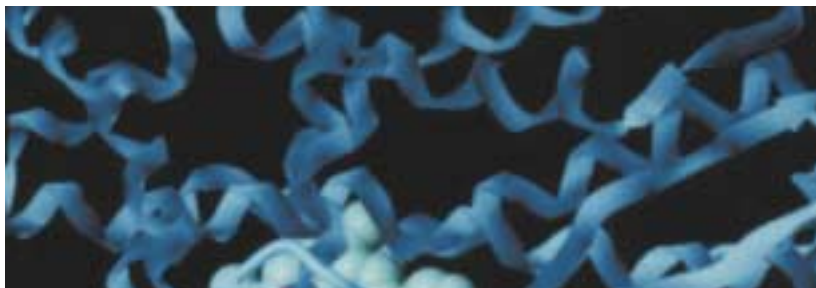
These are many. SBT assays take a few days on average, can be performed in large parallel screening set-ups (various cell types or doses, for instance), are relatively inexpensive, easy to standardise, and require tiny amounts of the xenobiotic (important in prescription drug testing). The results are quantitative (large range of linear dose-response), reproducible and, most importantly, are valid for the species under investigation. Furthermore, understanding the mechanism of the adverse effect may allow for the chemical modification of the xenobiotic, thereby facilitating its removal, or reducing its toxic potential (with the help of Structure-Activity-Relationship models, for example). Since SBT tests are easily standardised and are independent of subjective parameters, the tests remain valid across political borders. This allows for unrestricted circulation of SBT-tested goods among EU countries.

It is true, however, that advanced technical skill is required for most of the SBT methods, and that the lab equipment required is expensive. Against this, there are major efficiencies and economic gains to be had in the medium and long term.

SBT procedures apply to other species and can, therefore, be used to assess **environmental toxicity** in any animal or plant species.

Since SBT procedures necessarily avoid “model” species, they would **satisfy the demands of animal welfare organisations**.

Finally, resorting to SBT methods would improve the image of the EU, both inside the EU countries (consumers would be grateful for better protection of their health) and outside the EU. Thus, the EU could lead the way in effective improvement with respect to environmental health issues.



HOW TO PROCEED WITH THE RAPID AND PRACTICAL IMPLEMENTATION OF SBT IN THE EU



A strategy for the EU to hasten the introduction of SBT methods could be based on the following steps:

- Elaborate a detailed SBT programme. To this end, create and fund a board of specialists.
- Set up a European pilot lab in SBT (e.g. at the Joint Research Center, based at ISPRA, Italy).
- Fund and train SB Toxicologists. A six to eight month training course would be required in the pilot lab, with conferences attended by leading specialists in the field, for the benefit of graduate or postgraduate participants from all EU countries.
- Encourage all EU countries to set up SBT pilot labs, under the supervision of trained SB toxicologists. More SB toxicologists could subsequently be trained locally.
- Support industrial initiatives aimed at conversion to SBT methods.
- Issue EU directives stating that all new products brought to the market must have been tested as safe by SBT methods, namely at the molecular and cellular level for substances to which exposure is limited. For substances to which exposure is significant, tissue, organ (especially liver and kidney) and systemic level investigations would also be required. Such substances would include prescription drugs, food additives and pesticides. Products already on the market should be tested by SBT methods within three to five years; those failing to pass the test should be withdrawn and replaced with a safe equivalent.

Footnote: (e.g. First European Workshop in Molecular Toxicology, Sophia-Antipolis (France) 1996; Second European Workshop in Molecular Toxicology, Paris 1999) and published proceedings of these workshops (Molecular Toxicology (1997) VSP publishing, edited by Reiss, Parvez and Labbe, Molecular Responses to Xenobiotics (2001) Elsevier publishing; Parvez, Reiss and Labbe editors, Special Issue of TOXICOLOGY 153 (2003), n° 1-3, guest editors Parvez and Reiss).

Further technical information can be obtained from the website:
www.healthwithoutfrontiers.org



The precautionary principle:

Whenever reliable scientific evidence is available that a substance may have an adverse impact on human health and the environment but there is still scientific uncertainty about the precise nature or magnitude of the potential damage, decision-making must be based on precaution, in order to prevent damage to human health and the environment.



For further information see: www.healthwithoutfrontiers.org
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