Future of ToxicologyLow−Dose Toxicology and Risk−Benefit Analysis

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Perspective

Future of Toxicology—Low-Dose Toxicology and Risk−Benefit Analysis

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Toxicology historically has been directed at studying the mechanisms of adverse effects of isolated compounds on living organisms at high levels of exposure, forming the basis for risk and safety assessment. One way to refocus and mobilize new research funds would be to better match the priorities in regulatory issues and direct the research within the field of toxicology more to low-dose toxicology and risk−benefit analysis. Low-dose toxicology can only be developed when taking into account mechanistic insight and will require risk−benefit analysis and a definition of interactions between compounds at realistic doses of exposure, especially in the case of dietary constituents. This is because the biological effects at low levels of exposure not only may be adverse but also can be beneficial depending on the target organ, the actual end point studied, the receptors activated, and/or the gene expression, protein, and metabolite patterns affected. Toxicologists have the tools and knowledge to study mechanisms of biological effects of chemicals on living organisms, and they should redirect their focus from looking only at adverse effects at high levels of exposure to characterizing the complex biological effects, both adverse and beneficial, at low levels of exposure. This may even result in the notion that beneficial effects can be the result of reaction pathways that are generally considered adverse and vice versa. Low-dose toxicology not only will provide a significant research challenge for the years ahead but also should contribute to better methods for low-dose risk assessment for complex mixtures of chemical compounds. This refocusing from high- to low-dose effects turns the field from a science focusing on adverse effects into a science studying the biological effects of chemical compounds on living organisms, taking into account the realization that the ultimate biological effect of a chemical may vary with its dose, the end point or target organ considered, and/or the combined exposure with other chemicals. By defining the effects of chemicals on living organisms at physiologically relevant exposure levels, toxicologists may contribute not only to better risk and safety assessment but also to preventive medicine, generating knowledge on possible adverse and also beneficial effects of chemicals. In addition, it will result in an approach for food safety assessment more in line with that for drug safety assessment taking the risk−benefit balance into consideration.

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Introduction

The field of toxicology is at a crossroads. It faces reconsideration of its focus, which has been directed historically at studying the mechanisms of adverse effects of isolated compounds on living organisms at high levels of exposure. These studies formed the basis for risk and safety assessment.

Nowadays, this framework of risk and safety assessment by regulatory bodies is increasingly directed by the needs of the consumer, politics, industry, and society and requires a continuous education of experts in toxicology. However, the number of new students entering the field and funding the research training possibilities for young scientists have become major challenges.

One way to overcome these problems and to mobilize new research funds would be to better match the priorities in regulatory issues with a focus on research on low-dose toxicology, applying knowledge of toxicity mechanisms to predict low-dose risks. Because at low-dose the effects of chemicals on living organisms will be less pronounced, this low-dose toxicology will have to take into account the effects of different bioactive compounds simultaneously present and balance the adverse effects and beneficial effects of chemicals at physiologically relevant concentrations.

The present paper tries to illustrate why and how low-dose toxicology could be further developed, leading to mechanistically based risk−benefit concepts, improved possibilities for low-dose cancer risk extrapolation, and a contribution to what could be called preventive medicine.

Low-Dose Toxicology

The Need for Improved Low-Dose Cancer Risk Assessment

Current risk assessment paradigms for low-dose risk assessment generally apply extrapolation of experimental data from animal studies obtained with isolated chemicals at high doses (*1*−*3*). Models used for these extrapolations are based on safety factors or curve fitting, often assuming low-dose linearity. However, major criticisms of quantitative models for risk assessment have been put forward for some time stating that there are many unverifiable assumptions in these calculations (*4*−*6*). This especially holds for quantitative cancer risk assessment. Quantitative estimates for cancer incidences at realistic exposure regimens are extrapolated from cancer incidences in experimental animals exposed at relatively high doses. The outcomes of such extrapolations may, depending on the extrapolation model applied, vary by several orders of magnitude (*5*, *6*). However, given the ever decreasing detection limit of analytical methods—estimated to be a factor of 10 every 4 years—improved methods for low-dose cancer risk extrapolation become an important issue. Levels of ppb, ppt, and even lower amounts of toxic compounds, including genotoxic carcinogens, are likely to be found in a variety of foods, environments, and even human blood or tissue samples. Clearly, this mandates the need for better low-dose cancer risk extrapolation models that allow the definition of a virtual safe dose and an acceptable quantified additional cancer risk upon lifetime exposure of, for example, one in a million. This more accurate description of low-dose cancer risks can only be achieved on the basis of fundamental insights into the mechanisms of biological responses at very low doses. This implies insight in dose-related changes in toxicokinetic and toxicodynamic processes and insight in how DNA repair processes modulate the low-dose cancer risk. This requires research on mechanisms of toxicokinetics and toxicodynamics, specifically at low doses, in order to improve our current safety and risk assessment protocols.

The threshold concept and the low-dose linearity concept for genotoxic carcinogens are increasingly questioned, because, for example, bioactivation and DNA repair routes become saturated at relatively higher levels of exposure, resulting in nonlinear dose−response curves. Mechanistic studies on genotoxicity at low doses should allow for more accurate descriptions of the dose−response curves at low doses.

Physiologically Based Pharmacokinetic (PBPK) and Physiologically Based Pharmacodynamic (PBPD) Modeling for Extrapolation to Humans

A tool that would allow translation of the mechanistic insight in toxicokinetics and toxicodynamics of a toxic compound into mathematical models for description of its low dose effects can be found in PBPK and PBPD modeling. Recently, this approach has been selected for estimating the low-dose cancer risk for acrylamide. In an effort to improve risk-based decisions for acrylamide and glycinamide, its epoxide metabolite, PBPK models in the rat were developed (*7*−*9*). These models aim at predicting target tissue doses of acrylamide and glycinamide, linking them to known mechanisms of toxic effects. Extending these rat models to PBPK models for the human situation will improve the quantitative risk assessment of acrylamide as present in a variety of food items (*9*).

These types of approaches are necessary to allow more reliable low-dose predictions since they can take into account not only low-dose exposure regimens but also the effects of species differences, genetic polymorphisms, and saturation and, thus, nonlinear kinetics for biotransformation and repair processes. Although building these models may be time-consuming and has to be done for each chemical independently, the knowledge generated is essential to develop the field of low-dose toxicology and to perform risk and safety assessment on low-dose exposure regimens with higher levels of confidence.

Low-Dose Toxicology Requires Risk−Benefit Analysis

Another aspect of risk and safety assessment that needs to be updated within the field of modern toxicology, especially

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when developing low-dose toxicology, is the misunderstanding that bioactive compounds are either adverse or beneficial. This also implies extending the concept of Paracelsus that toxicity is a matter of dose. A chemical may have adverse effects at relatively high doses (as stated by Paracelsus) but no or even a beneficial effect at lower doses. This implies more emphasis on nonlinear dose−response curves, U-shaped dose−response curves, and the phenomenon of hormesis. Furthermore, the effect of a chemical may vary not only with the dose but also with the end point, cell type, tissue, or species considered.

In nutritional sciences, for example, it is well-established that vitamins have U-shaped dose−response curves with adverse health effects resulting from too low and too high levels of exposure, some doses having no effect and others having a beneficial health effect. Furthermore, quercetin inhibits tumor cell proliferation at physiologically relatively high concentrations. However, at lower, physiologically more relevant concentrations, the effect on tumor cells in vitro varies showing either no effect or stimulation of cell proliferation, the latter when the cells are estrogen receptor-positive (*10*, *11*). This example illustrates that the final biological effect of a chemical may vary with the dose and the characteristics of the target cells or tissue under investigation. This example also illustrates that insight into the mechanisms of action reveals that the characteristics of the target cells (estrogen receptor-positive or -negative) play a role.

In medicine, the risk−benefit concept is well-established and it is generally accepted that a drug, in addition to its beneficial pharmacological properties, also may have adverse side effects. These adverse effects are often considered acceptable when balanced against the positive effects of treatment and curing of the disease. In the field of toxicology, we advocate a comparable approach. The risk of adverse effects should be balanced against the positive nutritional and/or disease preventive effects. In practice, this means that a margin of safety or a dose−range of recommended daily intake has to be calculated based on the balance of demonstrated positive and the assessment of negative effects. An example in the field of food safety can be found in the use of nitrite, a substrate for the formation of carcinogenic nitrosamines in the stomach, in specific meat products to prevent botulism and formation of botulinum toxin.

Interestingly, botulinum toxin, one of the most toxic compounds known today, is also used at orders of magnitude dilutions as botox for its beneficial therapeutic effects in the treatment of spastic dysphonia, facial rejuvenation, and others (*12*, *13*). The mechanisms for the lethal effects and the beneficial applications of botulinum toxin are even based on the same principle—the inhibition of acetylcholine release. Other examples of drugs based on toxic chemicals include, for example, the use of the ergot alkaloid mycotoxins, causing St. Anthony's fire, for treatment of migraine (*14*), of digitalis alkaloids for treatment of heart disease, and the use of colchicine for treatment of gout. Clearly, these are only a few of the phytotoxins presently used in modern medicine. Insight into the mechanism of action and the exact definition of the low-dose to be applied is important to turn a compound with an adverse affect into a beneficial one.

Low-Dose Beneficial Effects of Toxic Compounds

When toxicology starts defining adverse and beneficial effects of chemicals at a low dose of exposure, the concept of hormesis comes into focus. Over the past years, there has been growing interest in the topic of hormesis, with some papers even stating that it is expected to have a profound impact on the practice of risk assessment (*15*−*17*). Hormesis has been defined as a dose−response phenomenon characterized by a stimulatory response at low doses and an inhibitory response at high doses (*15*−*17*). Hormesis appears to be primarily an adaptive response to stress. The stress triggers cellular repair and maintenance systems. A modest amount of overcompensation then produces the low-dose effect, which is often beneficial. This implies that, in theory, adverse effects of chemicals may be required to actually induce the beneficial effects.

In some cases, adverse effects are required to actually switch on the mechanisms underlying beneficial responses. An example of this can be found in the induction of electrophile-responsive element (EpRE)-mediated gene expression by polyphenols. EpRE-mediated expression of phase 2 enzymes, such as NAD(P)H:quinone oxidoreductase (NQO1) and glutathione S-transferases (GST), which are major defense enzymes against electrophilic toxicants and oxidative stress, is assumed to involve release of the transcription factor Nrf2 from a complex with Keap1 (*18*). A possible mechanism for polyphenol-induced release of Nrf2 from Keap1 includes their oxidation to reactive electrophilic quinones that are not only able to covalently bind to DNA and proteins but also may generate oxidative stress through redox cycling (*18*−*21*), thereby activating Nrf2 release from Keap1 and EpRE-mediated gene expression. Thus, the actual mechanism of toxic action of the polyphenols may be responsible and even a prerequiste for the induction of their beneficial effects.

Another example of an adverse effect that is required to induce the beneficial response can be found in the oxidative stress hypothesis for the beneficial effects of n-3 fatty acids protecting against colon cancer. Diets with high levels of n-3 polyunsaturated fatty acids are known to be protective against neoplastic lesions in the early stages of chemically induced colon carcinogenesis (*22*−*27*). Although the ultimate mechanism underlying the n-3 fatty acid-mediated protection against early stages of colon cancer development has not been fully elucidated, several mechanisms have been suggested that underlie the reported beneficial effects, one of these being the oxidative stress hypothesis (*27*−*29*). This hypothesis suggests that the anticarcinogenic effect of the n-3 polyunsaturated fatty acids is mediated by formation of oxidative toxic metabolites that may slow cell proliferation or stimulate apoptosis (*27*−*29*) or may have an effect on the induction of protective enzymes by activating EpRE-mediated gene expression (*30*, *31*). The more electrophilic a compound is, the larger is its effect on the EpRE (*30*−*32*). This indicates that an adverse effect and generation of reactive electrophilic intermediates at moderate or low doses may actually turn out to be a mechanism underlying a beneficial health effect.

A final example of toxic reactive compounds that switch on mechanisms underlying beneficial responses and cellular protection can be found in the gene expression profiles induced in human colorectal carcinoma (RKO) cells exposed to the reactive toxic product 4-hydroxy-2-nonenal, formed upon oxidation of polyunsaturated fatty acids (*33*). 4-Hydroxy-2-nonenal was shown to affect multiple stress signaling pathways, many of which represent potential mechanisms through which this toxic compound may alter cellular viability and the cellular response to damage. So at low, nontoxic doses of 4-hydroxy-2-nonenal, the major changes were in the induction of genes that play a role in protecting the cells from further damage, pointing at a beneficial effect of a supposed toxic intermediate.

Realizing that adverse effects can also be regarded as beneficial, depending on the target protein, cell, or organ, would

turn toxicology into a science, which not only focuses on adverse health effects but also focuses on the role of chemicals in preventive health care and mechanisms of beneficial effects. In addition, it will result in an approach for food safety assessment more in line with that for drug safety assessment taking the risk−benefit balance into consideration.

It will be clear that this shift from a negative to a more positive attitude toward the effects of chemicals on living organisms has to include mechanistic insight in the mode of action of the chemicals involved. Studying the actual mechanism of action, adverse effects may turn out to be beneficial.

Low-Dose Toxicology Requires Combination Toxicology

The effects of chemicals on living organisms will be less pronounced at low doses, so low-dose toxicology will also have to take into account the effects of different bioactive compounds simultaneously present and balance the adverse and beneficial effects of these different chemicals at physiologically relevant concentrations. A compound with a supposed beneficial health effect may even turn out to have an adverse effect or a toxic compound may appear to have a beneficial effect when their interactions with other compounds are taken into account.

This can be illustrated by the effects of supposed beneficial flavonoids on the transport of the procarcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) through differentiated Caco-2 monolayers, a model for uptake by the intestinal epithelium (*34*). Generally, flavonoids are considered to be beneficial food ingredients, and many epidemiological studies point at protective effects of flavonoids against cardiovascular diseases and certain forms of cancer (*35*−*38*). However, this view may be somewhat different when their effects on multidrug resistance proteins and thereby on the bioavailabilty of the chemical carcinogen, PhIP, is taken into account. In the Caco-2 model system, it was demonstrated that flavonoids inhibit ABC transporter-mediated excretion of PhIP from the intestinal cells back to the apical luminal side, resulting in an increased apical-to-basolateral transport, pointing at possible increased in vivo bioavailability of the procarcinogen PhIP and an unexpected adverse effect of supposed beneficial food ingredients (*34*). This example illustrates that mechanistic insight is important in the field of combination toxicology, not only to elucidate unexpected interactions but also to support future regulation of complex mixtures.

Similar modes of action may result in additive effects, but compounds with different modes of action on the same target organ may result in synergistic or antagonistic interactions. Although one may consider regulation of organophosphate esters and PCBs, dioxins, and furans by a cumulative approach based on additivity of the effects by using the toxic equivalence factor concept (*39*), the example of the effect of flavonoids on the epithelial transport of PhIP illustrates that combined exposure to compounds with different modes of action may result in unexpected adverse effects.

Conclusions

Altogether, it seems unrealistic to assume, as done in the field of high-dose toxicology, that a given chemical can have only adverse effects. It is more likely that chemicals with adverse effects can turn out to be chemicals with beneficial effects (and vice versa), depending on the dose, the target organ, the end point considered, or the combined exposure with other chemicals. To define the proper risk−benefit balance of a chemical compound in its complex biological matrix, it is obligatory to define its mechanisms of action at relevant and, thus, often low levels of exposure. This illustrates the need for improved low-dose toxicology, which can only be performed taking into account insight in the mechanisms underlying the adverse but also the beneficial effects of chemicals on living organisms. Low-dose toxicology is also required because of the need for definition of better models for low-dose cancer risk extrapolation and the identification of virtual safe doses for low levels of genotoxic carcinogens present in our environment and food.

Clearly, this implies that toxicologists should keep an open mind for the actual outcome of the effects of the chemicals they are studying, focusing not only on adverse effects but also on possible beneficial outcomes of the biological processes initiated at physiological dose levels. Especially at realistic low levels of exposure, biological effects generated may be not only adverse but also beneficial depending on the target organ, the actual end point studied, the receptors activated, and/or the gene expression, protein, and metabolite patterns affected. Toxicologists have the tools and knowledge to study mechanisms of biological effects of chemicals on living organisms, and they should redirect their focus from looking at adverse effects only to also characterizing the beneficial effects, including even the beneficial effects of supposed adverse effects. This will provide insight into the risk−benefit balance of exposure to mixtures of chemicals at realistic low levels of exposure. It may not only provide a significant research challenge for the years ahead but also contribute to better methods for low-dose risk assessment for complex mixtures of chemical compounds. This refocusing turns the field of toxicology from a science focusing on adverse effects into a science focusing on all of the biological effects of chemical compounds on living organisms taking into account that the ultimate biological effect of a chemical may vary with its dose, the end point or target organ considered, and/or the combined exposure with other chemicals.

By defining the low-dose biological effects of chemicals on living organisms, toxicologists may contribute not only to better risk and safety assessment but also to preventive medicine, generating knowledge of possible adverse and also beneficial effects of chemicals.

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