European Food Safety Authority

EFSA Scientific Colloquium XVII
On Low Dose Response in Toxicology and Risk Assessment

Parma, Italy, 14 -15 June 2012

BRIEFING NOTES FOR DISCUSSION GROUPS

These briefing notes are prepared to provide participants with the relevant background information so as to be prepared for an interactive exchange of views and expertise during the Colloquium.

BACKGROUND

«*All substances are poisons. It's the dose that makes the poison*". This famous statement by Paracelsus (1493-1541) is the basis for a fundamental concept in toxicology and risk assessment: the individual response of an organism to a chemical increases proportionally to the exposure (dose). Also, it is generally accepted that for most chemicals there is a threshold dose below which there is no adverse effect. In recent years the classical (monotonic) dose-response paradigm has been challenged by the so-called "low dose-hypothesis" particularly in the case of endocrine active substances. According to this hypothesis, hormonally active agents may exert "low dose effects", i.e. in the range of typical human exposure, which are not present at higher doses and which may display a non-monotonic dose-response profile, e.g. U-shaped, inverted U-shaped. According to this hypothesis a monotonic relationship between dose and effect would not allow, for a given effect, the extrapolation from high to low doses during risk assessment of those substances. This hypothesis challenges, moreover, a key assumption in toxicology: the existence of a threshold for some substances which is implicit in the current risk assessment process for most chemicals. Several chemicals that can be present in food have been claimed to possess endocrine active properties and to produce "low dose effects". These include some pesticides, dioxins, polychlorobiphenyls (PCBs), and bisphenol A. It follows that there is high scientific and public interest on how the low-dose response hypothesis can be taken into account when assessing chemical risk and food safety. This colloquium will offer a unique opportunity to international experts for an open scientific debate on the most recent scientific evidence of low dose response in toxicology, and current and future challenges for food and feed risk assessment in the European Union.

OBJECTIVE

The objective of this Colloquium is to bring together international experts from different sectors for an open scientific debate on key issues related to the current state of the art in low dose response in toxicology, to discuss current challenges and to identify ways of further enhancing the process of risk assessment in the European Union.

ORGANISING COMMITTEE

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DISCUSSION GROUP 1
Nature of an effect: adverse or non-adverse?

INTRODUCTION

"The dose alone makes a thing not a poison... ...it is possible to make evil out of good, it is also possible to make good out of evil (Paracelsus)". Based on this old concept, modern toxicology has traditionally used quantifiable outputs from toxicology studies, identifying exposure levels that either do not cause treatment-related effects or at which such effects begin to occur and which could be considered relevant to human risk assessment. In this context the No-Observed-Adverse-Effect-Level (NOAEL) and bench mark dose (BMD) represent a judgement based on a defined data set which impacts on the calculated margin of safety. Some (reversible) effects may be produced at these levels, but they are not considered to be adverse or precursors to adverse effects.

Lewis (Lewis et al., 2002) defined an adverse effect as "a biochemical, morphological or physiological change (in response to a stimulus, in this case the chemical substance) that either singly or in combination adversely affects the performance of the whole organism (the test species) or reduces the organism's ability to respond to an additional environmental challenge". The nature and extent of adverse effects can vary greatly with different types of chemicals, and it is anticipated that in many instances, experts will disagree on the characterization of effects as being adverse or not. However, the use of NOAEL/ BMD lower bound (BMDL) as a benchmark for setting the point of departure (POD) in the risk assessment of chemicals has traditionally been used to derive health-based reference values which are considered protective for target populations. The identification of the factors which determine whether effects are adverse or not is very complex and has provoked much debate, an adverse outcome being a variable highly influenced by the experimental model and study design.

An effect is less likely to be regarded as adverse if: 1) the effect is isolated or independent of dose, 2) there is no alteration in the general functions of the organism or organ/tissue affected (limited reduction in parameters), 3) it is transient and/ or reversible, 4) it is regarded as an adaptative response, 5) the severity of the effect is regarded as being below a threshold of concern (minimal and not related to changes in other parameters), 6) it is not a precursor to an accepted continuum of changes known to progress to an established adverse effect, 7) it is a consequence of experimental conditions e.g. restraining of the animals, palatability of the diet, etc.

These factors should be considered, individually or in combination, to differentiate adverse from non-adverse events in the overall weight of evidence approach. However, there are no exact biological definitions for "environmental challenge", "thresholds for concern", or "accepted continuum" and these represent important challenges in defining adversity. In the low dose response with non monotonic dose response curve the assessor may have problems in defining where on the dose response curve effects occur that could be considered potentially harmful to humans. It is also questioned whether the standard toxicological studies would be sensitive enough to provide confidence in identifying a given effect as adverse. If this is the case, the risk assessor should include in the weight of evidence approach additional complementary mechanistic evidence based on exploration of specific endpoints and specific experimental models.

DISCUSSION POINTS

1. What experimental evidence would be necessary to define adversity for low dose effects and non monotonic dose-responses?
2. Is the working definition of adversity for low dose effects, together with the factors to be considered, still valid?
3. Would the NOAEL/BMDL concept for defining a non-adverse POD still be applicable for low dose non-monotonic dose response effects?
4. Defining data gaps to be filled in order to establish a point of departure that can be used in the risk assessment of low dose non monotonic dose response effects.
5. What are the implications of using non validated experimental animal models in defining adversity for low dose, non monotonic dose response effects?

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DISCUSSION GROUP 2
Dose response relationships

INTRODUCTION

The dose-response relationship is a concept central to biological, pharmacological or toxicological research whether one is dealing with therapeutics or toxicants. The investigation of a dose-response requires an understanding of the biological/ physiological basis for the passage from an external dose to an internal dose, which elicits a biological or a toxicological response. In toxicological terms, an individual is exposed to a chemical (external dose) which is then absorbed, distributed and metabolised to a either a toxic species or detoxified form [parent compound or metabolites (s)] and excreted (toxicokinetics), the biologically active species of which then interacts at its target site to exert its toxicity (toxicodynamics) (Renwick, 1993). In hazard characterisation, the evaluation and quantification of the relationship between the administered dose and the toxicokinetic and toxicodynamic endpoints is performed in most cases using experimental animal data. This analysis constitutes a critical step to derive safe levels of exposure for chemicals (WHO, 2009). The contribution of toxicokinetic and toxicodynamic processes and their linearity, non-linearity and non-monotonicity has to be assessed in order to understand the resulting dose–response and to define at which dose/concentration a biologically/toxicologically significant effect may occur. Additionally, an understanding of interspecies differences and human variability and their impact on dose-response are critical to such an integrative approach. The influence of route of exposure on the toxicokinetic processes which determine the internal dose must also be taken into consideration, as must the possibility of particular windows of exposure/susceptibility for the target population. Differences in toxicokinetic and toxicodynamic processes during these windows of exposure/ susceptibility may be critical in determining the dose-response relationship.

Recently, the evaluation of low dose effects of chemicals has been discussed (Birnbaum, 2012) as well as non-monotonic dose responses such as "hormesis." (inverted U-shaped or a J-shaped dose–response curve) in which opposite effects have been observed at low, compared to high, doses for the same measured parameter (Connolly and Lutz, 2004; Calabrese and Blain., 2011; Vandenberg et al.,2012). Finally, physiologically-based toxicokinetic models have been proposed as tools to add to our understanding of toxicokinetic variability (Bois et al., 2010).

In this breakout session, a discussion of the biological/ toxicological basis of dose-response with its toxicokinetic and toxicodynamic elements will set the scene. In vitro and in vivo evidence for linear versus non linear dose-response relationships including low dose effects and non monotonic dose relationships; and physiologically-based models will be then discussed to provide a basis for further discussion on their potential impact on dose response modelling. The session will conclude with a final discussion on the integration of the quantitative aspects of toxicokinetic and toxicodynamic processes including interspecies differences, human variability and critical windows of exposure/susceptibility in dose response modelling and hazard characterisation.

DISCUSSION POINTS

1. A discussion of the toxicokinetic and toxicodynamic aspects of dose-response in biology and toxicology
2. How to implement variability in toxicokinetics and toxicodynamics. and also critical time windows of exposure/ susceptibility, in dose response modelling and hazard characterisation
3. Effects of routes of exposure on toxicokinetic and toxicodynamic processes
4. Integration of in vitro effects to in vivo whole body response
5. Physiologically-based models in dose-response assessment

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DISCUSSION GROUP 3
Low dose effects: is there sufficient evidence for non-monotonic dose response curves?

INTRODUCTION

Recently, the evaluation of low dose effects of chemicals has been discussed (Birnbaum, 2012) as well as non-monotonic dose responses such as "hormesis." (inverted U-shaped or a J-shaped dose–response curve) in which opposite effects have been observed at low, compared to high, doses for the same measured parameter (Connolly and Lutz, 2004; Calabrese and Blain., 2011; Vandenberg et al., 2012).

The so-called "low dose" hypothesis dates back to the late 1990s, on the basis of studies claiming that hormonally active environmental agents can cause a variety of effects, mainly reproductive and developmental, at "low doses" (vom Saal and Sheehan, 1998). Low dose effects have been suggested for a number of chemicals that mimic natural hormones, such as some pesticides, dioxins, polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs) and bisphenol A (BPA). What is exactly meant by "low-dose effects"? Different definitions of "low-dose effects" include effects that occur in the typical range of human exposures, or at environmentally-relevant doses, and/or effects observed at doses below those used for traditional toxicological studies, or at doses below the presumed NO(A)EL or BMDL expected by the traditional testing paradigm (Melnick et al., 2002). Supporters of this hypothesis do not consider the standard toxicology testing strategies as adequate to detect low dose effects.

A dose response curve is non monotonic when the slope of the curve changes sign somewhere within the range of doses examined. Non monotonicity is not synonymous with low dose, because there are low dose effects that follow monotonic dose response curves. The consequence of non-monotonic dose responses for toxicity testing is that a safe dose determined from high doses does not guarantee safety at lower untested doses that may be closer to current human exposure.

These two theories challenge key concepts in toxicology and risk assessment, such as the existence of a "safe" threshold dose for most (non genotoxic) chemicals, and the possibility to predict the effects of a chemical at low doses from its effects at higher doses. As yet, these claims are still highly controversial and the biochemical mechanisms by which these effects would occur are not well understood. Some expert panels in the US and EU that evaluated the underlying evidence for the low dose hypothesis in the early 2000s overall concluded that such "low dose" effects were not conclusively established (Kamrin, 2007). Proponents of the "low dose" and "non-monotonic dose responses" hypotheses assert that a large number of more recent studies now provide clear support for their hypothesis (Vandenberg, 2012). There is a great interest and debate within the scientific community concerning the scientific validity of these hypotheses. Basic precepts of scientific validity need to be applied to examination of the toxicological data. These include reproducibility of the data, consistency of the results, and proper conduct of the study. The degree to which these criteria are met is paramount to the critical assessment of the claims of "low dose" effects and non-monotonic dose responses and their applicability to humans.

DISCUSSION POINTS

1. Defining low dose effects and non-monotonic dose responses; what do they mean in the context of this Colloquium?
2. Is the current scientific evidence for low-dose effects and non-monotonic dose responses for endocrine-active chemicals convincing? (in vitro, in vivo /in mammalian species, epidemiological evidence).
3. If not, which data are necessary to provide conclusive scientific evidence for the occurrence of low dose effects and non monotonic dose response curves?
4. Are the current testing paradigms adequate to detect "low dose effects"? If not, how experimental design could be improved to address properly low-dose effects and non-monotonic dose responses?
5. How to model non-monotonic dose response in the context of a quantitative risk assessment?

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DISCUSSION GROUP 4
Impact for risk assessment

INTRODUCTION

The risk assessments of chemicals added either directly or indirectly to food, such as food additives, contaminants and chemicals used in food contact materials, carried out by EFSA and other international bodies are based on the classical risk assessment paradigm of hazard identification, hazard characterisation, exposure assessment and risk characterisation. The hazard characterisation step involves an evaluation of the relationship between the administered dose and the response of the organism (dose-response relationship), generally in studies in experimental animals. For those chemicals for which the critical effect is regarded as thresholded (i.e. a dose or exposure level can be identified below which no biologically significant effect has been identified), this exposure level (a No-Observed-Adverse-Effect-Level (NOAEL) or, more recently, a Benchmark Dose (Lower) (BMDL) can be used as a point of departure to derive (by application of an uncertainty factor) a health-based guidance value such as an Acceptable Daily intake. This low-dose extrapolation step provides an exposure level at which it can be concluded with reasonable certainty that no adverse effects will be induced in a human population exposed to the chemical for their lifetime. In contrast, for non-thresholded substances, e.g. genotoxic carcinogens, it is generally assumed that exposure to even very low levels can result in an adverse effect, and the low-dose extrapolation step aims to provide a quantitative estimate of the probability of risk at very low exposure levels. A Margin of Exposure approach can be applied to characterise the probability of risk, based on estimated exposure to the target population and the BMDL10 from the most sensitive/relevant animal carcinogenicity study.

Qualitative and quantitative risk assessment of low dose responses to chemicals added either directly or indirectly to food has been performed routinely using the above mentioned approaches. However, recent considerations of the impact of low dose responses, mainly arising from chemicals considered as endocrine active substances or endocrine disrupters has questioned the suitability of the risk assessment process for these type of substances. The acceptability of an alternative risk assessment process will require several considerations, amongst them (a) careful evaluation of the shape of the dose response curve at low doses (provided that a definition of a low dose is achieved), (b) scientifically-based decisions regarding the adverse nature of effects seen at low doses and their relevance to characterise the risk for humans. In the case of a number of chemicals showing endocrine-active or endocrine-disruptive activities a non-monotonic dose response curve has been reported to exist. For these substances, it has been argued that the traditional NOAEL/BMDL point of departure cannot be used to derive a health-based guidance value. According to the proponents of this view, for these substances uncertainties exist regarding identification of an exposure level at which it can be concluded with reasonable certainty that the risk for the exposed population is acceptable. Risk assessors have traditionally identified NOAELs/BMDLs in "gold-standard" animal toxicological studies, based on effects that have defined as adverse in terms of functional and morphological manifestations. If a scientific consensus can be reached that the effects recently reported at low doses for a number of chemicals showing endocrine-active activities are truly adverse, then the hazard characterisation approach used for monotonic non-thresholded substances could theoretically be applied to these substances and a quantitative estimate of the probability of risk for humans at very low exposure levels could be derived. This has significant implications in terms of risk management measures. An additional issue in considering the impact of monotonic low dose effects and non-monotonic dose response curves on current risk assessment approaches is the possibility that there may be critical windows of susceptibility for the population for these effects to be manifest. It may thus be difficult for risk assessors to identify a health-based guidance value that is appropriate for an entire population throughout a lifetime, taking into consideration potential critical windows of susceptibility. Alternatively it may also be possible, or even appropriate, to consider that a substance will only manifest its effects during this particular window of susceptibility and not outside those windows.

DISCUSSION POINTS

1. Assuming a general acceptance of the scientific validity of the low dose/non-monotonic dose response curve hypothesis, does this dictate a need for new risk assessment approaches?
2. Are different approaches already in use in risk assessment appropriate to deal with low-dose effects and non-monotonic dose-response curves (e.g. is there any need for additional uncertainty factors, does the Margin of Exposure approach covers these responses, can the TCC concept be applied to these responses), if not which data gaps would need to be filled to achieve a full risk assessment of this type of compound?
3. Assuming a general acceptance of the scientific validity of the low dose/non-monotonic dose response curve hypothesis, how to take critical windows of susceptibility into account in the risk assessment process of these compounds?
4. Can traditional "gold-standard" toxicology studies be coupled to targeted endpoint research studies to derive health-based guidance values for this type of compound?

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