Concept Article

The Use of Categorical Regression in the Assessment of the Risks of Nutrient Deficiency and Excess

Patrick J. Farrell[1,2], Peter Aggett[3], Brittany Milton[4], Siva Ramoju[4], Don Mattison[2,4], Nicolas Birkett[2,5] and Daniel Krewski[2,4,5]

[1]School of Mathematics and Statistics, Carleton University, Ottawa, Canada; [2]McLaughlin Centre for Population Health Risk Assessment, University of Ottawa, Ottawa, Canada; [3]Emeritus, Lancashire Postgraduate School of Medicine and Health, University of Central Lancashire, Preston, Lancashire, UK; [4]Risk Sciences International, Ottawa, Canada; [5]School of Epidemiology and Public Health, University of Ottawa, Ottawa, Canada

Abstract

Exposure-response assessment methods have shifted towards more quantitative approaches, with health risk assessors exploring more statistically driven techniques. These assessments, however, usually rely on one critical health effect from a single key study. Categorical regression addresses this limitation by incorporating data from all relevant studies – including human, animal, and mechanistic studies – thereby including a broad spectrum of health endpoints and exposure levels for exposure-response analysis in an objective manner. Categorical regression requires the establishment of ordered response categories corresponding to increasingly severe adverse health outcomes and the availability of a comprehensive database that summarizes all data on different outcomes from different studies, including the exposure or dose at which these outcomes are observed and their severity. It has found application in the risk assessment of essential nutrients and trace metals. Since adverse effects may arise from either deficient or excess exposure, the exposure-response curve is U-shaped, which provides a basis for determining optimal intake levels that minimize the joint risks of deficiency and excess. This article provides an overview of the use of categorical regression fit exposure-response models incorporating data from multiple evidence streams. An extension of categorical regression that permits the simultaneous analysis of excess and deficiency toxicity data is presented and applied to comprehensive databases on copper and manganese. Future applications of categorical regression will be able to make greater use of diverse data sets developed using new approach methodologies, which can be expected to provide valuable information on toxic responses of varying severity.

1 Introduction

The World Health Organization (WHO) categorizes a metal as essential when "*absence or deficiency of the element from the diet produces either functional or structural abnormalities and that the abnormalities are related to, or a consequence of, specific biochemical changes that can be reversed by the presence of the essential metal*" (WHO, 1996). However, too much of the metal can also produce undesirable effects. In the field of nutrient risk assessment, an adverse effect has a broad definition; it is "*a change in morphology, physiology, growth, development, reproduction or lifespan of an organism, system, or (sub)population that results in an impairment of functional capacity, and impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences*" (WHO/IPCS, 2002). The characterization of exposure-response relationships is important in estimating the risk of adverse health effects of essential elements arising from either excess or deficient oral intakes. These risk assessments determine the intakes of a nutrient that have a minimal risk of causing deficiency or of causing excess. Such assessments have typically been carried out independently by expert groups predominantly comprising nutritionists in the case of avoiding deficiency or toxicologists in the case of avoiding excess.

This content is part of the *Special Issue* Development of an Evidence-Based Risk Assessment Framework. doi:10.14573/altex.22S2

Received December 2, 2020; Accepted March 17, 2022;
Epub March 30, 2022;

ALTEX 39(4), 656-666. doi:10.14573/altex.2012022

Correspondence: Patrick J. Farrell, PhD
School of Mathematics and Statistics
Carleton University
1125 Colonel By Drive, Ottawa, Canada
(pfarrell@math.carleton.ca)

The risk assessments provide reference values for risk managers to use in emplacing policy and practice relating to public health nutrition, nutritional and diet surveillance, and the safety of foodstuffs, dietary intakes, nutrient supplements, and agri-food industry products. They provide guidelines relevant to populations rather than to individuals.

The process of risk assessment for non-nutrients applies also to nutrients. It comprises five stages (WHO, 2006). The first is problem formulation, in which the purpose of the risk assessment is defined and agreed upon with risk managers. The issues that need to be considered at this stage are inherent in the International Program on Chemical Safety (IPCS) definition of a risk assessment, which is "… *a process intended to calculate or estimate the risk to a given target organism, system, or (sub)population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system*" (WHO/IPCS, 2002, WHO, 2006).

The subsequent stages of assessment are hazard identification, hazard characterization, exposure assessment, and risk characterization. Hazard identification involves the identification of the type and nature of adverse effects that an agent has an inherent capacity to cause in an organism, system, or (sub)population (WHO/IPCS, 2002). Hazard characterization is a qualitative and, wherever possible, a quantitative description of the inherent property of an agent or situation having the potential to cause adverse effects. This should, where possible, include a dose-response assessment and its attendant uncertainties (WHO/IPCS, 2002). Exposure assessment is the evaluation of the exposure of an organism, system, or (sub)population to an agent and its derivatives (WHO/IPCS, 2002)), while risk characterization is the qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system, or (sub)population under defined exposure conditions (WHO/IPCS, 2002).

Historically, regulatory agencies have used benchmarks such as the no-observed-adverse-effects level (NOAEL), corresponding to the level of exposure that does not result in a significant increase in the risk of adverse effects in the exposed group when compared with controls. The NOAEL has served as a point of departure (PoD) on the exposure-response curve for establishing a reference dose (RfD) for human exposure through the application of appropriate adjustment factors (Barnes and Dourson, 1988). The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure in the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. However, since the RfD and benchmarks that preceded it did not consider nutrients, and this oversight could have implied that zero exposure poses no health risk, it was felt that an alternative measure should be used to designate the safe level of a lifetime's daily exposure to essential nutrients such as trace metals (Barnes and Dourson, 1988).

Considerable effort has been expended in assessments of adequate physiological nutrient intakes along with estimates of intakes which, if prolonged, would increase the risk of adverse effects, as defined above, indicative of deficient or excessive exposure to the nutrient of interest. There has been much effort to harmonize the concepts, approaches and terminology used in setting nutrient intake values (NIV) that would inform the development of policy and practice for public health nutrition (Lewis and Dwyer, 2020; King and Garza, 2007). NIVs vary according to the evidence and expert opinions used in their derivation. In principle, NIVs address a daily intake level for a defined population, thus (i) a low intake level would be compatible with health, however intakes below this have a risk of deficiency with adverse effects, (ii) an average intake would be expected to meet the needs of 50% of a population, (iii) a reference intake value would meet the requirements of nearly all (99 or 95% of the population), and (iv) an upper level (UL) of intake is a level above which there is a risk of adverse effects arising from excess exposure (Lewis and Dwyer, 2020; King and Garza, 2007). Procedures for establishing dietary reference intakes (DRIs), including recommended daily allowances (RDAs), of nutrients are discussed by the US National Research Council (NRC, 2000) and King and Garza (2007); more recently, procedures for setting ULs have been discussed by the European Food Safety Authority Panel on Nutrition, Novel Foods and Food Allergens (EFSA NDA, 2022). The assessment of these values is compromised by the limited quality and quantity of available data. Recently, the feasibility of using epidemiologically based assessments of chronic disease outcomes to derive NIVs, such as ULs for nutrients, has been explored (NRC, 2017; Yetley et al., 2017). However, such an approach involving a remote apical outcome would benefit from an appreciation of the processes involved in regulating a nutrient's homeostasis and the mechanisms of the dose-response relationships of effects of adequate, excessive, and inadequate intakes. Figure 1 illustrates this relationship further and describes the concept of an acceptable range of intake (AROI). In this figure, points A and B could be seen respectively as a value for a lower intake and as a possible marker for a reference intake for the population (i.e., (iii) above), or perhaps as an UL (i.e., (iv) above). These interpretations depend on an integration and analysis of the mechanisms of a nutrient's metabolism and of adverse effects at non-physiological intakes as is discussed below.

The benchmarks described above rely on a weight of evidence assessment for a relevant effect in humans and, to a considerable extent, on expert opinion. This led to the development of different human exposure guidelines by different regulatory bodies (US EPA, 1993, 1994; Health Canada, 1994; ATSDR, 2000, 2012; WHO, 2000; Lewis and Dwyer, 2020), including occupational exposure guidelines (Roels et al., 1992; Deveau et al., 2015). More recently, exposure-response assessment methods have shifted towards more quantitative methods, with health risk assessors exploring more statistically driven techniques such as the benchmark dose (BMD) (Crump, 1984) and the signal-to-noise crossover dose (SNCD) (Sand et al., 2011, 2017). Nonetheless, the RfD, SNCD, and BMD approaches all ultimately rely on

one critical health effect from a single key study rather than on an integrated analysis of adverse effects drawn from more than one objectively good-quality study.

Categorical regression addresses this limitation by allowing risk assessors to capture relevant health information across multiple studies and species, including a broad spectrum of health endpoints and exposure levels for exposure-response analysis in an objective manner. In addition, categorical regression also allows the inclusion of multiple independent variables, including level and duration of exposure, and variables that may modify the exposure-response relationship such as age and gender.

Hertzberg and Miller (1985) initially proposed the use of categorical regression methods in health risk assessment. Applications of such methods to exposure-response modeling include Allen et al. (2005), Gift et al. (2008), and Chambers et al. (2010). The foundation of categorical regression modeling is the establishment of ordered response categories corresponding to increasingly severe adverse health outcomes and the availability of a comprehensive database that summarizes ordered response categories for toxicity from deficiency or excess.

In 2006, the US EPA released a software program called CatReg, developed to perform categorical regression modeling and to calculate a benchmark level called the extra risk concentration (ERC_q) from an exposure-response model. The model builds a relationship describing the likelihood of a severity score in terms of the exposure information, for example, exposure level and duration (Haber et al., 2001). The ERC_q is the concentration that produces an increase of risk to a prescribed level known as the "extra risk". The extra risk, ER, for severity level $S_i = s$ at concentration $C_i = c_i$ and duration $T_i = t_i$ is defined as

$$ER = \frac{P(S_i \geq s \mid C_i = c_i, T_i = t_i) - P(S_i \geq s \mid C_i = 0, T_i = t_i)}{1 - P(S_i \geq s \mid C_i = 0, T_i = t_i)} \qquad \text{(Eq. 1)}.$$

The ERC_q at time $T_i = t_i$ is the concentration c_i that satisfies

$$\frac{P(S_i \geq s \mid C_i = c_i, T_i = t_i) - P(S_i \geq s \mid C_i = 0, T_i = t_i)}{1 - P(S_i \geq s \mid C_i = 0, T_i = t_i)} = \frac{q}{100} \qquad \text{(Eq. 2)},$$

where $q/100$ is the probability of an adverse effect of level s or higher due to exposure for time t_i given that the adverse effect would not have occurred from other causes during this time. Applications of CatReg include studies focusing on hydrogen sulfide (Strickland and Foureman, 2002; Brown and Strickland, 2003; Brown and Foureman, 2005), phosgene (Gift et al., 2008), and acrylamide (Allen et al., 2005), where excess exposure-toxicity curves were fit to exposure-response data.

Risk assessments for the presence of essential metals have typically been carried out independently by nutritionists, who focus on a minimum necessary sufficiency of the metal (avoiding deficiency), or by toxicologists, who are concerned with the adverse effects that may result from too much of the material (avoiding excess). Studies that focus simultaneously on excess and deficiency have begun to emerge. Such investigations have centered on modeling the exposure-response curve describing the probability of an adverse effect (deficiency or excess) as a function of the concentration of the metal possessed by a subject according to a U-shaped curve.

Chambers et al. (2010) used CatReg to perform an exposure-response analysis on a copper database (Krewski et al., 2010), creating separate excess and deficiency exposure-response models for oral intake. Since CatReg cannot analyze both the deficiency and excess data together in one analysis, the authors spliced the excess and deficiency curves together to create a U-shaped curve, and then estimated the exposure level at the trough of the curve.

Milton et al. (2017a) proposed a new method for defining U-shaped exposure-response curves based on categorical regression that does not require splicing excess and deficiency curves together. The authors applied their methods to the same copper database used by Chambers et al. (2010) and obtained a smooth, continuous non-symmetric U-shaped exposure-response curve that achieves balance between copper excess and deficiency. They also identified two potential benchmark levels: the equiprobable crossover point (EPCP), which corresponds to the level of exposure, where the risk of toxicity due to excess is equal to the risk of toxicity due to deficiency, and $x_{MIN\ DUE}$, which represents the level of exposure at the bottom of the U-shaped curve that minimizes the overall risk due to excess or deficiency (or both). Application of these techniques to a manganese database are discussed in Milton et al. (2017b).

This paper is devoted to a discussion of the simultaneous characterization of the adverse events associated with deficiency and excess of essential nutrients in the modelling of U-shaped exposure-response curves, and specifically the use of categorical regression for this purpose. The paper concludes with a summary of the current status of categorical regression in evidence integration and discusses how future applications could make use of data derived from new approach methodologies to obtain novel data on outcomes of varying degrees of severity to supplement or replace data from traditional toxicity tests.

2 U-shaped exposure-response curves for essential nutrients

The concept of using a U-shaped exposure-response curve addresses the fact that both excessive and deficient intakes of essential nutrients result in adverse events, the population prevalence of which increases as nutrient deficiencies or excesses become more severe. The principal public health focus has been on avoiding excessive intakes. However, the approaches taken in risk assessment of excess nutrient exposures might involve using large uncertainty factors that reduce a RfD to a value that lies below the range of dietary reference values set by nutritionists as being physiologically necessary. This is inherently illogical, and it arises from the differing approaches to risk assessment adopted by toxicologists and nutritionists (Mertz, 1993). To avoid such a clash, this prob-

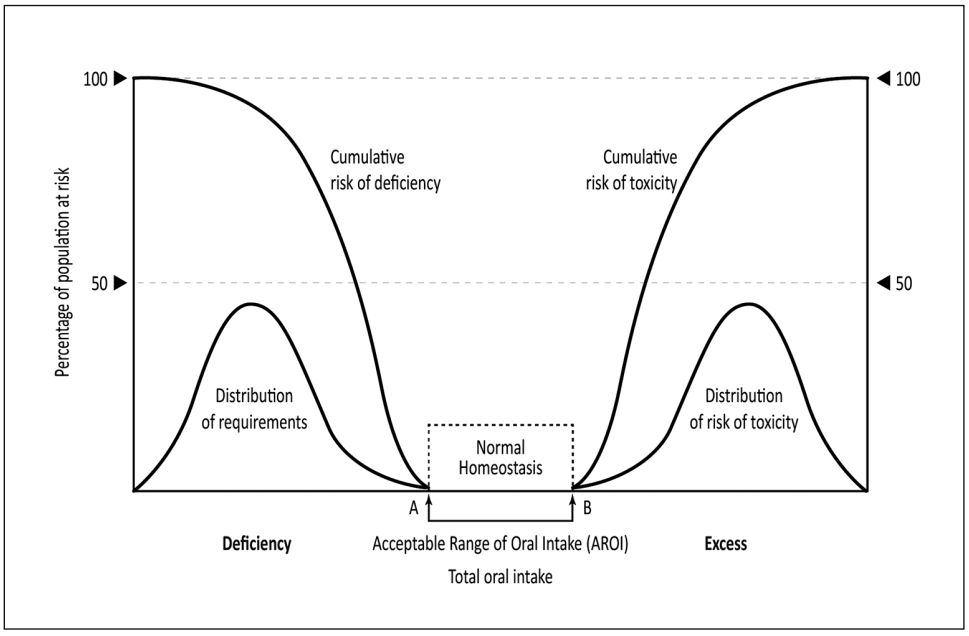

Fig. 1: The percentage of population at risk of deficiency and toxicity effects associated with inadequate and excess oral intakes

As intake drops below the lowest extent of normal homeostasis (indicated by A), an increasing proportion of the population will be at risk of deficiency. As the intake increasingly exceeds the upper limit of efficient homeostasis (indicated by B), an increasing proportion of the population will be at risk of toxicity.

lem has usually been solved by reducing an uncertainty factor, a *modus operandi* that has, as a generalization, been widely accepted. However, this process is not forensically robust, and, despite the acceptance of the derived values and their use, it would be difficult to defend them if they were to be challenged. Many who have been involved in deriving such health-based guidance values (HBGVs) for nutrients appreciate that, even if the current values serve well for risk management, there is a residual discomfort about their security. This arises from caution about or limited confidence in (i) the identification of appropriate critical events as adverse effects that could be used for PoDs or RfDs (intriguingly, nutritionists and toxicologists alike fail to appreciate the breadth of events that constitute an adverse effect), (ii) the determination and use of appropriate uncertainty factors to derive a UL that does not trespass on dietary reference values (DRVs), and (iii) the lack of transparency associated with these issues. This is not a recent dilemma; it has been recognized both in human nutrition and in animal husbandry for over half a century (Mertz, 1993). There has been an increasing appreciation by nutritionists and toxicologists that they had a common interest in the dose-response curve of nutrient intakes embracing toxicity from deficiency and excess and in the sharing of a broader database to explore this further.

In 1998, the IPCS convened a working group on the "Principles and Methods for the Assessment of Risk from Essential Trace Elements" (WHO/IPCS, 2002). Figure 1 is derived from the WHO/IPCS task group report. It illustrates the notion of a U-shaped response curve represented as cumulative incidence of population risks of toxicity and deficiency associated with increasing and decreasing intakes of essential trace elements. At the center of this exposure-response relationship, the working group envisaged the existence of an AROI.

The WHO/IPCS working group disaggregated the cumulative risks of deficiency and toxicity to show a broad categorization of the increasingly severe adverse effects as the related risks increased with diminishing and increasing exposures or intakes. These are shown in Figure 2, which also displays an AROI in which there is in a population minimal risk of adverse effects due either to deficiency or toxicity, and the physiological elements absorption, deposition, and excretion which collectively maintain homeostasis of a nutrient. The term homeostasis applies to the maintenance of the constancy of systemic physiology and metabolism, i.e., the "milieu interieur" as conceived by Claude Bernard (Davies, 2016). The IPCS activity therefore called the systemization illustrated in Figure 2 a homeostatic (based) model (WHO/IPCS, 2002). Subsequently, the terms biologically based and physiologically based (pharmacokinetic) models have been used.

Although the WHO/IPCS report focused on essential trace metals, it appreciated that the model in this figure is applicable to all essential nutrients and that it provides the opportunity to use physiological events at the extremes of homeostatic control as the bases of identifying the lower and upper intake boundaries of the AROI for a nutrient as well as in the risk assessments of markers for deficiency and excess exposures for nutrients. Thus, the WHO/IPCS report endorsed and revived the concept that biologically based homeostatic models should be adopted in the risk assessment of deficiency and excess rather than using the customary toxicological approaches (Mertz, 1993; WHO/IPCS, 2002). However, the spectrum of outcomes extending from grossly deficient through to grossly excessive dietary intakes is not necessarily U-shaped. It is more likely that there is a J-shaped asymmetry in this range of exposure, given that the range of exposures encompassing dietary deficiency is smaller than that involved with excess exposure and subsequent toxicity.

A refined and more metabolically-based categorization summarized in Table 1 has been used to perform a categorical regression-based risk assessment and severity scoring of defi-

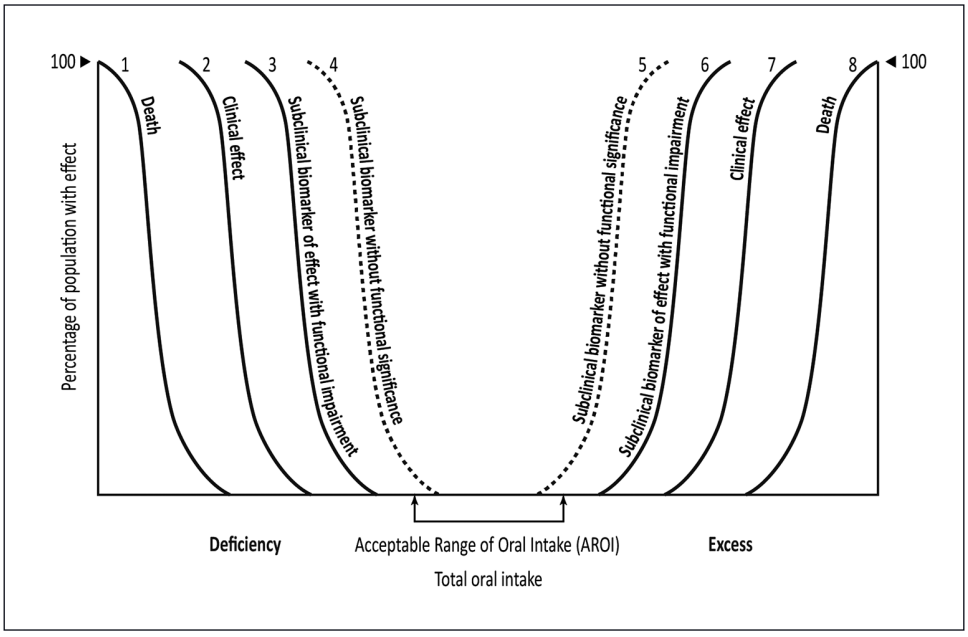

Fig. 2: The categorization of response to deficient and excess intakes of essential trace metals developed by the WHO/IPCS Working Group, and the relationship of homeostatic and adaptive response to decreasing and increasing intakes of the nutrient

Tab. 1: Thirteen-point severity scoring system for copper (Chambers et al., 2010)

Outcome	Score (S)	Physiological response
Deficiency	-6	Death
	-5	Serious irreversible gross deficiency
	-4	Reversible gross deficiency
	-3	Metabolic perturbation
	-2	Early biological indicators of deficient Cu levels
	-1	Homeostatic adaptation to low intakes
Homeostasis	0	No effect
Excess	1	Homeostatic adaptation to high intakes
	2	Early biological indicators of accumulated Cu
	3	Metabolic perturbation
	4	Reversible gross excess
	5	Serious irreversible gross excess
	6	Death

cient and excess exposure to copper (Chambers et al., 2010). A similar, more detailed categorization (presented in Tab. S1[1]) was used in a study of manganese (Mattison et al., 2016). Both studies exemplify an extensive use of biological or physiological events in addition to pathophysiological events in exposure-response modeling. In both studies, the ordered response categories corresponding to increasingly severe adverse health outcomes to deficient and excess intakes were related as much as was possible to the underlying biological mechanisms, as well as to the severity of the consequences of deficient or excess exposures. It is noteworthy that these studies made use of a comprehensive database from human and animal model sources to create ordered response categories for regression analysis. However, it was evident that, despite having a broader database, the variable and limited quality of the available information made it difficult to support approaches such as CatReg for risk assessment of nutrients, not least because there were more data available at the extremities of the exposure-response curves and categories than at levels representative of physiological intakes. This is because human and animal research have focused more on severe deficiency and gross excess than on customary physiological levels of intake. Both scoring matrices ascribed homeostasis as Category 0, which was essentially because there were few dose-response data relevant to the homeostasis of manganese, whereas those for homeostasis of copper were obscured by regressions that were strongly influenced by data from using extremely high and low exposures. It is expected that the advent of systems biology will improve the amount of data for categorization of events in homeostasis and the determination of the bounds of an AROI (Fig. 3).

[1] doi:10.14573/altex.2012022s

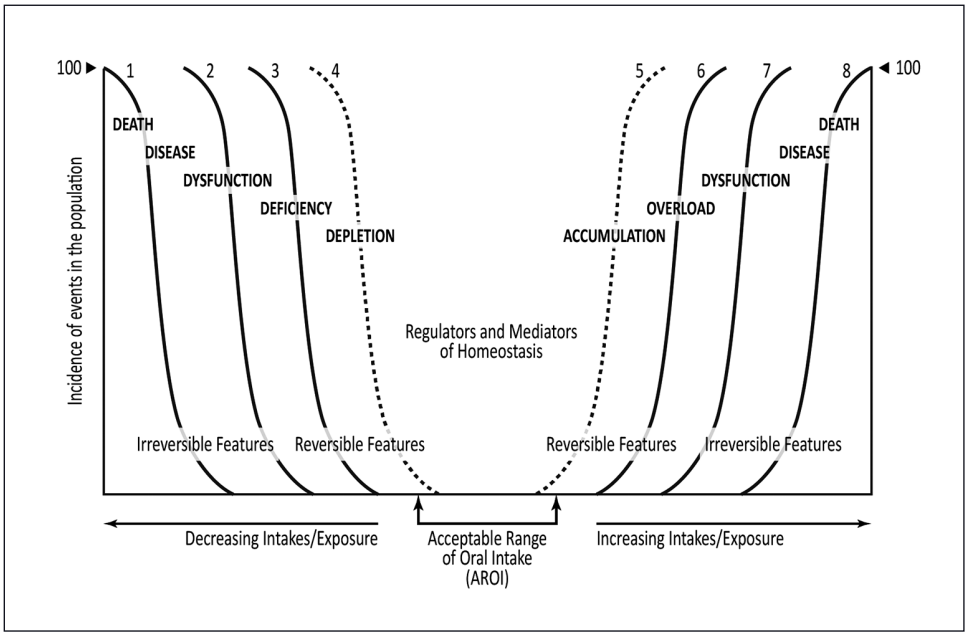

Fig. 3: The spectrum of homeostatic, adaptive, and toxic responses to intakes of an essential nutrient ranging between grossly deficient and grossly excessive

The underlying assumption in the above discussion is that the risk assessment seeks the limits of an AROI. On the other hand, if the objective of a CatReg is to identify, for example, a tolerable UL or a similar HBGV or even a threshold value to avoid deficiency, the available data might already be sufficient to generate a comprehensive database that could be used to explore the integration of information from studies on humans, animal models, and *in vitro* models.

In any case, there is a need to contextualize the public health objective within the spectrum of available data that would enable the development of a strategic and targeted risk assessment. This spectrum is illustrated in Figure 3, which outlines the range of responses to intakes of an essential nutrient extending from deficiency to excess. This figure is intended to demonstrate a basis for a risk assessment using existing data. For example, in studies of copper homeostasis, evidence of ectopic deposition and of increased urinary excretion have not been used in risk assessment. Similarly, earlier studies of sodium risk assessment data indicative of failure of sodium homeostasis have been relatively ignored but in fact produced ULs for sodium consumption that were subsequently corroborated by detailed analysis of epidemiological data (EFSA NDA, 2019).

It is not recommended that one set of information should be considered better than another but that the complementarity of available data should be appreciated and used more efficiently in risk assessment. This can be achieved by setting data against categories that are based broadly on the sequential progress of homeostasis to adaptation and the development of increasingly serious adverse effects; these categories match the severity-based categorization of data (Renwick et al., 2004) as is described below. In this approach, categorical regression enables a more specific focus on the underlying biological events that fit into mechanistic chains or pathways of critical/adverse events that are potential sources of markers relevant to risk assessment. Each pathway may provide discrete reliable events that can be validated as existing or predictive markers of impending overload and deficiency. The latter predictive markers may be identified and validated, not just as a single adverse effect, but as a pathway of events known to presage excess or deficient intakes.

All events shown in Figure 3 on either side of the central AROI zone are potential markers of effect. As noted previously, markers or biomarkers of effect have classically focused on their perceived impact. Thus, the selection of adverse effects used in the assessment of excess intakes has focused on obvious and irreversible toxicity. In fact, the sequence of events shown in Figure 3 provides a causal pathway for the adverse effects of diminishing or increasing exposures to the nutrients of interest. This means that the risk assessment should include markers of the associated homeostatic and adaptive responses and adverse effects. Thus, based on Figure 3, identified events (or biomarkers) are categorized according to whether or not they are reversible or irreversible. This classification arises from that of Renwick et al. (2004), who ranked potential biological and toxicological endpoints according to their potential value in risk assessment as follows:

1) Biochemical changes within the homeostatic range and without indication of adverse sequelae.
2) Biochemical changes outside the homeostatic range without known sequelae.
3) Biochemical changes outside the homeostatic range that represent a marker of potential adverse effects due to excess.
4) Clinical symptoms indicative of a minor but reversible change.
5) Clinical symptoms of significant but reversible effects.
6) Clinical signs indicative of significant but reversible organ damage; and
7) Clinical signs indicative of irreversible organ damage.

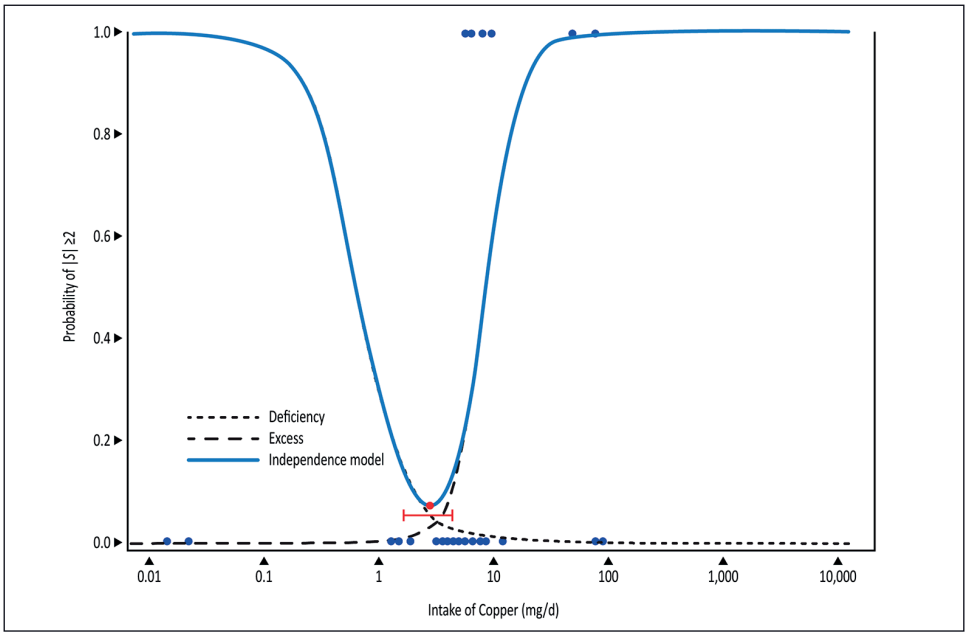

Fig. 4: U-shaped exposure-response curve for copper exposure-response assessment for humans fitted from the species-stratified JMED and IM models
The underlying JMED curves are shown as dashed black lines. The estimate for $x_{MIN\ DUE}$ is indicated by a red dot, while its 95% bootstrap confidence interval is indicated by the red line segment. The human data used to fit this model are indicated by blue circles.

This ranking is useful in interpreting the events summarized in Figure 3. The markers at levels 1-3 and, possibly, those at level 4 are of value to a biologically based approach to nutrient risk assessment; they represent homeostatic and adaptive responses. However, markers beyond level 4 can be regarded as toxicological. There is an ongoing debate about which responses constitute homeostasis and which constitute adaptation. This arises because risk assessors might not be inclined to regard biomarkers of adaptation as relevant to assessments of deficiency or toxicity. However, in a regression analysis, biomarkers and an understanding of their dose-responses are integral to a sound risk assessment and the construction of a mechanistic pathway of adverse effects. Here, again, it is worth noting that alterations in the adaptive responses would comply with the definition of adverse effects.

As such, it is possible that a reversible marker could be validated as a critical endpoint to support a traditional approach to risk assessment dependent on a single "adverse effect", particularly if it can be validated as a predictive marker of the development of irreversible outcomes if intake or exposure is not reduced or increased. Although it is difficult philosophically to determine a boundary between homeostasis and adaptation, this is probably unimportant in the context of overall risk assessment. Homeostatic mechanisms can be envisaged to control the body burden of a nutrient as well as the milieu interieur. Thus, if the body burden is increasing or decreasing, the metabolic responses should arguably be regarded as adaptive. However, the responses to deficiency occur over a narrower range of intakes and are therefore more compacted than those to excess intakes. This contributes to the nonsymmetric or asymmetric U-shaped curve of responses to deficiency and toxicity.

It is difficult currently to see how biologically based markers can be identified and validated as critical events to use in developing an RfD for risk assessment (WHO, 2006; Aggett, 2007). However, for most nutrients there is much available information that could be configured as in Figure 3 and subjected to categorical regression focusing on data supporting markers such as those of levels 1 to 4 above (i.e., biologically based markers). The general commonality of homeostatic mechanisms and their regulation amongst human and animal models offers opportunities to integrate or explore existing data from different species to inform CatReg analyses and mechanistic approaches to risk assessment, as has been shown recently in evaluating the safety of exposure to phosphorus for which a UL or health-based guidance value had not been determined. In this evaluation, data derived from rat studies using nephrocalcinosis as an endpoint were used to derive a chemical-specific adjustment factor rather than the customary default factor of a hundred to extrapolate data from an animal model to humans (Smeraldi et al., 2020).

The advantages of using a biologically based homeostatic model include enabling a focus on modes and mechanisms of action, the ability to use nutrient ADME and nutrikinetics, a less conservative derivation of uncertainty factors and ULs than those derived by single event-based analyses such as the NOAEL approach, consideration of predictive indicators for short and long term effects along with a consideration of the latency of effects and duration of exposures, and the facilitation of using a mechanistic analysis of a pathophysiological pathway to work back from a significant adverse effect or critical event to identify a convenient and predictive marker (Krewski et al., 2020). Since the WHO/IPCS report in 2002, advances in molecular biology and computational modelling have enhanced the ability to develop the homeostatic based model. It is now possible to characterize the regulation and mechanisms involved in homeostasis and thereby to explore their use in risk assessments of inadequate and excess exposures to essential trace metals as well as other nutrients. It is also feasible to explore the homeostatic and metabolic

mechanistic pathways and networks involved at subcellular, cellular, organ, and systemic levels through the integrated use of genomics, transcriptomics, proteomics, and metabolomics, to explore the dynamics and systemic kinetics of nutrients and how these are associated with intakes or exposure at both the individual and population level if this is needed (Edwards, 2017).

The effective exploitation and analysis of such complex data requires computational support to enable the capture of data from such diverse sources (see below) and their integration. These approaches comprise systems biology, which is foreseen to enhance toxicological risk assessment (Krewski et al., 2020) and which is equally applicable to nutrient needs and risk assessment. The use of such databases would enable deeper exploration of the interconnectivity at the biological levels involved in the reactions to deficient and excess intakes of nutrients and, one would expect, enable the identification via cell culture and *in vitro* studies of markers that could be used in human studies. An example of the use of systems biology in the investigation of the metabolism of essential trace metals is available for iron (Chifman et al., 2014). Here, known features of iron metabolism and its inborn errors were used to develop a computational model to describe the regulation of iron metabolism. Another, more recent example is a detailed systems biology study of the molecular aspects of the cellular handling and redistribution of copper; it focuses on inherited defects that cause significant disturbances in the homeostatic excretion of the element (Magistrato et al., 2019).

As stated above, a systems biology approach to the homeostatic model can use data from many sources (Edwards, 2017), including all information that would be used in identifying environmental causes of disease including dietary and adventitious occupational or industrial exposure to nutrients and other environmental chemicals (Academy of Medical Sciences, 2007). This includes evidence from human studies such as randomized controlled trials, intervention studies in which experimental and reference groups have well characterized intakes, and relevant and validated endpoints, as well as observational studies in human populations. There are many experimental studies on animal models targeting the effects of high and low intakes on specific organs and functions from which it might be possible to derive data on homeostatic and adaptive responses as body burden increases and on the sequential development of toxicological endpoints, which might enable the characterization of pathogenic events in the physio-pathological pathway (Fig. 3). Epidemiological studies in livestock and reports including cases of high intake and toxicities affecting humans and animals can also be helpful. Inborn errors of metabolism in humans and animals contribute to the understanding of underpinning genetic and consequent metabolic defects leading to toxicity. The quality of such data needs to be critically assessed for biologically based endpoints as would be the case for hazard identification and characterization.

Milton et al. (2017a) proposed a method based on logistic regression for simultaneously modeling excess and deficiency data that results in non-symmetric U-shaped exposure-response curves based on logistic regression. The model, referred to as a joint model for excess and deficiency (JMED), is based on a binary scale for the severity response; namely $Y = 0$ for a homeostatic response and $Y = 1$ for an adverse response (excess or deficiency). The JMED expresses the probability of an adverse response as a function of an excess/deficiency study indicator, a continuous covariate representing the level of exposure (transformed on the logarithm base ten scale), and its interaction with the indicator described above. Specifically, if Y_i is a random variable for the i-th observation ($i = 1,\ldots, n$), where

$$Y_i = \begin{cases} 1 \text{ for an adverse response (excess or deficiency)} \\ 0 \text{ for a homeostatic response} \end{cases} \quad \text{(Eq. 3)},$$

the JMED describes the probability of an adverse response, $P(Y_i = 1)$, as

$$P(Y_i = 1) = \frac{\exp(\beta_0 + \beta_1 \, x_{i1} + \beta_2 \, x_{i2} + \beta_3 \, x_{i1} \, x_{i2})}{1 + \exp(\beta_0 + \beta_1 \, x_{i1} + \beta_2 \, x_{i2} + \beta_3 \, x_{i1} \, x_{i2})} \quad \text{(Eq. 4)},$$

where x_{i1} is the exposure concentration expressed in mg/kg-bw/d on the logarithm base ten scale and x_{i2} is an indicator variable for the type of adverse event study (excess or deficiency); namely

$$x_{i2} = \begin{cases} 1 \text{ for an excess study} \\ 0 \text{ for a deficiency study} \end{cases} \quad \text{(Eq. 5)}.$$

By specifying the type of departure from homeostasis (excess or deficiency), the JMED describes the probability of an adverse response at a specified exposure level. The inclusion of an interaction permits the relationship between the probability of an adverse response and exposure concentration to be different depending on the type of departure from homeostasis (Milton et al., 2017a). When $x_{i2} = 1$, the JMED provides the probability of an adverse response due to excess, $P(Y_{Ei} = 1)$, which is given by:

$$P(Y_{Ei} = 1) = \frac{\exp[(\beta_0 + \beta_2) + (\beta_1 + \beta_3) \, x_{1i}]}{1 + \exp[(\beta_0 + \beta_2) + (\beta_1 + \beta_3) \, x_{1i}]} \quad \text{(Eq. 6)}.$$

When $x_{i2} = 0$, the JMED gives the probability of an adverse response due to deficiency, $P(Y_{Di} = 1)$, as

$$P(Y_{Di} = 1) = \frac{\exp(\beta_0 + \beta_1 \, x_{1i})}{1 + \exp(\beta_0 + \beta_1 \, x_{1i})} \quad \text{(Eq. 7)}.$$

The expressions for $P(Y_{Ei} = 1)$ and $P(Y_{Di} = 1)$ differ due to the presence of the parameters β_2 and β_3 in the former. The parameter β_2 allows for the possibility that $P(Y_{Ei} = 1)$ and $P(Y_{Di} = 1)$ are not equal when the exposure concentration on the logarithm base ten scale $x_{i1} = 0$. Further, β_3 acknowledges that the shapes of the deficiency and excess curves will not be the same. A potential benchmark level called the equiprobable crossover point (EPCP) was identified that corresponds to the level of exposure where the risk of toxicity due to excess is equal to the risk of toxicity due to deficiency. The authors show that the EPCP is equivalent to the ratio β_2/β_3, providing a simple closed-form solution for its estimation.

Assuming that the excess and deficiency data are independent, Milton et al. (2017a) subsequently used the JMED to propose a model that they referred to as the independence model (IM). This model allows for the construction of a single non-symmetric U-shaped curve to characterize the excess and deficiency data simultaneously. Assuming the presence or absence of an excess condition is independent of a deficient one (and *vice versa*), the probability that an individual will experience an adverse outcome due to either excess or deficiency, or both, is

$$P_{D\cup E} = P_D + P_E - P_D P_E \quad \text{(Eq. 8)},$$

where $P_E = P(Y_{Ei} = 1)$ and $P_D = P(Y_{Di} = 1)$. Using the excess and deficiency probability equations from the JMED, the IM provides a means of modeling excess and deficiency simultaneously under the assumption of independence of these two outcomes. The graphical representation of $P_{D\cup E}$ is a nonsymmetric U-shaped curve, since the JMED acknowledges that the shapes of the excess and deficiency curves will not necessarily be the same. Milton et al. (2017a) identified a second potential benchmark level as the exposure level at the trough of this curve, as this is the exposure level that minimizes overall risk due to excess or deficiency. They referred to this point as $x_{\mathrm{MIN}D\cup E}$, and developed an approach based on bootstrapping to determine a confidence interval for this quantity. Unlike the EPCP, there is no closed form solution for $x_{\mathrm{MIN}D\cup E}$, so that numerical search methods are needed for its estimation.

Krewski et al. (2010) discuss the development of a comprehensive copper toxicity database designed for the application of categorical regression, whereby relevant exposure-response information, including species, age, sex, route of exposure, concentration, and duration of exposure, was abstracted from select studies and stored in a digital database. This database serves as an organizational platform to summarize a large number of studies of the health effects of varying copper intake levels. These studies have accrued because of the continuing interest in assessing the potential risks of human exposure to copper arising from its widespread use in the built environment, food processing, feed production, and in agriculture, especially horticulture and viniculture and associated occupational exposures. Milton et al. (2017a) applied the JMED/IM approach to this database. They dichotomized the 13-point severity scoring system using the absolute value of 2 as a cut-off point between homeostatic and adverse responses; that is, they set $Y_i = 1$ if $S_i < -2$ or if $S_i > 2$ to reflect an adverse response (deficiency or excess), and $Y_i = 0$ if $-2 \leq S_i \leq 2$ to represent a homeostatic outcome.

The non-symmetric U-shaped exposure-response curve for humans that resulted from fitting the JMED is presented in Figure 4. The point estimate for the exposure level at the $x_{\mathrm{MIN}D\cup E}$ for a human weighing 70 kg was 2.73 mg/day. This is interpreted as the exposure level that minimizes the overall risk of an adverse response due to either excess or deficiency or both, with an associated 95% bootstrap confidence interval of 1.54 to 4.48 mg/day. These values compare favorably with recommendations of the US Institute of Medicine Panel on Micronutrients (US NRC, 2001), which established an RDA of 0.9 mg/day (based on plasma copper and ceruloplasmin concentrations, erythrocyte superoxide dismutase activity, and platelet copper concentration in controlled human depletion/repletion studies) and a UL of 10 mg/day (based on protection against liver damage as the critical adverse effect). The Panel further noted that the median intake of copper in the US ranges from 1.0-1.6 mg/day.

Building on the experience with CatReg in using the JMED model for describing the U-shaped exposure-response curve for copper discussed above, Milton et al. (2017b) conducted a similar analysis for manganese, which is also essential for various physiological processes such as metabolism and brain function. This analysis involved a more refined 18-level severity scoring system for manganese toxicity due to deficiency and excess developed by Mattison et al. (2016) specifically for manganese. However, the human data was quite sparse and not amenable to inclusion in CatReg. As detailed in Figure S1[1], the JMED model provided a good fit to the animal data, which provided a precise estimate of $x_{\mathrm{MIN}D\cup E}$ as the exposure minimizing the risk of adverse effects due to deficiency and excess combined. Ramoju et al. (2017) undertook another application of CatReg involving a combined analysis of eight epidemiological studies of the neurotoxic effects of manganese following inhalation exposure in occupational environments. This analysis was particularly useful in reconciling differences in environmental and occupational exposure limits set by different regulatory agencies that relied on a single key study, rather than a combined analysis of relevant epidemiological studies. Ramoju et al. (2017) further employed a physiologically-based pharmacokinetic model for manganese developed by Schroeter et al. (2011) to predict manganese concentrations in the globus pallidus, thereby allowing for the description of saturable kinetic processes that affect neural tissue concentrations and the characterization of dose-dependent transitions in manganese neurotoxicity.

3 Conclusion and discussion

Determining optimal intakes of essential elements that can cause toxicity due to both deficiency and excess requires careful consideration of the exposure-response relationships for both deficiency and excess in order to determine an AROI that meets nutritional requirements without exceeding a UL at which toxicity occurs. The modeling of exposure-response relationships that simultaneously characterize the adverse events of deficiency and excess of essential nutrients can be accomplished with nonsymmetric U-shaped exposure-response curves, using recent advances in categorical regression to describe such curves. By using ordered severity categories to calibrate all relevant exposure-response data on multiple outcomes from all available human, animal, and experimental studies on a common severity scale, CatReg is able to define an optimal intake for essential elements that minimizes the joint risks associated with deficiency and excess, based on a single categorical regression model fit to these data.

Looking to the future, new approach methodologies such as those described by Andersen et al. (2019) and Tyshenko (2022) could prove to be of value in strengthening applications of cate-

gorical regression by providing novel data on outcomes of varying severity, including outcomes of moderate to low severity that may be difficult to evaluate in traditional toxicological bioassays. From a 3Rs perspective (Krewski et al., 2022), this could also lessen the reliance on the use of animals in toxicity testing. As a tool designed specifically for the quantitative integration of data on diverse endpoints from multiple evidence streams through the use of an appropriate severity scoring scale, data derived from new approach methodologies would naturally fit within the scope of categorical regression. Sensitive methodologies able to identify biological markers of moderate and low severity would be of particular value in characterizing subtle effects that might be difficult to detect with traditional mammalian toxicity testing approaches, thereby enhancing our ability to define the AROI of essential nutrients.

References

Academy of Medical Sciences (2007). Identifying the environmental causes of disease: How should we decide what to believe and when to take action? https://acmedsci.ac.uk/file-download/34590-IECDWebD.pdf

Aggett, P. J. (2007). Nutrient risk assessment: Setting upper levels and an opportunity for harmonization. *Food Nutr Bull 28, Suppl 1*, S27-S37. doi:10.1177/15648265070281s104

Allen, B., Zeiger, E., Lawrence, G. et al. (2005). Dose-response modeling of in vivo genotoxicity data for use in risk assessment: Some approaches illustrated by an analysis of acrylamide. *Regul Toxicol Pharmacol 41*, 6-27. doi:10.1016/j.yrtph.2004.09.006

Andersen, M. E., McMullen, P. D., Phillips, M. B. et al. (2019). Developing context appropriate toxicity testing approaches using new alternative methods (NAMs). *ALTEX 36*, 523-534. doi:10.14573/altex.1906261

ATSDR – Agency for Toxic Substances and Disease Registry (2000). Toxicological Profile for Manganese (Update). U.S. Department of Health and Human Services, Atlanta (GA). doi:10.15620/cdc:95222

ATSDR (2012). Toxicological Profile for Manganese. U.S. Department of Health and Human Services, Atlanta (GA).

Barnes, D. and Dourson, M. (1988). Reference dose (RfD): Description and use in health risk assessments. *Regul Toxicol Pharmacol 8*, 471-486. doi:10.1016/0273-2300(88)90047-5

Brown, K. G. and Strickland, J. A. (2003). Utilising data from multiple studies (meta-analysis) to determine effective dose-duration levels: Example: Rats and mice exposed to hydrogen sulfide. *Regul Toxicol Pharmacol 37*, 305-317. doi:10.1016/S0273-2300(03)00007-2

Brown, K. G. and Foureman, G. L. (2005). Concentration-time-response modelling for acute and short-term exposures. *Regul Toxicol Pharmacol 43*, 45-54. doi:10.1016/j.yrtph.2005.06.002

Chambers, A., Krewski, D., Birkett, N. et al. (2010). An exposure-response curve for copper excess and deficiency. *J Toxicol Environ Health B Crit Rev 13*, 546-578. doi:10.1080/10937404.2010.538657

Chifman J. and Laubenbacher, R. (2014). A systems biology approach to iron metabolism. *Adv Exp Med Biol 844*, 201-225. doi:10. 1007/978-1-4939-2095-2_10

Crump, K. S. (1984). A new method for determining allowable daily intake. *Fundam Appl Toxicol 4*, 854-871. doi:10.1016/0272-0590(84)90107-6

Davies, K. J. A. (2016). Adaptive homeostasis. *Mol Aspects Med 49*, 1-7. doi:10.1016/j.mam.2016.04.007

Deveau, M., Chen, C. P., Johanson, G. et al. (2015). The global landscape of occupational exposure limits-implementation of harmonization principles to guide limit selection. *J Occup Environ Hyg 12, Suppl 1*, S127-S144. doi:10.1080/15459624.2015.1060327

Edwards, L. M. (2017). Metabolic systems biology: A brief primer. *J Physiol 595*, 2849-2855. doi:10.1113/JP272275

EFSA NDA Panel – EFSA Panel on Nutrition, Novel Foods, and Food Allergens (2019). Scientific opinion on the dietary reference values for sodium. *EFSA J 17*, e05778. doi:10.2903/j.efsa.2019.5778

EFSA NDA Panel (2022). Guidance for establishing and applying tolerable upper intake levels for vitamins and essential minerals. *EFSA J 20*, e200102. doi:10.2903/j.efsa.2022.e200102

Gift, J. S., McGaughty, R., Singh, D. V. et al. (2008). Health assessment of phosgene: Approaches for the derivation of reference concentration. *Regul Toxicol Pharmacol 51*, 98-107. doi:10.1016/j.yrtph.2008.03.004

Haber, L., Strickland, J. A. and Guth, D. J. (2001). Categorical regression analysis of toxicity data. *Comments Toxicol 7*, 437-452.

Health Canada (1994). Risk assessment for the combustion products of methylcyclopentadienyl manganese tricarbonyl (MMT) in gasoline.

Hertzberg, R. C. and Miller, M. A. (1985). A statistical model for species extrapolation using categorical response data. *Toxicol Ind Health 1*, 43-57. doi:10.1177/074823378500100405

King, J. C. and Garza, C. (eds) (2007). International harmonisation of approaches for developing nutrient-based dietary standards. *Food Nutr Bull 28, Suppl 1*, S1-S151. doi:10.1177/15648265070281S101

Krewski, D., Chambers, A., Ransom Stern, B. et al. (2010). Development of a copper database for exposure-response analysis. *J Toxicol Environ Health A 73*, 208-216. doi:10.1080/15287390903340815

Krewski, D., Andersen, M. E., Tyshenko, M. G. et al. (2020). Toxicity testing in the 21st century: Progress in the past decade and future perspectives. *Arch Toxicol 94*, 1-58. doi:10.1007/s00204-019-02613-4

Krewski, D., Saunders-Hastings, P., Baan, R. A. et al. (2022). Development of an Evidence-Based Risk Assessment Framework. *ALTEX 39,* 667-693. doi:10.14573/altex.2004071

Lewis, J. and Dwyer, J. T. (2020). Establishing nutrient intake values. In B. P. Marriott, D. F. Birt, V. A. Stallings et al. (eds), *Present Knowledge in Nutrition* (267-287). Volume 2. London, UK: Elsevier. doi:10.1016/B978-0-12-818460-8.01001-7

Magistrato, A., Pavlin, M., Qasem, Z. et al. (2019). Copper trafficking in eukaryotic systems: Current knowledge from exper-

imental and rotational efforts. *Curr Opin Struct Biol 58*, 26-33. doi:10. 1016/j.sbi. 2019.05.002

Mattison, D., Milton, B., Krewski, D. et al. (2016). Severity scoring of manganese health effects for categorical regression. *Neurotoxicology 58*, 203-216. doi:10.1016/j.neuro.2016.09.001

Mertz, W. (1993). Essential trace metals: New definitions based on new paradigms. *Nutr Rev 51*, 287-295. doi:10. 1111/j.1753-4887.1993.tb03057.x

Milton, B., Farrell, P. J., Birkett, N. et al. (2017a). Modeling U-shaped exposure-response relationships for agents that demonstrate toxicity due to both excess and deficiency. *Risk Anal 37*, 265-279. doi:10.1111/risa.12603

Milton, B., Krewski, D., Mattison, D. R. et al. (2017b). Modelling U-shaped dose-response curves for manganese using categorical regression. *Neurotoxicology 58*, 217-225. doi:10.1016/j.neuro.2016.10.001

NRC – National Research Council (2000). DRI Dietary Reference Intakes: Applications in Dietary Assessment. National Academies Press, Washington, D.C.

NRC (2001). Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academies Press, Washington, D.C. doi:10.17226/10026

NRC (2017). Guiding Principles for Developing Dietary Reference Intakes Based on Chronic Disease. National Academies Press, Washington, D.C. doi:10.17226/24828

Ramoju, S. P., Mattison, D. R., Milton, B. et al. (2017). The application of PBPK models in estimating human brain tissue manganese concentrations. *Neurotoxicology 58*, 226-237. doi:10.1016/j.neuro.2016.12.001

Renwick, A. G., Flynn, A., Fletcher, R. J. et al. (2004). Risk-benefit analysis of micronutrients. *Food Chem Toxicol 42*, 1903-1922. doi:10.1016/j.fct.2004.07.013

Roels, H. A., Ghyselen, P., Buchet, J. P. et al. (1992). Assessment of the permissible exposure level to manganese in workers exposed to manganese dioxide dust. *Br J Ind Med 49*, 25-34. doi:10.1136/oem.49.1.25

Sand, S., Poirier, C. J. and Krewski, D. (2011). A signal-to-noise crossover dose as the point of departure for health risk assessment. *Environ Health Perspect 119*, 1766-1774. doi:10.1289/ehp.1003327

Sand, S., Parham, F. C. J., Portier, C. J. et al. (2017). Comparison of points of departure for health risk assessment based on high-throughput screening data. *Environ Health Perspect 125*, 623-633. doi:10.1289/ehp408

Schroeter, J. D., Nong, A., Yoon, M. et al. (2011). Analysis of manganese tracker kinetics and target tissue dosimetry in monkeys and humans with multi-route physiologically-based pharmacokinetic models. *Toxicol Sci 120*, 481-498. doi:10.1093/toxsci/kfq389

Smeraldi, C., Giarola, A., Aggett, P. J. et al. (2020). Use of mechanistic information to derive chemical specific adjustment factors – Refinement of risk assessment. *Regul Toxicol Pharmacol 117*, 104776. doi:10.1016/j.yrtph.2020.104776

Strickland, J. A. and Foureman, G. A. (2002). US EPA's acute reference exposure methodology for acute inhalation exposures. *Sci Total Environ 288*, 51-63. doi:10.1016/S0048-9697(01)01114-7

Tyshenko, M. G., Willhite, D. Levy, L. et al. (2022). Evolution of the use of toxicological data in evaluating chemical safety. In W. Farland and D. Paustenback (eds.), *Patty's Toxicology*. 7th edition. Wiley, New York, USA.

US EPA – U.S. Environmental Protection Agency (1993). Integrated Risk Information System (IRIS). 1.B. Reference concentration for chronic inhalation exposure (RfC). US EPA (U.S. Environmental Protection Agency) Substance Name: Manganese (CASRN – 7439-96-5).

US EPA – U.S. Environmental Protection Agency (1994). Re-evaluation of inhalation health risk association with methylcyclopentadienyl manganese tricarbonyl (MMT) in gasoline. Office of Research and Development, Environmental Criteria and Assessment Office, Research Triangle Park, NC (EPA 600/R-94/062).

WHO – World Health Organization (1996). Trace elements in human nutrition and human health. Geneva: World Health Organization.

WHO (2000). Manganese, Air Quality Guidelines for Europe. 2nd edition WHO Regional Office for Europe. Copenhagen, Denmark European Series No. 91, 154-156. ISBN 92 890 1358 3.

WHO/IPCS – WHO/International Program on Chemical Safety (2002). Principles and methods for the assessment of risk from essential trace elements (No. 228), World Health Organization. https://apps.who.int/iris/handle/10665/42416WHO

WHO (2006). A model for establishing upper levels of intake for nutrients and related substances. Report of a Joint FAO/WHO Technical Workshop on Nutrient Risk Assessment, World Health Organization.

Yetley, E. A., MacFarlane, A. J. Greene-Finestone, L. S. et al. (2017). Options for basing dietary reference intakes (DRIs) on chronic disease endpoints: Report from a joint US/Canadian-sponsored working group. *Am J Clin Nutr 105*, 249S-285S. doi:10.3945/ajcn.116.139097

Data availability statement

No data sets were generated or analyzed in this study.

Conflict of interest

No external funding was provided for this work. Although not a conflict of interest, three authors (DK, DM, and SR) are affiliated with Risk Science International (www.risksciences.com), which has conducted work on metals (copper and manganese, used as examples of previous applications of categorical regression) for public and private sector clients.