Background Information
IOM/FNB Workshop on Dietary Reference Intakes
The Development of DRIs 1994-2004: Lessons Learned & New Challenges
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Purpose: To Provide Useful/Relevant Information for
Workshop Participants and Attendees

Opportunity for interested parties to comment electronically through August 11, 2007:
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DOCUMENT:

Approximating Dose-Response in the Face of Limited Data

Developed by:
IOM Staff Based on Brainstorming Session

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Background Information: Workshop Topic 2.3
Dose-Response Data: Are There Options for Dealing with Limited Data?

Brainstorming Session
Are There Strategies/Techniques Used for Non-Nutrient Substances That May be Useful in Approximating Dose-Response Relationships for Nutrients?

OVERVIEW

One of the key aspects of the development of Dietary Reference Intake (DRI) values is the determination of the relationship between the level of intake of a nutrient substance and the advent of the "response" of interest, generally either (i) a sign of deficiency or increased risk of a chronic disease, or (ii) an adverse or toxic effect. In short, the goal is to ascertain the "dose-response curve" that reflects the levels of intake at which undesirable conditions occur. With this information, DRI study panels can move on to specify Estimated Average Requirements and Tolerable Upper Intake Levels. One unique aspect of nutrition versus non-nutrient assessment of exposures is that either too little or too much exposure may be cause harm. Thus, there is a need to set both minimal and maximal exposure limits. This could be conceptualized as two dose-response curves or relationships: one for the probability of healthy function (low-nutrient intake D-R) and another for the probability of adverse response (high-nutrient intake D-R).

Often data concerning dose-response relationships for nutrient substances are limited or less than ideal. Since DRI values are needed to help guide policy makers and others in real time, awaiting a complete data set before articulating some type of guidance is often not an option or at best leaves a void into which others may input less scientifically-based values. Therefore, there is considerable interest in ensuring that DRI study panels make the best use of available data and as appropriate incorporate cutting-edge strategies and methodologies for approximating dose-response relationships when data are less than ideal.

Risk assessment for on non-nutrient substances, such as environmental contaminants, pesticides and food additives, have been addressing dose-response relationships for many years. These fields have actively worked to consider strategies for approximating or estimating dose-response relationships with limited data and have led the way in new techniques and approaches. Moreover, fields such as statistics, computer design and data analysis have focused on data enhancement strategies such as meta-analysis for combining results across multiple studies. The expertise and experiences available from these fields of study are likely to have direct application to the enhancement of the DRI development process.

This paper summarizes an informal 4-hour 'brainstorming' session held to highlight the thoughts of toxicologists, biostatisticians/quantitative risk assessors and related scientists concerning strategies they use in the face of limited dose-response data that may be useful to the process of DRI development for nutrient substances. The informal session was organized by Institute of Medicine (IOM) staff; seven persons participated (see Appendix A). This summary of the brainstorming discussion will be used as a background document for an IOM workshop, "*The Development of DRIs: Lessons Learned and New Challenges,*" to be held in September 2007 (www.iom.edu/driworkshop2007).

GENERAL CONSIDERATIONS HIGHLIGHTED BY PARTICIPANTS

● After reviewing the nature of nutrient substances, the participants noted that the study of dose-response relationships gives rise to basically the same questions regardless of whether the focus is endpoints germane to deficiency and reduction of risk for chronic disease or, alternatively, endpoints relative to toxic-type effects. They suggested that the steps of scientific analysis (evaluation and weighing of data, addressing data uncertainties, extrapolation to unstudied groups) would, generally, be **equally applicable** to either set of dose-response situations – determining levels of adequacy or determining levels of excess.

● Given the premise of limited data, it seemed that the term "modeling" dose-response was not entirely appropriate in that modeling generally requires more data than is implied by the "limited data" encountered by DRI study panels. Rather, the preferred term for this purpose would be "approximating" dose response. The participants suggested that approximating dose-response relationships as commonly practiced in the face of limited data is dependent upon the use of **scientific judgment** and that the goal is to ensure that this scientific judgment is **(i) fully informed, (ii) well-reasoned and transparent, and (iii) based on agreed-upon general principles to the extent possible**. Scientific judgment is considered an extremely useful approach for developing good estimates of dose-response relationships given limited data with the proviso that it is recognized to rest on assumptions and that these assumptions must be specified using a rigorous process.

● Participants underscored the importance of working from a **"mode of action" framework** when exercising the needed scientific judgment associated with approximating dose-response relationships. Consistent with a just-released report from the National Research Council of the National Academy of Sciences' (NAS)[1] – a report that the brainstorming participants then used to further outline approaches and strategies for approximating dose-response relationships – participants pointed out that the understanding of mode of action involves studying the mechanistic pathways by which toxic effects are induced, including the key molecular and other biologic targets in the pathways. Some indicated that the cutting-edge aspects of non-nutrient fields of study

[1] National Research Council. 2007. Toxicity Testing in the Twenty-first Century: A Vision and a Strategy. Washington, DC: The National Academies Press (prepublication copy, June 12, 2007).

may include a shift away from the traditional toxicity testing that focuses on demonstrating adverse effects in experimental animals toward a deeper understanding of biological perturbations in key pathways that lead to the adverse outcome

● Nonetheless, participants emphasized that the field of nutrition would initially benefit from increased emphasis on animal models. At a minimum, nutrition might benefit from an incorporation of animal studies in addition to human studies when developing DRIs. Participants noted that in non-nutrient fields the development of animal models to address limited data is advancing rapidly, is reasonably well supported and have proven extremely useful in lieu of human data. They noted that while nutrition science has historically rested on animal and related *in vitro* studies, use of these approaches has slowed noticeably in the last 15-20 years. Participants strongly recommended **increased development of animal models** for the study and articulation of nutrient relationships, and specifically for those related to dose response. It was noted that the use of human data, including observational studies if clinical data are limited, is desirable but that an increased emphasis on animal models and related studies can be highly beneficial to the process. It was recognized that much of the needed data for dose-response relationships for nutrient substances are not easily obtained via human studies given ethical considerations. In particular, it was noted that animal data may be the only available data for conducting assessments. This further highlighted the critical need to focus on other approaches.

POTENTIALLY RELEVANT METHDOLOGIES OUTLINED BY PARTICIPANTS

Some participants highlighted the recent report from NAS entitled ***"Toxicity Testing in the 21st Century: A Vision and a Strategy,"*** specifically Chapter 4 (see Footnote 1 above) as a possible source of methodologies that might be examined in more depth for their relevance to nutrient substances and DRI development in general. The report was in prepublication format at the time of the brainstorming session. It was recognized that while some of the methods may have relevance some, such as the in vitro methods known as high-throughput screening, are likely to be of less value but are included for the purposes of comprehensiveness. A description of methodologies taken from the text of the report is in Appendix B, along with the reference listing for Chapter 4 of the report. Many of the references were considered useful for future considerations for approximating dose-response relationships for nutrient substances, so the entire reference section has been included.

NEXT STEPS

Participants acknowledged that limited data for dose-response relationships are not unique to the field of nutrition. However, it would seem that non-nutrient fields of study have been relatively active in targeting non-human methodologies for addressing dose-response relationships. At the same time efforts are being made in these fields to put in

place recognized principles to make use of scientific judgment to outline dose-response relationships so that public health can be protected even in the face of limited data.

It is therefore logical to first make the standard recommendation that seeking more data is desirable relevant to nutrient substance dose-response relationships. But, participants pointed out the wisdom of specifically directing efforts toward work relative to animal and in vitro methodologies as an important path to the future for nutrient does-response relationships.

Just as importantly, it was recognized that an immediate need is the development of a general set of principles that can be used by persons working to articulate dose-response relationships for nutrients with limited and for whom scientific judgment must come into play. Such principles would be fleshed out over time, but critical first steps can be taken now to outline practical and accountable general strategies germane to nutrition and DRI development. Participants further acknowledged the point made in a 2005 joint report[2] from the Food and Agriculture Organization and the World Health Organization which highlighted the value of combining nutrition science with other disciplines such as toxicology so as to enhance scientific decision-making in the field of nutrition – and that this is best accomplished when a meaningful dialogue among scientists with different expertise is allowed to take place. To that end, participants suggested that a useful next step would be a meeting of scientific experts from nutrition and toxicology and other related non-nutrient fields of study. The goal would be to specifically focus on relevant approximating methodologies for dose-response relationships for nutrient substances, taking into account the needs relevant to determining levels of adequacy as well as levels of excess. The tasks of such a meeting would be first to begin the process of developing general guidelines for approximating dose-response under various relevant scenarios encountered during DRI development and then to focus specifically on methodologies such as those described above with the goal of considering their current relevance to nutrition dose-response approximation and in turn identifying research needs.

[2] Food and Agriculture Organization and World Health Organization, 2005. A Model for Establishing Upper Levels of Intake for Nutrients and Related Substances. Geneva, World Health Organization. http://www.who.int/ipcs/methods/en/.

Appendix A

IOM/FNB Brainstorming Session
June 18, 2007

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Appendix B

Methodologies That Could be Studied for Relevance to Nutrient Substances

Participants highlighted the following as potentially useful in providing information needed for the scientific judgment considerations relevant to approximating dose-response relationships for nutrient substances. Participants stressed that not all the methodologies may be equally applicable to nutrition and substantial further consideration is needed. The descriptions given are from a recent report from NAS entitled ***"Toxicity Testing in the 21st Century: A Vision and a Strategy,"*** specifically Chapter 4[3].

Tools and Technologies for Improved Understanding

♦ Mapping Pathways

The evaluation of perturbations in relevant biological pathways is an important component of newer technologies. "Many tools and technologies are available that can aid in the identification of biologic signaling pathways and the development of assays to evaluate their function. Recent advances in cellular and molecular biology, -omics technologies, and computational analysis have contributed considerably to the understanding of biologic signaling processes (Daston 1997; Ekins et al.
2005). Within the last 15 years, multiple cellular response pathways have been evaluated in increasing depth as is evidenced by the progress in the basic knowledge of cellular and molecular biology (Fernandis and Wenk 2007; Lewin et al. 2007). Moreover, systems biology constitutes a powerful approach to describing and understanding the fundamental mechanisms by which biologic systems operate. Specifically, systems biology focuses on the elucidation of biologic components and how they work together to give rise to biologic function. A systems approach can be used to describe the fundamental biologic events involved in toxicity pathways and to provide evolving biologic modeling tools that describe cellular circuits and their perturbations by environmental agents (Andersen et al. 2005a). A longer-term goal of systems biology is to create mathematical models of biologic circuits that predict the behavior of cells in response to environmental agents qualitatively and quantitatively (Lander and Weinberg 2000). Progress in that regard is being made in developmental biology (Cummings and Kavlock 2005; Slikker et al. 2005)."

The brief listing that follows outlines tools and technologies that will most likely be used to elucidate the critical pathways and to develop assays to evaluate them. Many aspects could be relevant to nutrient substance dose-response relationships.

[3] National Research Council. 2007. Toxicity Testing in the Twenty-first Century: A Vision and a Strategy. Washington, DC: The National Academies Press (prepublication copy, June 12, 2007).

♦ In Vitro Tests

In vitro assays will make up the bulk of the toxicity tests for those fields focused on toxicity. These assays may have relevance for nutrient substances as well. "In vitro tests are currently used in traditional toxicity testing and indicate the success of developing and using in vitro assays (Goldberg and Hartung 2005). In vitro tests include the 3T3 neural red uptake phototoxicity assay (Spielman and Liebsch 2001), cytotoxicity assays (O'Brien and Haskins 2007), skin-corrosivity tests, and assays measuring vascular injury using human endothelial cells (Schleger et al. 2004). Many tests have been validated by the European Centre for the Validation of Alternative Methods. The committee[4] notes that the current in vitro tests originated as alternatives to or replacements of other toxicity tests. In the committee's vision, in vitro assays will evaluate biologically significant perturbations in toxicity pathways and thus are not intended to serve as direct replacements of existing toxicity tests.

The committee envisions the use of human cell lines for the in vitro assays. Cell lines have been used for a long time in experimental toxicology and pharmacology. Human cell lines are readily available from tissue-culture banks and laboratories and are particularly attractive because they offer the possibility of working with a system that maintains several phenotypic and genotypic characteristics of the human cells in vivo (Suemori 2006). Differentiated functions, functional markers, and metabolic capacities may be altered or preserved in cell lines, depending on culture conditions, thereby allowing testing of a wide array of agents in different experimental settings. Other possibilities include using animal cells that are transfected to express human genes and proteins. For example, various cell lines—such as V79, CHO, COS-7, NIH3T3, and HEPG2—have been transfected with complementary DNA (cDNA, DNA synthesized from mature mRNA) coding for human enzymes and used in mutagenesis and drug-metabolism studies (Potier et al. 1995). Individual enzymes have also been stably expressed to identify the major human isoenzymes, such as cytochromes P-450 and UDP-glucuronosyltransferases, responsible for the metabolism of potential therapeutic and environmental agents. The metabolic in vitro screens with human enzymes are usually conducted as a prelude to clinical studies.

A major limitation of using human cell lines is the difficulty of extrapolating data from the simple biologic system of single cells to the complex interactions in whole animals. Questions have also been raised concerning the stability of cell lines over time, the reproducibility of responses over time, and the ability of cell lines to account for genetic diversity of the human population. Nonetheless, cell lines have been used as key tools in the initial screening and evaluation of toxic agents and the characterization of properties of cancer cells (Suzuki et al. 2005) and in gene profiling with microarrays (Wang et al. 2006). The high-throughput methods now becoming more common will allow the expansion of the methods to larger numbers of end points, wider dose ranges, and mixtures of agents (Inglese 2002; Inglese et al. 2006)."

[4] The term "committee" here and elsewhere in this section refers to The Committee on Toxicity and Assessment of Environmental Agents, National Research Council; this committee is the author of the report.

♦ High-Throughput Methods

High-throughput methods can allow economical screening of large numbers of substances in a short period. Its direct application to nutrients needs to be explored. "The pharmaceutical industry provides an example of the successful use of high-throughput methods. Optimizing drug-candidate screening is essential for timely and cost-effective development of new pharmaceuticals. Without effective screening methods, poor drug candidates might not be identified until the preclinical or clinical phase of the drug-development process, and this could lead to high costs and low productivity for the pharmaceutical industry (Lee and Dordick 2006). Pharmaceutical companies have turned to high-throughput screening, which allows automated simultaneous testing of thousands of chemical compounds under conditions that model key biologic mechanisms (Fischer 2005). Such technologies as hybridization, microarrays, real-time polymerase chain reaction, and large-scale sequencing are some of the high-throughput methods that have been developed (Waring and Ulrich 2000). High-throughput assays are useful for predicting several important characteristics related to the absorption, distribution, metabolism, excretion, and toxicity of a compound (Gombar et al. 2003). They can predict the interaction of a compound with enzymes, the metabolic degradation of the compound, the enzymes involved in its biotransformation, and the metabolites formed (Masimirembwa et al. 2001). That information is integral for selecting compounds to advance to the next phase of drug development, especially when many compounds may have comparable pharmacologic properties but differing toxicity profiles (Pallardy et al.1998). High-throughput assays are also useful for rapid and accurate detection of genetic polymorphisms that could dramatically influence individual differences in drug response (Shi et al. 1999)."

♦ Microarrays

"Microarray technologies have allowed the development of the field of toxicogenomics, which evaluates changes in genetic response to environmental agents or toxicants. These technologies permit genomewide assessments of changes in gene expression associated with exposure to environmental agents. The identification of responding genes can provide valuable information on cellular response and some information on toxicity pathways that might be affected by environmental agents. Some of the tools and technologies are described below.

Microarrays are high-throughput analytic devices that provide comprehensive genome-scale expression analysis by simultaneously monitoring quantitative transcription of thousands of genes in parallel (Hoheisel 2006). The Affymetrix GeneChip Human Genome U133 Plus 2.0 Array provides comprehensive analysis of genomewide expression of the entire transcribed human genome on a single microarray (Affymetrix Corporation 2007). Whole-genome arrays are also available for the rat and mouse. The use of the rat arrays will probably increase as the relationships between specific genes and markers on the arrays become better understood.

Protein microarrays potentially offer the ability to evaluate all expressed proteins in cells or tissues. Protein-expression profiling would allow some understanding of the relationship between transcription (the suite of mRNAs in the cell) and the translational

readout of the transcripts (the proteins). Protein microarrays have diverse applications in biomedical research, including profiling of disease markers and understanding of molecular pathways, protein modifications, and protein activities (Zangar et al. 2005). However, whole-cell or tissue profiling of expressed proteins is still in the developmental stage. These techniques remain expensive, and the technology is in flux.

Differential gene-expression experiments use comparative microarray analysis to identify genes that are upregulated or downregulated in response to experimental conditions. The large-scale investigation of differential gene expression attaches functional activity to structural genomics. Wholegenome- expression experiments involve hundreds of experimental conditions in which patterns of global gene expression are used to classify disease specimens and discover gene functions and toxicogenomic targets (Peeters and Van der Spek 2005). Gene-expression profiling will have a role in identifying toxicity pathways in whole-animal studies but is not expected to be the staple technology for identifying and mapping the pathways."

♦ Computational Biology

"Computational biology uses computer techniques and mathematical modeling to understand biologic processes. It is a powerful tool to cope with the ever-increasing quantity and quality of biologic information on genomics, proteomics, gene expression, gene varieties, genotyping techniques, and protein and cell arrays (Kriete and Eils 2006). Computational tools are used in data analysis, data mining, data integration, network analysis, and multiscale modeling (Kitano 2005). Computational biology is particularly useful for systems biology in understanding structural, regulatory, and kinetic models (Barabasi and Oltvai 2004); in modeling signal transduction (Eungdamrong and Iyengar 2004); and in analyzing genome information and its structural and functional properties (Snitkin et al. 2006). Furthermore, computational biology is used to predict toxic effects of chemical substances (Simon- Hettich et al. 2006), to understand the toxicokinetics and toxicodynamics of xenobiotics (Ekins 2006), to determine gene-expression profiling of cancer cells (Katoh and Katoh 2006), to help in the development of genomic biomarkers (Ginsburg and Haga 2006), and to design virtual experiments to replace or reduce animal testing (Vedani 1999). In drug design and discovery, novel computational technologies help to create chemical libraries of structural motifs relevant to target proteins and their small molecular ligands (Balakin et al. 2006; O'Donoghue et al. 2006).

Cellular signaling circuits handle an enormous variety of functions. Apart from replication and other functions of individual cells, signaling circuits must implement the complex logic of development and function of multicellular organisms. Computer models are helpful in understanding that complexity (Bhalla et al. 2002). Recent studies have extended such models to include electrical, mechanical, and spatial details of signaling (Bhalla 2004a,b). The mitogen-activated protein kinase (MAPK) pathway is one of the most important and extensively studied signaling pathways; it governs growth, proliferation, differentiation, and survival of cells. A wide variety of mathematical models of the MAPK pathway have led to novel insights and predictions as to how it functions (Orton et al. 2005; Santos et al. 2007).

Predictive computational models derived from experimental studies have been developed to describe receptor-mediated cell communication and intracellular signal

transduction (Sachs et al. 2005). Physicochemical models attempt to describe biomolecular transformations, such as covalent modification and intermolecular association, with physicochemical equations. The models make specific predictions and work mostly with pathways that are better understood. They can be viewed as translations of familiar pathway maps into mathematical forms (Aldridge et al. 2006). Integrated mechanistic and data-driven modeling for multivariate analysis of signaling pathways is a novel approach to understanding multivariate dependence among molecules in complex networks and potentially can be used to identify combinatorial targets for therapeutic interventions and toxicity-pathway targets that lead to adverse responses (Hua et al. 2006)."

Tools and Technologies for Dose-Response and Extrapolation Modeling

♦ Physiologically Based Pharmacokinetic Models

"Assessing the risk associated with human non-nutrient exposure has traditionally relied on the extrapolation of data from animal models to humans, from one route of exposure to another, and from high doses to low doses. "Such extrapolation attempts to relate the extent of external exposure to a toxicant to the internal dose in the target tissue of interest. However, differences in biotransformation and other pharmacokinetic processes can introduce error and uncertainty into the extrapolation of toxicity from animals to humans (Kedderis and Lipscomb 2001).

PBPK models provide a physiologic basis for extrapolating between species and routes of exposure and thus allow estimation of the active form of a toxicant that reaches the target tissue after absorption, distribution, and biotransformation (Watanabe et al. 1988). However, PBPK results can differ significantly in the hands of different modelers (Hattis et al. 1990), and improved modeling approaches for parameter selection and uncertainty analysis are under discussion. PBPK models might also be useful for estimating the effect of exposure at different life stages, such as pregnancy, critical periods of development, and childhood growth (Barton 2005). Interindividual differences can be incorporated into PBPK models by integrating quantitative information from in vitro biotransformation studies (Bois et al. 1995; Kedderis and Lipscomb 2001).

The more pervasive use of PBPK approaches in the new strategy for toxicity testing will be in basing dosimetry extrapolations on estimates of partitioning, metabolism, and interactions among chemicals derived from in vitro measurements or perhaps even from SAR or QSAR techniques. Those extrapolations will require some level of validation that might require data from kinetic studies in volunteers or from biomonitoring studies in human populations. In the committee's vision for toxicity testing, the development of PBPK models from SAR predictions of partitioning and metabolism would decrease animal use, and continued improvements in in vitro to in vivo extrapolations of kinetics will support the translation from test-tube studies of perturbations to predictions."

♦ Dose-Response Models of Pathways

"Dose-response modeling of toxicity pathways involves the integration of mechanistic and dosimetric information about the toxicity of a chemical into descriptive mathematical terms to provide a quantitative model that allows dose and interspecies extrapolation (Conolly 2002). New techniques in molecular biology, such as functional genomics, will play a key role in the development of such models because they provide more detailed information about the organization of toxicity pathways and the dose response relationships of perturbations of toxicity pathways by environmental agents. Dose-response models have been developed for cell-signaling pathways and used in risk assessment (Andersen et al. 2002). They have found important applications in studying chemical carcinogenesis (Park and Stayner 2006). In particular, models of cancer formation have been developed to describe the induction of squamous-cell carcinomas of the nasal passage in rats exposed to formaldehyde by inhalation, taking into account both tissue dosimetry and the nonlinear effects of cellular proliferation and formation of DNAprotein cross-links (Slikker et al. 2004a, 2004b; Conolly et al. 2004). However, alternative implementations of the formaldehyde model gave substantially different results (Subramaniam et al. 2006). Emerging developments in systems biology allow modeling of cellular and molecular signaling networks affected by chemical exposures and thereby produce an integrated modeling approach capable of predicting dose-response relationships of pathway perturbations by developmental and reproductive toxicants (Andersen et al. 2005b).

In the next decades, the dose-response modeling tools for perturbations should progress relatively rapidly to guide low-dose extrapolations of initial interactions of toxic compounds with biologic systems. The quantitative lineage of early perturbations with apical responses is likely to develop more slowly. For the foreseeable future, the continued refinement of biologic models of signaling circuitry should guide the extrapolation approaches necessary for conducting risk assessment with the toxicity-pathway tests as the cornerstone of toxicity-testing methods."

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