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Modernizing problem formulation for risk assessment necessitates articulation of mode of action

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ABSTRACT

The process of scientific hypothesis formulation affects the experimental designs, methods and interpretations applied, but to be testable, the hypotheses posed must conform to the state of scientific knowledge and available technology. An analogous situation exists in risk assessment, where the questions addressed are typically articulated in the problem formulation phase. Decades ago, regulatory agencies couched problem formulation according to the questions answerable by the science of the day. As regulatory requirements for risk assessment became codified, so too did the rudiments of problem formulation. Unfortunately, codifying problem formulation prevented it from evolving to keep pace with scientific advancements. Today's more advanced science is not always being used effectively and efficiently in risk assessment because the risk assessment problem formulation step still typically poses antiquated questions. Problem formulation needs to be improved so that modern science can inform risk considerations. Based on recent developments in the Human Relevance Framework and using well-studied example chemicals – chloroform and carbon tetrachloride – an approach is proposed for focusing problem formulation on human-relevant hypotheses. We contend that modernizing problem formulation in this way will make risk assessment more scientifically accurate, more practical, and more relevant for protecting human health and the environment.

1. Introduction and problem statement

As all scientists recognize, the hypotheses addressed in a scientific investigation influence the experimental design. In pharmacology and toxicology, experimental design determines from which organs, tissues, or in vitro systems specific types of measurements are made, as well as the conditions under which the measurements are taken, (e.g., route and duration of administration), the necessary control groups, and a variety of other factors. The experimental design also determines the methods used to record and analyze the data and the context in which the results are interpreted. Thus, the results obtained from a scientific investigation are largely dependent upon the questions posed, i.e., the process of hypothesis generation. Fig. 1a provides a simple conceptual diagram of this process.

The questions asked in an experimental investigation are both constrained and empowered by the methodologies and technologies available to address them. As knowledge and technology improves, the hypotheses that can be addressed by experimental science gain sophistication. In pharmacology, for example, X-ray crystallography, computer-assisted molecular modeling, site-directed mutagenesis, pharmacogenomics, bioinformatics, and other technological advancements have expanded drug development beyond the observational screening of natural products to include targeted molecular design based on mechanisms of action and conformational knowledge of receptor and enzyme active sites. By taking advantage of advancements in technology, pharmacologists can now pose and answer questions far more relevant to understanding and treating disease processes than was possible just a few decades ago. In similar fashion, toxicological research has shifted rapidly since the 1960s from a science able to focus only on the descriptive characterization of adverse effects to one capable of probing the mechanisms underlying them (Hodgson, 2012). Toxicological research is now focused, more than ever before, on applying knowledge of potential modes of action (MoAs) to predict the types of adverse effects possible, or

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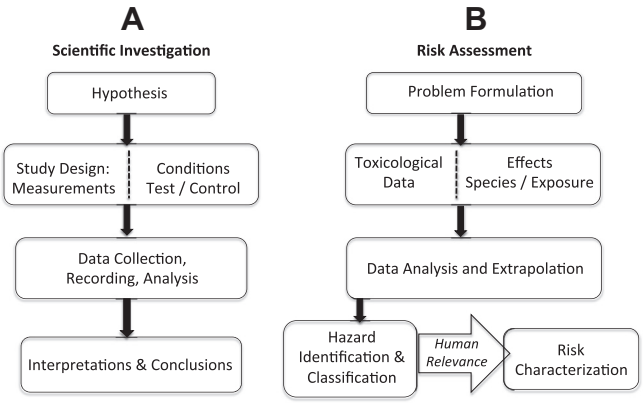

Fig. 1. Logic sequence used for scientific investigations (A) versus for hazard identification and risk assessment (B).

impossible, under various exposure conditions (Hodgson, 2012; Meek et al., 2014a).

The importance of hypothesis generation and study design is well recognized in experimental sciences, but these concepts are less well appreciated as organizing principles in risk analysis despite their critical role in determining how information is evaluated and integrated to reach overall conclusions regarding hazard and risk. As illustrated in Fig. 1b, toxicological risk assessment employs a process analogous to experimental hypothesis testing whereby the questions to be addressed are identified in an initial step called "problem formulation." Components of problem formulation include determining the assessment objectives, defining endpoints to be evaluated, developing a conceptual model and integrating these into the protocol that will guide the assessment itself. Similar to experimental hypotheses, the questions posed in problem formulation determine the data evaluated, which in turn influences the methods used to analyze the data and the interpretations drawn from those analyses.

Recognizing that the scientific quality and applicability of information produced by risk assessment depends on the problem formulation step, the U.S. National Academy of Sciences recently recommended that problem formulation also include consideration of the available risk mitigation strategies for exposures of concern (NAS, 2009). Just as the available technology influences the hypotheses that can be addressed in experimental sciences, the NAS recommendations implicitly recognize that the available risk mitigation technologies influence the problem formulation step of risk characterization. The NAS recommendations also indicate the need for problem formulation to involve a comparison of competing or alternative hypotheses regarding how best to evaluate potential risks and to mitigate potential risks, given the available technologies and approaches.

Although the NAS recommendations encourage a modernization of the risk characterization phase of risk assessment, the initial step of risk assessment, hazard characterization (hazard identification and dose response analysis), has not been explicitly addressed. For the most part, current processes used by regulatory agencies for identifying chemical hazards are still focused on broad, qualitative questions about chemical effects that occur up to the Maximum Tolerated Dose (MTD), consistent with the scientific methods developed when hazard identification methods were established and standardized for regulatory purposes nearly 40 years ago. To streamline the discussion here, human carcinogenic risk assessment will be highlighted, but the conceptual principles apply to all types of toxicological risk assessment, regardless of the type or mechanism of toxicity.

In its 1976 Interim Cancer Assessment Procedures (EPA, 1976), the U.S. Environmental Protection Agency (EPA) considered a two-step process with regard to the regulation of a potential carcinogen, the first being the decision as to whether a particular substance poses a cancer risk. To decide that question, the Agency specified that a substance would be considered a presumptive cancer risk when it causes a statistically significant excess incidence of benign or malignant tumors in humans or animals, and acknowledged that in most instances, the evidence is limited to animal studies. This approach still drives the determination of a cancer hazard by the International Agency for Research on Cancer (IARC, 2006). In fact, many programs, including EPA's Integrated Risk Information System (IRIS), the National Toxicology Program (NTP) Report on Carcinogens and IARC formulate the toxicological problem (hypothesis) in very broad terms. For example, the current NTP classification criteria state that a chemical is reasonably anticipated to be a human carcinogen based on "... *sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset,* ..." (NTP, 2015).

Problem formulations framed in this way allow focusing on a default, surrogate test species rather than on the species of concern. Furthermore, even though humans are the species of concern, the conditions of human exposure to chemicals are not considered explicitly in formulating the critical question for hazard characterization. The methods that logically follow from questions posed in this way – lifelong exposure near the MTD – were developed to enhance statistical power, but employed the overly simplistic, and arguably demonstrably false, assumption that administration of high doses of a chemical to maximize tumor incidence among a small number of animals (e.g., 50 animals per treatment group) is a scientifically valid substitute for using sufficient group sizes to detect low-incidence events from lower, environmentally relevant chemical doses (Gaylor, 2005; Freedman and Zeisel, 1988).

The case can be made that such practices were excusable before probative mechanistic studies and exposure modeling techniques were available. However, modern experimental science continues to reveal that those methods are largely, or in many cases, entirely irrelevant for the risk assessment goal of quantifying human health risk at environmentally relevant levels of exposures. To list just a few of the reasons: lifetime administration of the MTD is typically thousands to millions of times higher than environmental exposures; toxic effects in rodents can be due to species-specific mechanisms; and cross species predictability may be poor even among rodents (Freedman et al., 1996), much less between rodent and humans (Goodman and Wilson, 1991; Freedman and Zeisel, 1988). Mechanisms operating at high doses may not be occurring at lower, environmentally relevant doses, and detoxification processes operating at lower doses may be overwhelmed at higher doses. Opportunities for DNA repair that are abundant at lower doses may be lost as toxicity impairs normal cellular processes at high doses.

Furthermore, were group sizes increased to 200 animals, nearly all chemicals would be expected to produce cancer in some organ or tissue in standard rodent carcinogenicity tests (Gaylor, 2005). Rather than distinguishing between true rodent carcinogens and non-carcinogens, these bioassays are simply failing to detect the weaker carcinogens at the MTD with 50 animals per dose group (Gaylor, 2005). Consequently, so-called rodent carcinogenicity tests are more likely to identify the dose of a chemical at which toxicity produces tumors secondary to the cascade of cell damage-replication-repair rather than detecting chemicals with a unique carcinogenic property (Goodman et al., 1991; Gaylor,