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An ongoing journey toward a risk-based testing in genetic toxicology

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Abstract

The primary focus of genetic toxicology testing has been to predict rodent carcinogens using test batteries to qualitatively bin substances into genotoxic or non-genotoxic categories. There has been little interest in understanding the full dose response in order to identify a point of departure (PoD) value for quantitative risk assessment. This is due to the prevailing paradigm that mutagens have no thresholds. Furthermore, mutagenicity in and of itself was largely ignored as a relevant endpoint for risk assessment purposes. In recent years, however, genetic toxicologists have embarked on a journey to explore opportunities to broaden the utility of genetic toxicology information for human safety assessment. This commentary examines some of these opportunities, including the potential establishment of permitted daily exposures based on the PoD estimated from the mutagenicity data.

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Dose-response, Risk assessment, Mutagen, Carcinogen, Genetic disease.

1. Introduction

Genetic toxicology testing is an essential component in the safety evaluation of substances belonging to various classes such as pharmaceuticals, pesticides, industrial chemicals, food contact materials, and personal care products. The primary goal of this testing is to protect humans from exposure to environmental mutagens. The emphasis has historically been on testing synthetic substances with relatively little attention to naturally occurring chemicals such as toxicants in the plants. Results from the testing strategies have been utilized to qualitatively bin substances into genotoxic and non-genotoxic categories. In general, there has been relatively little consideration to define a no-adverse-effect-level (NOAEL) or acceptable dose level because genotoxicity was believed to have no thresholds. The following commentary addresses a few opportunities in the testing philosophy that need re-examination/refinement as the field moves ahead into the next decade. This commentary is meant to be a forward look rather than finding fault with the past. Genetic toxicologists made significant contributions in preventing the entry of mutagens into the human environment. In this process, they have gained considerable experience on the strengths and limitations of the testing approaches. However, along the way, there was also a recognition of the missed opportunities because of the narrow fixation to use the testing strategies to predicting carcinogens.

2. Genotoxicity and thresholds

Over the years, it has become increasingly apparent that genotoxicity is not an exclusive property of certain manmade substances, but that this activity is also widely distributed among naturally occurring chemicals [1]. Furthermore, extensive testing experience has shown that genotoxicity is often a conditional property and most substances demonstrate genotoxicity under certain set of experimental conditions. Such conditions include the choice of the test system, cell cultures used, endpoint enumerated, dose levels tested, and the external metabolic activation system employed. Thus, it was no longer a simple exercise of preventing the introduction of a few genotoxicants into the environment, instead having to deal with a deluge of data, often contradictory, in making the qualitative decision concerning genotoxicity.

In recent years, a section of genetic toxicologist community started questioning the practice of qualitative data assessment and initiated efforts to examine opportunities for better utilization of the information from these studies to enable human risk assessment [2,3]. In particular, the belief that genotoxicants have no thresholds was re-examined following the demonstration of biologically plausible threshold mechanisms for spindle poisons and chemicals that induce nucleotide pool imbalances. A look back into the history reveals that Dr. John Ashby, one of the thought leaders in the field, pondered on this issue several decades ago and he speculated the existence of threshold for organic genotoxic chemicals; he further thought that there would be a dose below which the risk implicit in exposure becomes negligibly small, or even zero [4]. He further

envisioned that the threshold values should be an integral part of the risk estimation process. He realized that the above concepts were not sustainable at the time and hoped that they would have a role to play in the future.

The future is already here, and nothing is likely to change unless there is a concerted data-driven effort to bring about the type of change envisioned above. Demonstrating thresholds is a very difficult task, especially for DNA reactive genotoxicants, no matter how extensive the database is, since the response observed at the lower end of the dose-response curve is compatible with both threshold and non-threshold models [5,6]. As pointed out in 1978 by Gehring and Blau [7], this threshold debate is not likely to be resolvable in the near future since "proponents and opponents will argue their cases similar to those arguing religion."

3. Quantitative risk assessment for mutagenicity

Quantitative risk assessment approaches provide the opportunity to establish acceptable exposure levels, without having to prove threshold values. The first question in this context is whether genotoxicity indeed is an adverse outcome to qualify for risk assessment. In answering this question, it is important to distinguish between genotoxicity and mutagenicity. Mutagenicity is a permanent and heritable change in the structure and content of the genetic material. Genotoxicity, on the other hand, includes a wide range of indicator endpoints (e.g., DNA adducts, DNA strand breaks, sister chromatid exchanges, DNA repair, etc.) which may or may not lead to mutagenicity. Thus, only certain genotoxicity tests are capable of assessing mutagenicity. From a risk assessment standpoint, mutagenicity should be the endpoint of concern. An adverse outcome can be defined as "a specialized type of key event that is generally accepted as being of regulatory significance on the basis of correspondence to an established protection goal or equivalence to an apical endpoint in an accepted regulatory guideline toxicity test" [8]. There is little doubt that somatic and germ cell mutations in certain genes are key events along the pathway to carcinogenesis. There is also a large body of evidence implicating mutations to other disease outcomes. Thus, mutations mostly are adverse, so this endpoint could be used for assessing risk. Currently, however, qualitative mutagenicity information is used in deciding whether to use a linear or non-linear risk assessment approach for carcinogens. The dose-response is seldom taken into consideration in the above assessment. This is one of the opportunities for a change and considerable collaborative efforts are currently in progress. It is hoped that these efforts would have an impact on the use of genetic toxicology data in a regulatory context.

In order to realize the above objective, the purpose of genetic toxicology studies should no longer be just to predict cancer outcomes in animal bioassays. Such predictions have mostly yielded rather disappointing results anyway and the result is unlikely to change with further tweaks to the testing battery. Instead, genetic toxicology studies should be designed to facilitate risk assessment to protect human population from somatic and heritable adverse effects. A look back into the history reveals that the roadmap for this type of approach was already laid out by the pioneers in the field. The International Committee for Protection against Environmental Mutagens and Carcinogens (ICPEMC) attempted to address the above issue almost 40 years ago by establishing a committee to evaluate risk estimation procedures for mutagenic chemicals and to develop practical exposure limits [9]. At their first meeting in 1978, the committee identified the following priority tasks: a) dose-response relationships, definition of dose, and pharmacokinetics, b) approaches for species to species and cell type to cell type extrapolation, c) methods for the identification of genetically significant dose, and d) estimation of resultant genetic damage. The concepts espoused by the leaders in the field are as valid then as they are today, although not much has changed in the ensuing 40 years and the field continued to operate in a screen and bin mode.

As stated earlier, there is now an interest to instill some fundamental changes to the philosophy of genetic toxicology testing so that the field stays current vis-à-vis scientific developments in genomic sciences and remains relevant to protecting human health. This next generation strategy envisions that tests for genotoxicity, or more broadly genomic damage, should refocus on assessing risk rather than simply evaluating the hazard [10]. As is the case with several other toxicology disciplines, identification of a point-of-departure (PoD) or NOAEL should be one of the objectives of the study design. The PoD is then used to determine permitted daily exposures (PDE) by applying appropriate uncertainty factors [5,6,11,12]. The PoD can also be used to determine margin of exposure (MoE) in order to set priorities for further testing or regulatory action. Further investigations to establish the mode of action responsible for mutagenicity could in some cases lead to the refinement of the uncertainty factors in calculating the PDE/MoE.

In vivo mutagenicity studies are ideal for establishing PDE/MOE. There are currently several validated protocols whereby the mutagenicity endpoints (e.g., Pig-a mutations and cytogenetic abnormalities) can be integrated into a repeat dose toxicology study, thus avoiding or minimizing the use of animal resources. It is acknowledged that stand-alone in vivo mutagenicity