Genetic Toxicology at the Crossroads – From Qualitative Hazard Evaluation to Quantitative Risk Assessment.

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Abstract

Applied genetic toxicology is undergoing a transition from qualitative hazard identification to quantitative dose-response analysis and risk assessment. To facilitate this change, the Health and Environmental Sciences Institute (HESI) Genetic Toxicology Technical Committee (GTTC) sponsored a workshop held in Lancaster, UK July 10-11, 2014. The event included invited speakers from several institutions and the contents was divided into 3 themes – 1, Point-of-departure Metrics for Quantitative Dose-Response Analysis in Genetic Toxicology; 2, Measurement and Estimation of Exposures for Better Extrapolation to Humans; and 3, The Use of Quantitative Approaches in Genetic Toxicology for Human Health Risk Assessment (HHRA). A host of pertinent issues were discussed relating to the use of in vitro and in vivo dose response data, the development of methods for in vitro to in vivo extrapolation, and approaches to use in vivo dose-response data to determine human exposure limits for regulatory evaluations and decision-making. This Special Issue, which was inspired by the workshop, contains a series of papers that collectively address topics related to the aforementioned themes. The Issue includes contributions that collectively evaluate, describe, and discuss in silico, in vitro, in vivo and statistical approaches that are facilitating the shift from qualitative hazard evaluation to quantitative risk assessment. The use and application of the benchmark dose (BMD) approach was a central theme in many of the workshop presentations and discussions and the Special Issue includes several contributions that outline novel applications for the analysis and interpretation of genetic toxicity data. Although the contents of the Special Issue constitute an important step towards the adoption of quantitative methods for regulatory assessment of genetic toxicity, formal acceptance of quantitative methods for HHRA and regulatory decision-making will require consensus regarding the relationships between genetic damage and disease, and the concomitant ability to use genetic toxicity results *per se*.

Genetic toxicity assessment for regulatory evaluations and decision-making has traditionally been restricted to hazard identification, with test results only used to identify genotoxic substances via the establishment of dichotomous substance groupings (i.e., genotoxic or non-genotoxic). This so-called "screen and bin" approach to applied genetic toxicology has been criticised and many researchers have called for recognition of genetic toxicity as a relevant *bona fide* toxicological endpoint, transition from qualitative hazard identification to quantitative dose-response analysis that employs mathematical point-of-departure (PoD) determination and, finally, extrapolation below the PoD for human health risk assessment (HHRA) and regulatory decision-making (1-6). Indeed, many members of the applied genetic toxicology community worldwide now agree that genetic toxicology should move away from qualitative hazard identification and embrace quantitative approaches to enhance the interpretation of test results. More importantly, many stakeholders agree that the genetic toxicology community needs to establish a framework to facilitate quantitative use of genetic toxicity test results in regulatory evaluations, HHRA, and regulatory decision-making. Although recent publications (7-10) constitute substantial steps towards establishing a sound premise for the development of quantitative methods and, moreover, towards advancement of quantitative methods in genetic toxicology, there is general agreement that acceptance of quantitative methods will require periodic evaluations of the state of the science to identify knowledge gaps and to delineate steps towards the development of a formal framework for quantitative use of genetic toxicity dose-response data in a regulatory setting.

In late 2013 several Genetic Toxicology Technical Committee (GTTC) members, including the chairs of the GTTC Quantitative Analysis Workgroup (i.e., Johnson, Gollapudi, White), agreed that it would be prudent to hold an international workshop to bring together experts from several toxicology sub-disciplines (e.g., genetic toxicology, molecular toxicology, applied toxicology and risk assessment, computational toxicology) to survey the state of the science regarding quantitative analyses of genetic toxicity dose-response data, to identify and evaluate noteworthy knowledge gaps and related requirements for further research and, finally, to issue recommendations for a path towards adoption of quantitative methods. The workshop, which was sponsored by the Health and Environmental Sciences Institute (HESI) of the International Life Sciences Institute (ILSI) in Washington, DC (USA), was held in Lancaster, UK July 10-11, 2014. The workshop included invited speakers from several institutions, and the contents divided into 3 themes – 1, Point-of-departure Metrics for Quantitative Dose-Response Analysis in Genetic Toxicology; 2, Measurement and Estimation of Exposures for Better Extrapolation to Humans; and 3, The Use of Quantitative Approaches in Genetic Toxicology for HHRA. Each thematic portion of the workshop included a plenary presentation, several short invited presentations and, finally, a discussion period to address targeted questions that were distributed in advance. The discussion questions addressed pertinent issues pertaining to the selection and use of PoD metrics for regulatory evaluations of genetic toxicity dose-response data, the utility of in vitro dose-response data and the development of effective methods for in vitro to in vivo extrapolations, and extrapolations from in vivo dose-response data to determine human exposure limits for regulatory decision-making. The complete workshop prospectus is available online at http://www.hesiglobal.org/i4a/pages/index.cfm?pageID=3647.

This Special Issue of Mutagenesis, which addresses a variety of topics related to the quantitative analysis of genetic toxicity dose-response data, was inspired by the aforementioned workshop. This collection of 13 manuscripts is not meant to be a workshop report *per se*, but rather contains a series of papers that collectively address topics related to the workshop themes. Authors of several of the papers attended the workshop and their contributions are based on their invited

presentations (11-16). Other authors' contributions constitute thematic variations on material presented at the workshop (17) with the remaining articles comprising invited works that address issues related to quantitative analysis of genetic toxicity dose-response data (18), applications of quantitative methods for data analysis and interpretation (17,19-22) and the development of tools for quantitative analysis of dose-response data (23).

Several statistical approaches can be used to define dose-response PoD metrics, and the works by Gollapudi et al. (7), Johnson et al. (8) and MacGregor et al. (9,10) used published in vitro and in vivo dose-response data to assess the suitability of various approaches. Gollapudi et al. (7) and Johnson et al. (8) noted the limitations of the bilinear model approach used by other authors (24,25), and Johnson et al. (8) also noted the limitations of other non-linear modelling approaches that employ R algorithms (26). In contrast, the BMD (Benchmark Dose) approach, which has been employed for analysis of toxicological dose-response data for over 30 years (27), consistently provided suitable metrics. The Johnson et al. work (8) extended the analyses by illustrating how the BMD lower confidence limit (i.e., the BMDL) could be used to define a human exposure limit. This framework for quantitative use of genetic toxicity data in a regulatory context, which is based on the previously published AI (acceptable intake) and PDE (permitted daily exposure) concepts (28,29) is extensively discussed in MacGregor et al. (10). Although that work primarily focused on in vivo endpoints, the authors acknowledge the utility of in vitro and in silico data for certain applications.

There is now general acceptance that the BMD approach constitutes the most effective and flexible strategy for deriving dose-response PoDs. This acceptance, which necessitates rejection of threshold-based approaches that have been the subject of much debate, allows genetic toxicologists to benefit from recent advances in BMD modelling, such as the BMD covariate approach that employs simultaneous non-linear analysis of several dose-response functions with conserved shape. Nevertheless, determination of the appropriate benchmark response or critical effect size (i.e., BMR or CES) for each genetic toxicity endpoint will be required prior to routine application of BMD-based approaches. In their contribution to this Special Issue, Zeller et al. (18) acknowledge the need to address BMR determination, and propose a method that incorporates the strategies currently employed to define positive responses into the determination of endpoint-specific BMRs. Although their work initiates important discussions related to BMR definition, it will be necessary to carefully consider the influence of dose-response shape, and concomitant variations in non-linear parameters across endpoints and compounds, on the determination of appropriate BMRs for genetic toxicity endpoints. The noteworthy work of Slob and Setzer (30) suggests that determinations of endpoint-specific BMRs must consider the dynamic range and maximum response for each endpoint. Since the establishment and adoption of a framework for adoption of quantitative methods in genetic toxicology requires definition of endpoint-specific CES/BMR values, there is a critical need to design and implement research activities that address this issue. Moreover, there is a pressing need for research aimed at defining uncertainty factors for genetic toxicity endpoints that can be employed to calculate human exposure limits analogous to metrics such as the TDI (Tolerable Daily Intake).

A noteworthy benefit of the BMD approach for chemical evaluation, and more specifically, the aforementioned covariate approach for simultaneous analyses of numerous dose-response functions, is the ability to rank potencies, delineate compound groupings, and/or evaluate MOA (mode of action) hypotheses. The first Wills et al. contribution (22) in the Special Issue employs analyses of in vitro dose-response data to illustrate these applications, with the follow-up contribution illustrating how use of the covariate approach, and concomitant improvements in BMD precision, can permit more efficient use of experimental animals in genetic toxicology.

The acceptance and established utility of the BMD approach necessitates the creation of a user-friendly platform for dose-response analyses and determination of PoD metrics. The Avancini et al. contribution (23) describes an online platform and associated tools that readily permit the determination of several PoDs metrics. The tools, which are available at MutAIT.org, are easily implemented using a web browser and alleviate the need to employ R scripting (23). The improvement in accessibility and ease of use constitutes an important step towards the adoptions of quantitative dose-response analyses in genetic toxicity and, moreover, the eventual use of PoD metrics, such as the BMDL, for HHRA and regulatory decision-making.

The aforementioned MacGregor et al. works (9,10) note that, at least for the time being, convincing extrapolations from genetic toxicity PoD metrics for HHRA and regulatory decision-making are restricted to in vivo endpoints such as micronuclei or gene mutation. Indeed, as already noted, in vivo genetic toxicity dose-response data are already being used to determine human exposure limits (i.e., AI or PDE) for pharmaceutical impurities. An analogous approach is being employed to evaluate the MOE (Margin or Exposure) for carcinogens in food. The contribution by Benford (11) indicates that for evaluations of substances in food, PoD metrics (e.g., BMD) derived from carcinogenicity dose-response data are routinely compared with human exposure estimates to determine MOEs that form the basis for regulatory action. Although the approach is currently limited to rodent carcinogenicity data, Benford comments on the benefits of conducting similar analyses with genetic toxicity dose-response data, particularly in cases where carcinogenicity data are not available. This pragmatic extension of the paradigm already employed to assess the likelihood of carcinogenic effects will be particularly valuable when regulatory decisions are urgently required and additional testing is not practical or permissible. The contribution by Soeteman-Hernandez et al. (15) also highlights the regulatory utility of carcinogenicity assay results, and employs quantitative comparisons of in vivo genotoxic potency (i.e., BMDL) to carcinogenic potency to develop empirical models to predict the latter from the former. Although the relationships presented are based on a restricted set of substances, the work provides a promising empirical framework to predict carcinogenic potency in instances where only in vivo genetic toxicity dose-response data are available. The related contribution by Fukushima et al. (13) discusses the empirical trend in PoD values across a series of temporally-arrayed endpoints between genetic damage and cancer. Although the analysis is restricted to only 3 hepatocarcinogens and it is not clear whether cross-endpoint comparisons of PoDs associated with the same BMR/CES is mathematically robust, the analyses present a compelling pattern of effect progression from initial genetic damage through mutation, preneoplastic lesion and hepatocellular carcinoma. Thus, the Soeteman-Hernandez et al. and Fukushima et al. contributions (13,15) provide a context for the interpretation of genetic toxicity PoDs; and moreover, regulatory metrics (e.g., human exposure limits or MOEs) derived from genetic toxicity PoDs. Although the latter cannot be used to evaluate the likelihood that an apical endpoint of concern (e.g., cancer) will be manifested, the establishment of robust quantitative relationships between genotoxic potency and the ability to induce disease endpoints should facilitate adoption of regulatory metrics derived from genetic toxicity data and, furthermore, their use to reduce the risk of undesirable genetic effects and their consequences. Although these types of analyses need to be expanded, it is reasonable to assert that regulatory decisions that effectively reduce the likelihood of undesirable genetic damage will concurrently reduce the risk of frank adverse effects such as cancer and other diseases linked to genetic damage.

In contrast to in vivo data, current use of PoD metrics from in vitro dose-response data are largely restricted to potency comparisons and evaluations of MOA hypotheses. Nevertheless, there is

general recognition that ongoing developments of in silico and in vitro methods; and moreover, approaches for in vitro to in vivo extrapolations (IVIVE) are poised to have an important impact on the utility of in vitro endpoints for HHRA and regulatory decision-making (31-34). Researchers affiliated with the Hamner Institute, an institution with an established reputation in the development of in silico tools for regulatory toxicology, are refining IVIVE approaches that will ultimately permit the use of in vitro dose-response data to establish human exposure limits and/or margins or exposure (12). The benefits of these approaches are obvious, and indeed, they can be applied to many quantitative aspects of regulatory toxicology (e.g., determination of Cmax from in vitro data).

In vitro-in vivo correlations (i.e., IVIVC) that relate in vitro properties to in vivo effects have been used for drug development for almost 20 years (35) and their applications in applied toxicology are undergoing rapid development (36,37). The contribution by Sahota et al. (14) provides an update on the latest in vitro to in vivo extrapolations and pharmacokinetic and pharmacodynamic (PBPK) modelling, emphasising recent improvements in in silico modelling. Although there is increasing acceptance of *in silico* approaches, particularly for genotoxic impurities, general acceptance by regulatory authorities is lacking. Therefore, although in silico tools are now available, their perceived complexity has limited their current use in regulatory submissions. The inherent complexity of current in silico tools can alternatively be viewed as a strength, and more uniform adoption, which is likely unavoidable, will require effective knowledge transfer, training and education.

Although several noteworthy publications already profess that genetic damage is a relevant toxicological endpoint that can be quantitatively employed for HHRA and regulatory decision-making and, moreover, that the ability to induce genetic effects such as mutation and chromosomal abnormalities in somatic and/or germ cells is empirically and mechanistically related to adverse (i.e., disease) outcomes such as cancer and heritable genetic diseases, it is important to acknowledge herein that genetic damage in and of itself is not an apical endpoint. Rather, using the recently-developed AOP (Adverse Outcome Pathway) terminology (38), genetic effects such as mutation and chromosomal damage are key events that precede apical disease endpoints. Thus, prior to routine implementation of quantitative analyses of genetic toxicity dose-response data for HHRA and regulatory decision-making, it will be necessary to convince the regulatory community that decisions based on genetic toxicity data can effectively reduce the risks of adverse outcomes known to be associated with genetic effects such as mutation and chromosome damage (e.g., cancer, heritable genetic diseases, and diseases associated with somatic mosaicism). Such diseases, which themselves would be considered apical endpoints, are known to be mediated by the aforementioned types of genetic damage, as well structural chromosome alterations that cause copy number variants (CNV) and copy-neutral rearrangements such as inversions and translocations (39-42). Nevertheless, moving forward with formal adoption of quantitative analyses of genetic toxicity dose-response data for regulatory decision-making will require rigorous consideration of several issues. For example, if quantitative analysis of genetic toxicity data is to be used to effectively reduce the risks of adverse human effects, how many tissues need to be examined in experimental models? Moreover, if the risk of diseases associated with somatic mosaicism are to be minimised, will this necessitate in utero assessments to capture sensitive developmental stages? Finally, what assessment endpoints would be required to effectively capture all events associated with disease outcomes (e.g., mutations, CNVs, recombination, LOH, etc.)? Critical discussions of these issues are already underway, and several of the authors featured in the Special Issue (e.g., Johnson, White, Heflich) will be presenting an elaboration in a forthcoming publication.

Although the preceding discussions and, indeed, the content of the Special Issue in general, describe and discuss the use of quantitative dose-response data for recognised genotoxic endpoints such as

DNA damage, mutation and chromosomal aberrations, formal adoption of an AOP approach to rigorously connect genetic damage and disease may provide an opportunity to employ a broader range of endpoints for quantitative assessment of relevant effects. These may include changes in gene expression or other high-content molecular phenotypes that are currently being employed for high-throughput chemical screening. Such methods are currently undergoing rapid development and validation (36,43-45).

Moving forward, advancement of the transition of applied genetic toxicology from hazard evaluation to risk assessment will necessitate engagement of academics, industrial and governmental research scientists, and regulatory authorities in several countries (e.g., Canada, USA, European Union, Switzerland, Japan) to identify impediments to routine quantitative use of genetic toxicity dose-response data for regulatory evaluations, HHRA and regulatory decisions. Under the auspices of the GTTC, plans are already underway for a series of workshops to address remaining issues, identify persistent knowledge gaps, and delineate steps to establish a formal framework for quantitative use of genetic toxicity data for regulatory evaluations and decisions.

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