Need for a New Approach to Genetic Toxicity Assessment: Lessons Learned and New Opportunities

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Regulatory Genetic Toxicology Begins in the 1970’s

- EPA and FDA testing batteries adopted
- Need for *in vivo* risk assessment recognized
- **DHEW Subcommitte on Environmental Mutagenesis (1974-77)**
  - “It is not sufficient to identify substances which may pose a genetic hazard to the human population.”
  - “…it is necessary to obtain quantitative data from relevant animal model systems from which extrapolation to humans can be made to predict virtually safe or tolerable levels of exposure.”
Thinking changed significantly by the end of the 1970’s

McCann and Ames (PNAS, 72: 5135-9, 1975):
Carcinogens are mutagens
- Perception that mutagens & carcinogens are rare & simple screening can identify them & eliminate exposure
- Cancer became the main health consequence of concern, especially at FDA
- Regulatory testing and decision-making based largely on qualitative test outcomes
Only recently has it been recognized that regulation based on qualitative test outcomes is inadequate

- Thybaud et al., Mutation Research 633: 67-79, 2007: We need to move to a more quantitative risk assessment paradigm
- FDA, 2006: overall weight of evidence emphasized, but quantitative approach still not endorsed
- CHMP, 2006: Threshold of toxicological concern recognized for genotoxic pharmaceutical impurities
- ICH, S2(R1) proposed genetic toxicology testing revision: more weight on *in vivo* outcomes
Regulation based on qualitative outcomes of *in vivo* tests is also inadequate

- For example, limiting exposure of agents giving positive mutagenic effects *in vivo* would lead to the following case
Fig. 1. Mutation frequency of \textit{cII} in liver (\(\square\)) and colon (\(\blacklozenge\)) in the four groups. \textit{Bars} represent means, and \textit{lines} denote 1 SD. Groups that are significantly different (\(P < 0.05\)) from the control group (group 1) are denoted with a \textit{star}. 

mutation frequency (per \(10^6\) phages)

\begin{tabular}{lcccc}

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Group & Ctlr & low & med & high \\
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Note that, in the preceding example:

- The route is relevant (dietary administration at a non-toxic level)
- The result clearly shows genotoxicity under “relevant” test conditions
- The test substance is sucrose¹

The HESI IVGT Initiative

• Recognition that *in vitro* hazard screening is insufficient led to HESI IVGT initiative in 2005

• Early consensus (June 2006): Quantitative dose-response & exposure information could contribute to better risk categorization. Useful to determine:
  • If *in vitro* potency in mammalian cell assays and projected human exposure can be used to categorize risk into broad levels
    – For direct-acting agents only, or can metabolism can be taken into account? Only for agents with expected thresholds? etc.
  • If analysis of dose-response parameters and/or benchmark doses *in vivo* can be used to identify acceptable margins of exposure
    – Does this depend on mechanism of action (*e.g.*, DNA-reactive agents vs. “indirect” mechanisms)?
June 2007: work group formed to develop a decision tree based on the existing 2007 IWGT framework

- “Quantitative Subgroup” charged to develop quantitative approaches to support the decision tree

- Need for support to develop a database for the needed quantitative analyses led to application to Health Canada to support a collaboration to:
  - Develop the database, conduct analyses, and support additional laboratory work
  - Grant was approved and funded in August 2008
Health Canada Grant

• 3 yr grant to develop improved understanding of the relationship between *in vitro* and *in vivo* genetic toxicology assays
  – HESI/IVGT database in year 1
    Database manager: Beth Julien

• Co-PIs: Paul White, George Douglas


• Steering Committee: B. Gollapudi, P. Kasper, D. J.-Kram, J. MacGregor, V. Thybaud
One important question: Are there practical thresholds for genotoxicants?

- Initial consensus was yes, for some classes of non-DNA-reactive genetic toxicants (e.g., many aneugens, disturbance of nucleotide pool balance, glutathione depletion, DNA synthesis inhibitors)

- Need a systematic compilation and analysis of data, including DNA-reactive mutagens, that examines the dose response and modes of action, including the presumption of low dose linearity

- Need consensus on appropriate methods (and parameters) to describe the dose-response curve
Other Questions:

Can “negligible risk” be defined as an exposure that does not increase the already-present spontaneous rate more than a defined increment?

- Appropriate metrics? NOGEL, benchmark dose, MOE
- Can response curves be normalized across test systems?
  - Can exposure parameters (e.g., Cmax, AUC) or adducts (DNA, protein) be used to normalize across systems? When?
  - Can it be assumed that the ratio of toxicity to mutagenicity is similar in vitro and in vivo, and in different tissues?
  - Can a virtually safe dose be defined in terms of a fraction of the toxic dose?
When dose is expressed as fraction of TD50:

- MN and lacZ mutation freqs behave similarly
- EMS & ENU similar at toxic dose but EMS is at baseline at 1/10 toxic dose & ENU more mutagenic
- EMS clearly non-linear; ENU data insufficient to determine
  - If linear, will rapidly become insignificant relative to spontaneous
- Do in vitro results show similar dose-responses?

Data courtesy of E. Gocke Roche Pharmaceuticals
Data courtesy of B. Gollapudi, Dow Chemical
Data from ED01 study and IPCS short-term test studies (Tates, 1988; Dean, 1981)
Summary of IVGT Status

- HESI has established project committee to implement improved approaches to genetic toxicology assessment
- Health Canada grant and HESI contract are in place
- Database is under construction
- Meeting to discuss initial analyses will be held in mid-2010
  - It is likely that experimental work will be needed to generate additional dose-response data
- Recommendations to supplement decision tree will be made as data analysis permits consensus on approaches
Where Might the Field Go From Here?

Is it practical to re-structure genetic toxicology testing to include risk assessments based on exposure-response information using existing methodologies?
Key Questions

• Are assays available and sufficiently sensitive to identify relevant risks?
• Is it economically feasible to measure them during toxicity testing?
• How many tissues/endpoints need to be monitored?
  - E.g., are there enough “genotoxic carcinogens” specific for tissues other than liver, lung, kidney, bone marrow, intestine, urinary bladder to be of concern?
  - If there are, how can we expect one or two in vitro systems to identify them?
• If an objective is to identify “genotoxic carcinogens”, can insignificant risk be defined as a specified increment of the spontaneous background mutagenicity?
Tumor or Mutant Frequency
2,4- or 2,6-Diaminotoluene

% Liver Tumors or mutants/10^6

ppm in diet

In vivo mutation correlates with in vivo tumorigenesis: Data from Nohmi et al., Tox. Sci. 114: 71-78, 2010 and NTP Technical Reports 162 and 200
What is the cost of current vs. alternative screening practices?

- ICH 3-test battery is at least $55,000 to $65,000 (if cleanly negative)
- What would be the cost of integrating relevant *in vivo* endpoints into toxicology studies?
  - an integrated design would allow measurement during a GLP repeat-dose toxicology protocol
  - cost would then be dependent on only genetic endpoint measurement & be independent of other study costs
One Hypothetical Battery

- Bacterial mutagenicity to flag potential hazard ($6000)
- Micronuclei in reticulocytes and pig-a mutations in RBC (and/or lymphocytes?) ($10,000 - $15,000 ??)
- Screening for base change and deletion mutations in 5-6 tissues—e.g., gpt-Δ rat using liver, lung, intestine, kidney, urinary bladder using a selectable marker
  (Cost uncertain but 4-5 weeks of technical time at $60,000/yr salary x10% 4x OH multiplier = ~$24,000)

Sum of above =~ $40,000 to $45,000
Strategic considerations

- A battery such as described would be far more comprehensive than current practice, and no more expensive (after initial validation and adoption into practice)

- Site specific analysis could be added or substituted for local exposures (e.g., comet, MN, or transgene analysis at exposure site for dermal, inhalation, etc.)

- Specific gene analysis could be used when MOA is known or hypothesized (e.g., ras or other oncogenes)
Conclusions

Technical tools for a better approach are available

- An integrated scheme might be very cost effective, if the required “up-front” assessments are made and agencies commit to a new approach
  - Analysis of tumor site specificity needed
  - Commitment to the use of animals with appropriate genetic markers in routine toxicology needed
    - NTP could develop path/clin chem. bkgrd. if animal model could be agreed upon (e.g., $gpt\Delta$ Spi\(^{-}\) selection?)
    - Mass breeding would dramatically ↓ cost of transgenic animals

- We should make these commitments and move toward a better testing paradigm