
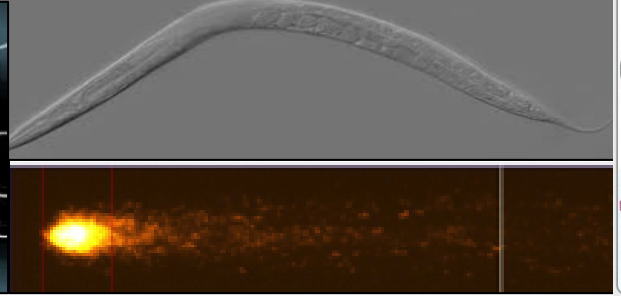
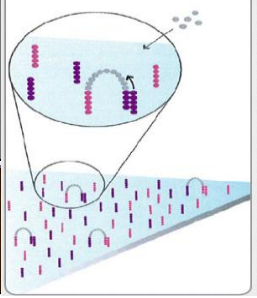


IWGT Workshop on Germ Cell Assays

Chair: George Douglas (Health Canada); Co-chair: Carole Yauk (Health Canada); Rapporteur: Jan van Benthem (RIVM, The Netherlands)

Government:

Jack Bishop (NIEHS); Kerry Dearfield (US FSIS); David DeMarini (US EPA); Francesco Marchetti (Health Canada); Francesca Pacchierotti (ENEA, Italy); Masa Honma (NIHS, Japan);

Academia:

James Lupski (Baylor, USA); Marvin Meistrich (M.D. Anderson Cancer Center, USA); Yuri Dubrova (Leicester, United Kingdom); Bernard Robaire (McGill, Canada)

Industry:

Michael Waters (Integrated Laboratory Systems, Inc., USA); Jane Stewart (AstroZeneca, UK); Marilyn Aardema (BioReliance)

Workshop mandate

- To achieve scientific consensus on issues surrounding the use of germ cell tests in regulatory assessments.
- Review the current science, recommend the best evidence-based approaches and future prospects.

Core Questions

- Do somatic genotoxicity tests predict germ cell effects?
- Do current reproductive toxicology tests provide relevant data on germ cell genotoxicity?
- When do you do germ cell tests and when do you NOT do them?
- What new assays should be implemented and how?

Do *in vivo* somatic tests predict germ cell mutagenicity?

- **Agreed that the currently available data (1994) suggest that somatic cell tests detect germ cell mutagenicity quite well. (14/14)**

- **However, there are notable exceptions and gaps that should dictate caution, and which may result in a different conclusion when more data becomes available.**

Somatic *vs* germ cells: cautions, gaps, and exceptions

- The existing database is small and biased towards alkylating agents and a few specific mechanisms, and the data are skewed towards chemicals that were FIRST positive in a somatic cell test.
  - Predictivity likely to decline as it did for *in vitro* prediction of carcinogenicity.
- A number of qualitative and quantitative exceptions to the above have been identified.
  - N-Hydroxymethylacrylamide, cigarette smoke, zygote chromosomal aberrations
- There are unique germ cell targets (e.g., meiotic machinery, protamines) and features (haploidy, DNA repair deficiency).
- Female mutagenesis is a major gap.
- Analysis of genomic effects arising post-fertilization is a critical gap. These may be more sensitive to chemical exposure and are relevant to human disease.
- There are many genomic changes that we don't currently assess (e.g. CNVs). New tests needed.

Do current reproductive toxicology tests provide relevant data on germ cell mutation?

- Current reprotox tests can provide relevant data on germ cell concerns (14/14)
- Tests that should be considered: 1-generation and multi-generation studies (14/14)
- Following reprotox endpoints may indicate germ cell concerns (14/14)
  - Effects on fecundity/fertility (e.g., # successful litters, # pups)
  - Severely reduced sperm count
  - Testicular histopathology
  - Testicular weight – decrease only
- RECOMMENDED: if there is reduction in male reproductive performance, then the Dominant Lethal Test following male exposure should be conducted. (14/14)
- SPERM MORPHOLOGY does not inform germ cell genotoxicty (14/14)

When do you do germ cell mutagenicity testing?

In vivo Somatic Mutation Result	Clear Exposure of germ cells[a]	Further Germ Cell Testing Required	Probable Germ Cell Call
-	-	No	-
-	+[b]	?[b]	±
+	-	No	-
+	+	Yes[c]	+

10/11

[a]Includes detection of compound or metabolites in relevant testicular compartments. Also includes measures of concern resulting from reprotox and genotoxivity experiments.

[b]NEXT SLIDE.

[c]It is not necessary to do the germ cell tests with +/+ result if it is assumed that a test substance is a germ cell mutagen and regulatory decisions are based accordingly. If it is not assumed to be a germ cell mutagen, then germ cell testing should be conducted. Also required if there is a need for quantitative risk assessment.

Considerations for somatic mutagenicity negative, germ cell exposure positive findings

In vivo Somatic Mutation result	Clear exposure of germ cells	Further germ cell testing required	Probable germ cell call
-	+	?	±

- If compound or metabolites found in gonadal compartments, evaluate result in combination with other measures of male/female germ cell toxicity.
- If there is clear evidence for gonadal toxicity, further germ cell testing should be considered (case-by-case).
- This is because the existing information suggests that there are exceptions where germ cell effects occur in the absence of, or at lower doses, than somatic cell mutagenicity.
- Still controversial and needs more supporting data.

Integration of germ cell genotoxicity endpoints into current regulatory tests

- **KEY opportunities for integration (13/14)**
  - Transgenic rodent mutation reporter assays (test both somatic and germ cells)
  - Spermatid micronucleus assay integrated with somatic micronucleus test
- **Complementary tests** (12/14)
  - Sperm Chromatin Structure Assay (SCSA), Comet, Tunel in sperm cells

URGENT NEED FOR GERM CELL TESTS TO BE CONDUCTED IN PARALLEL WITH SOMATIC CELL GENOTOXICITY TO ADDRESS THE GAPS IN THIS FIELD.

Priorities and Actions

- **IMMEDIATE PRIORITIES/ACTION**
  - Conduct both TGR and MN assays in rodent germ cells to fill gaps.
  - Implement C. elegans aneuploidy assay (Allard et al, EHP), 2013 with ToxCast battery.
- **PRIORITIES/ACTION for the future**
  - IMMEDIATE research on the use of whole genome sequencing in heritable mutation analysis.
  - Standardize the protocol for the sperm comet assay.
  - Further develop the spermatid MN flow cytometry.

If these recommendations are fully implemented, we would capture all known genomic effects that can be inherited.

We need renewed attention in this field

- New clinical data clearly demonstrate that *de novo* germline mutations are critically important to human health.
- Must focus on environmental exposures that can cause *de novo* mutations is required. New types of genomic changes need to be carefully considered.
- Induced mutations that do not cause a phenotype in the first generation must be considered; these 'silent' mutations may not be manifest as a phenotype until future generations.
- Applying new genomics technologies to evaluate animals, and particularly humans, exposed to mutagens should be a priority.

Thank You!