Health Canada
and Alternative Methods

Tim Singer
Director
Environmental Health Science and Research Bureau

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What is Health Canada

• Health Canada is the Federal department responsible for helping Canadians maintain and improve their health.

• Broad responsibilities as a regulator of foods, biologics, consumer products, medical devices, natural health products, pesticides, pharmaceuticals, and chemical substances
Research at Health Canada

- Focused on developing the scientific evidence base to support the department’s regulatory activities
  - Identifying and responding to health risks posed by diseases, environmental chemicals, food contaminants and other threats;
  - Verifying that the drugs, food, medical devices and other therapeutic products available to Canadians are safe and effective; and
  - Providing information to help people make informed decisions about their health.
Health Canada and ICATM

- Health Canada is a signatory to the 2009 ICATM Memorandum of Cooperation

- There is no Canadian center for alternative test method validation

- We remain committed to sharing our expertise and collaborating to develop and implement non-animal alternatives for regulatory testing
Past recent ICATM updates

• Alternative safety testing strategies for acellular Pertussis vaccines

• Cell transformation assays pre-validation study

• Biochemical and cell-based methods for detecting and quantifying botulinum neurotoxin activity

• Chemical method of detection for paralytic shellfish toxins
Reduction of Animal Use Through Validation of a Chemical Method of Detection for Paralytic Shellfish Toxins

Our vision:
To excel as a science-based regulator, trusted and respected by Canadians and the international community.

Our mission:
Dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy.

Wade Rourke
Canadian Food Inspection Agency
Dartmouth Laboratory

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Paralytic shellfish toxins

- Produced by algae and accumulate in filter-feeding shellfish
- Potent human neurotoxicants, often causing death by respiratory paralysis
- The Canadian Food Inspection Agency is responsible for monitoring levels in molluscan shellfish – Canadian Shellfish Sanitation Program
Test methods

- Historical gold standard is the mouse time-to-death bioassay
  - Three mice are used per sample analyzed
  - A mouse unit [MU] is defined as the minimum amount of extract needed to cause the death of an 18 to 22 g white mouse in 15 minutes
  - Cannot provide toxin profiles
  - Expensive
Development of an alternative

• Collaboration
  • CFIA Dartmouth Laboratory
  • National Research Council of Canada Institute for Marine Biosciences

• Post-column oxidation (PCOX) HPLC method
  • July to October 2006 every sample analyzed by both methods in parallel
  • Single laboratory validation study (2009)
  • Multi-laboratory international study according to AOAC International protocols (15 labs, 11 countries)
Intermediate outcome

- PCOX acceptance by Health Canada and the CFIA in August 2009
- Followed by Interstate Shellfish Sanitation Conference acceptance
  - Use as routine monitoring for import/export samples
- Implemented as screening tool in two CFIA labs in November 2009
  - Follow up in vivo testing for regulatory confirmation
  - Led to a 75% reduction in animal use
Final outcome

- Final approval when AOAC International elevated the method to official method of analysis status in spring 2011
- Eliminated all animal shellfish toxin testing in four CFIA labs
- Annual savings of 40,000 animals
Health Canada and test methods

• Active involvement in the OECD Working Party of National Coordinators of the Test Guidelines Programme
  • Validation Management Group for Non-animal Testing
  • Advisory Group on Endocrine Disrupter Testing and Assessment
  • Advisory Group on Molecular Screening and Toxicogenomics
  • Various expert groups, including genotoxicity and cell transformation assays
Health Canada’s ICATM role

• Continue to contribute expertise, principally through the review of documentation, towards:
  • Validation study design
  • Validation studies
  • Peer review
  • Recommendations on suitability and limitations for use of alternative methods

• Pre-validation research
In Vitro Versions of the Muta™Mouse Transgenic Rodent Mutation Assay for Hazard Identification of Chemicals

Paul A. White, Christine L. Lemieux, Alexandra S. Long

Genetic Toxicology Group, Environmental Health Sciences & Research Bureau
Transgenic Animals in Mutagenesis Research

BigBlue® rat – cII, lacI (lac repressor)
BigBlue® mouse – cII, lacI (lac repressor)
Muta™ Mouse – cII, lacZ (β-galactosidase)
LacZ plasmid mouse – lacZ (β-galactosidase)
Gpt delta rodents – gpt, gam, redBA

New OECD Guideline TG 488
July 28, 2011.
The Muta™Mouse Transgenic System for Mutation Scoring

In vitro “rescue”, packaging, *E. coli* infection, and scoring.

Development of a Transgenic-derived Cell Line

Development and Characterization of a Stable Epithelial Cell Line from Muta™Mouse Lung

Paul A. White,* George R. Douglas, John Gingerich, Craig Parfett, Phil Shwed, Vern Seligy, Lynda Soper, Lynn Berndt, Janet Bayley, Shelley Wagner, Kathleen Pound, and David Blakey

Mutagenesis Section, Safe Environments Program, Health Canada, Ottawa, Ontario
Minced in 0.25% trypsin, overnight at 4ºC & 45-90 min at 37ºC

Processing of Muta™ Mouse lung tissue and isolation of cells with epithelial morphology

Mean doubling time = 18.7 ± 1.2 hrs
Cloning efficiency = 16.8 ± 1.2%
Mitotic index (sub-confluent) = 14.1 ± 2.4%

Culture in DMEM:Ham’s F12 (1:1) with 2% FBS + 1ng/mL murine EGF
37 ºC, 95% RH, 5%CO₂

Repeated subculture on polystyrene plates coated with covalently linked collagen (rat tail type I)
Regulatory Problem: Low specificity of \textit{in vitro} mammalian genetic toxicity tests

\textbf{Sensitivity}

The proportion of carcinogens determined to be positive

\textbf{Specificity}

Proportion of non-carcinogens determined to be negative

**“Irrelevant” positive compounds – existing results**

<table>
<thead>
<tr>
<th>Compound</th>
<th>CAS #</th>
<th>In Vivo Genotoxicity Test Results</th>
<th>In Vitro Mammalian Genotoxicity Results</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>MN&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CA&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Phthalic anhydride</td>
<td>85-44-9</td>
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<td>Tertiary-butylhydroquinone</td>
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<tr>
<td>1,2-Dihydroxybenzene (resorcinol)</td>
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<td>Curcumin</td>
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<tr>
<td>Propyl gallate</td>
<td>121-79-9</td>
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<tr>
<td>p-Nitrophenol</td>
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<tr>
<td>Ethyl acrylate</td>
<td>140-88-5</td>
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<td>+/-</td>
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<td>Eugenol</td>
<td>97-53-0</td>
<td>E&lt;sup&gt;e&lt;/sup&gt;/-</td>
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</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>120-83-2</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup>Micronucleus assay, <sup>b</sup>chromosomal aberration assay, <sup>c</sup>mouse lymphoma assay, <sup>d</sup>inconclusive, <sup>e</sup>equivocal, * these compounds have been subsequently re-evaluated and determined to be unclassifiable [5].

Adapted from Kirkland et al., 2005
Evaluation of Muta™Mouse FE1 for regulatory use

- 9 non-DNA-reactive (i.e., Salmonella negative) chemicals
  - Non-carcinogens
  - Previously elicited irrelevant positives in in vitro assays for gene mutation or chromosome damage

- Identical to compounds being assessed in the current COLIPA trial
  - Same chemical lots wherever possible

A tiered approach to the use of alternatives to animal testing for the safety assessment of cosmetics: Genotoxicity. A COLIPA analysis

Stefan Pfuher a, Annette Kirst b, Marilyn Aardema a,¹, Norbert Banduhn c, Carsten Goebel d, Daisuke Araki e, Margit Costabel-Farkas f, Eric Dufour g, Rolf Fautz b, James Harvey h, Nicola J. Hewitt i, Jalila Hihatallah j, Paul Carmichael k, Martin Macfarlane k, Kerstin Reisinger c, Joanna Rowland l, Florian Schellauf m, Andreas Schepky n, Julia Scheel c,*
“Irrelevant” positive compounds – FE1 results

- Each compound tested in FE1 cells up to 10mM/5mg/plate, or to toxicity
- All compounds tested with and without rat liver S9

Without S9
- 7 of 9 compounds were negative overall
- 2 compounds (resorcinol and eugenol) positive only at very high concentrations

With S9
- All compounds were negative
“Irrelevant” positive compounds – FE1 results

- True positives (BaP, ENU) tested, as well as simultaneous BaP control (0.1ug/plate) – consistently positive

- True negatives (ampicillin trihydrate and D-mannitol) also tested – both negative

- Results indicate that the *in vitro* FE1 Muta™Mouse TGR assay may be useful for regulatory testing of chemicals
  - Useful in confirming a negative Salmonella finding in mammalian cells, thus preventing unnecessary follow-up *in vivo* testing.
P450 isoforms like 1A2 are almost exclusively hepatic.

Several aromatic amines NOT active in FE1 cells (e.g., PhIP, NNN in cigarette smoke condensate).

More difficult to obtain & culture, but suitable methods exist.

Future priorities

• Continue to develop, promote, investigate and/or implement alternative toxicity methods where we have scientific expertise
• Active involvement in OECD Test Guidelines Programme
• Continue engagement as an ICATM partner
  • Facilitator of linkages between groups to advance alternative methods