Health Canada and Alternative Methods

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SACATM Advisory Committee Meeting
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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

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Originally presented at WC8
Montreal, QC
August, 2011

Canadian Food Inspection Agency

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.

Reduction of Animal Use Through Validation of a Chemical Method of Detection for Paralytic Shellfish Toxins

Wade Rourke

Canadian Food Inspection Agency

Dartmouth Laboratory

Canada

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

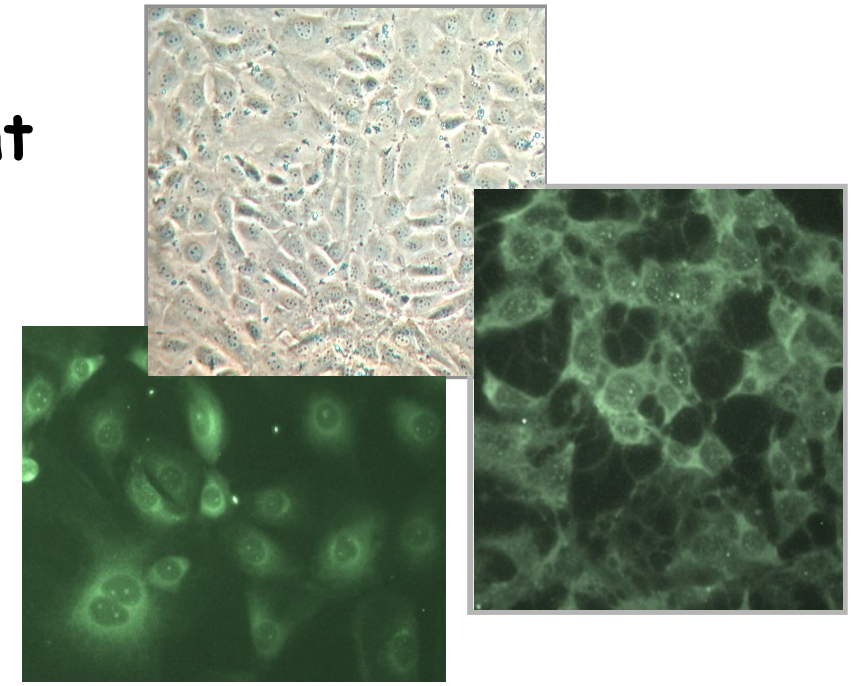
- Active involvement in the OECD Working Party of National Coordinators of the Test Guidelines Programme
  - Validation Management Group for Non-animal Testing
  - Advisory Group on Endocrine Disrupter Testing and Assessment
  - Advisory Group on Molecular Screening and Toxicogenomics
  - Various expert 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

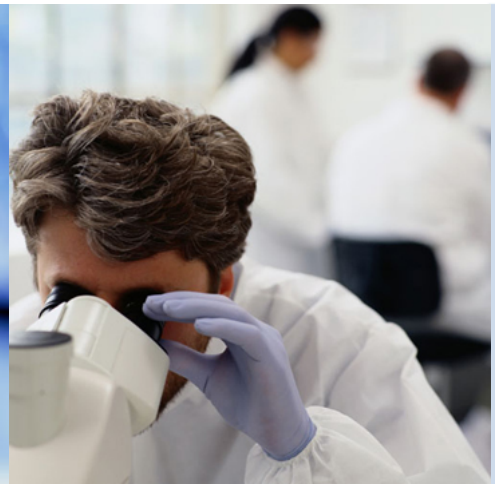
In Vitro Versions of the Muta™Mouse Transgenic Rodent Mutation Assay for Hazard Identification of Chemicals



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

Genetic Toxicology Group,
Environmental Health Sciences & Research Bureau





Canada

Transgenic Animals in Mutagenesis Research

BigBlue® rat – *cII, lacI* (lac repressor)

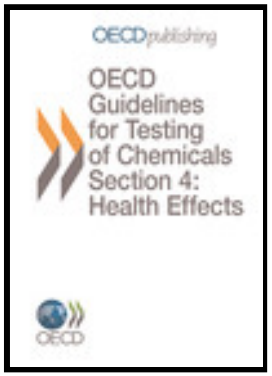
BigBlue® mouse – *cII, lacI* (lac repressor)

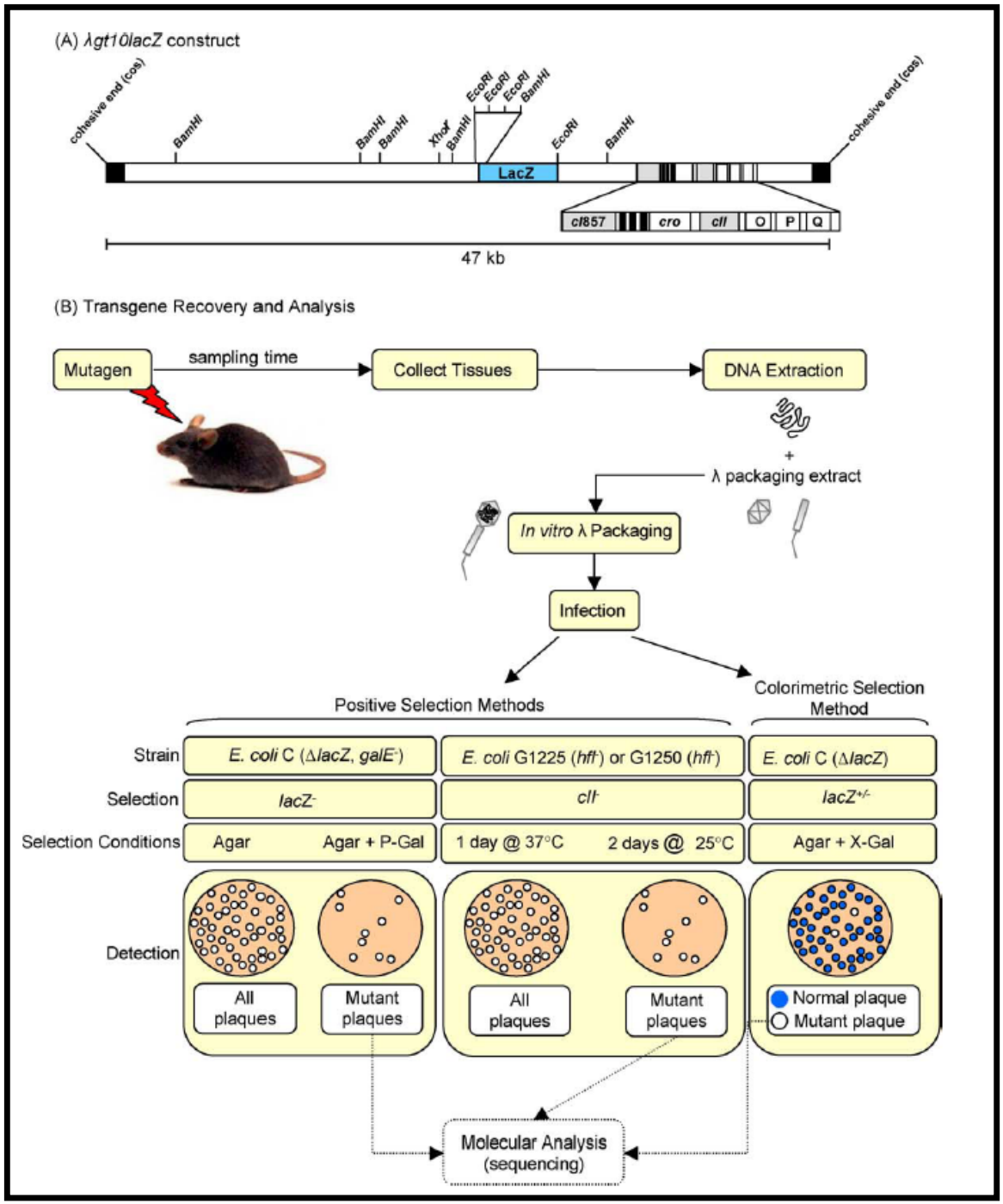
Muta™Mouse – *cII, lacZ* (ß-galactosidase)

LacZ plasmid mouse – *lacZ* (ß-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.**

Source: Lambert et al. 2005. *Mutat Res* 590:1-280.

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

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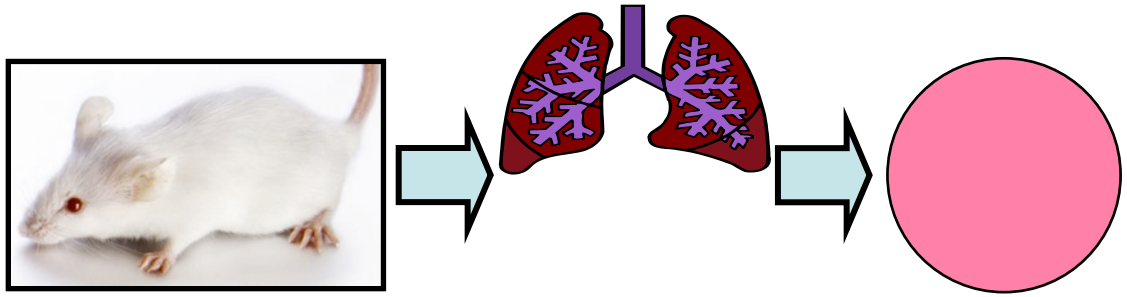
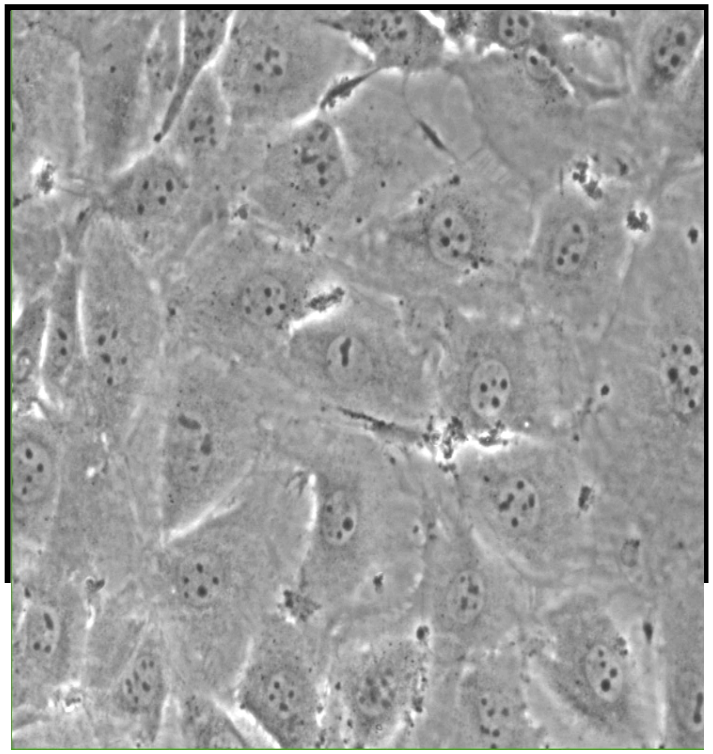


Minced in 0.25% trypsin, overnight at 4ºC & 45-90 min at 37ºC

Culture in DMEM:Ham's F12 (1:1) with 2% FBS + 1ng/mL murine EGF 37 ºC, 95% RH, 5%CO_2

Repeated subculture on polystyrene plates coated with covalently linked collagen (rat tail type I)



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%.

Regulatory Problem: Low specificity of *in vitro* mammalian genetic toxicity tests

Sensitivity

The proportion of carcinogens determined to be positive

Specificity

Proportion of non-carcinogens determined to be negative

Sources: Kirkland et al. 2005. *Mutat Res*. 584:1-256; Kirkland et al. 2007. *Mutat Res* 628:31-55.

"Irrelevant" positive compounds – existing results

Compound	CAS #	*In Vivo* Genotoxicity Test Results			*In Vitro* Mammalian Genotoxicity Results		
		MN[a]	*CA*[b]	*Other*	*MLA*[c]	*CA*	*MN*
Phthalic anhydride	85-44-9			- (gene mut.)	+*	+	
Tertiary-butylhydroquinone	1948-33-0	-	-			+	
1,2-Dihydroxybenzene (resorcinol)	108-46-3	-			+	+	
Curcumin	458-37-7						+
Propyl gallate	121-79-9	-	?		+	+	
p-Nitrophenol	100-02-7				+		Inc[d]
Ethyl acrylate	140-88-5	+	+/-		+	+	
Eugenol	97-53-0	E[e]/-			+	+	
2,4-Dichlorophenol	120-83-2	+			+*	+	

[a]Micronucleus assay, [b]chromosomal aberration assay, [c]mouse lymphoma assay, [d]inconclusive, [e]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

Contents lists available at ScienceDirect

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

Stefan Pfuhler [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 Hibatallah [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.

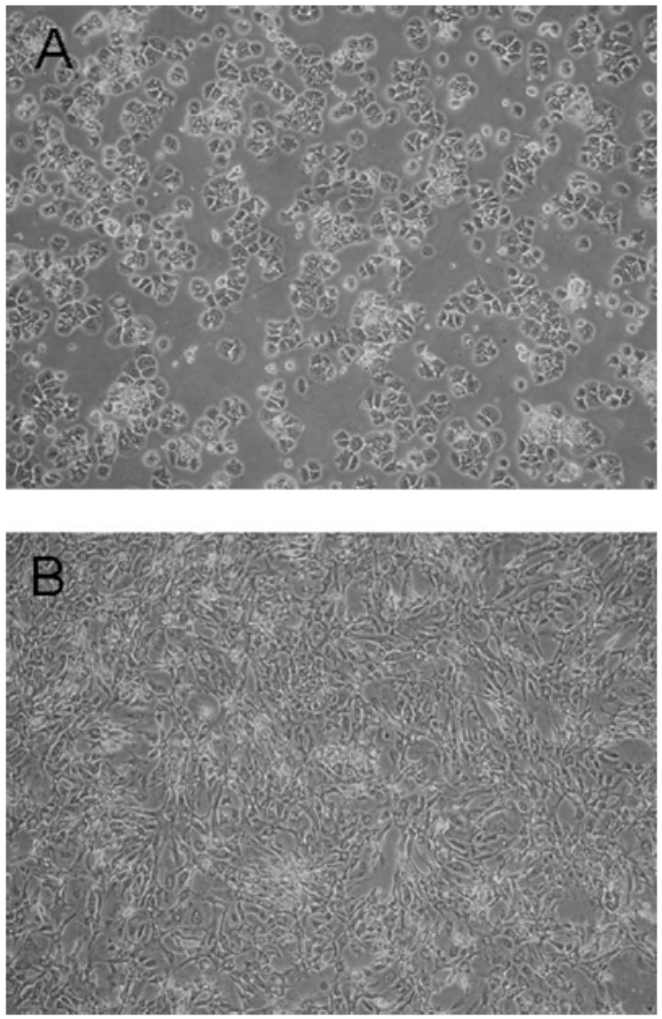


Fig. 2. Phase-contrast photomicrographs of cultured primary hepatocytes. (**A**) Typical cubic hepatocytes shortly after isolation; (**B**) scattered elongated hepatocytes after 48 hr (magnification, 40×).

Induction of *lacZ* Mutations in Muta™ Mouse Primary Hepatocytes

Guosheng Chen, John Gingerich, Lynda Soper, George R. Douglas, and Paul A. White*

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- 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.

Source: Chen et al. 2010. *Environ Molec Mutagen*. 51:330-337.

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