Pollution-Induced Germ-line Mutations in Gull and Mouse Tandem Repeat DNA

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McMaster University
DNA Damaging Agents
In the Environment:

- heritable mutations?
Hamilton Harbour Remedial Action Plan:

- Designated area of concern under IJC in 1978
- Remediation goals established for chemical and biological state of the area
- Our lab contributed with an attempt to establish a Method to monitor germ-line mutations

Heritable Mutations?
Contaminants Can Damage DNA

body cells
Mutation
Cancer

Sex cells
Mutation
??

Exposed
Unexposed offspring
Outline

• Germline mutation monitoring
• Pollution and minisatellite mutations
• Experimental studies of ESTR mutations in mice
• Implications and reactions
• What can be done and is often not done (Hamilton’s legacy).
• Future mutation work
Contaminant-Induced Heritable Mutations: Difficult to study

Natural Populations
• Comparisons between exposed and unexposed populations lack statistical power
• Causal links between exposure and mutation difficult

Laboratory Studies
• Enormous sample sizes and high treatment doses
• Do not reflect exposure under ambient conditions

Few attempts have been made to study mutations induced under ambient environmental conditions
Part 1: Germline mutation screening in herring gulls (*Larus argentatus*) on the Great Lakes
Mutation Detection:

- Pedigree analysis
- Multilocus DNA fingerprinting
- Minisatellite loci
- Non-parental bands = mutations

Minisatellite DNA:
- Non-coding, repetitive DNA
- High spontaneous mutation rate
Germline Minisatellite Mutation Rates in Herring Gulls:

Study Sites:
Table 3
Results of Nested ANOVA

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>$F_s$</th>
<th>$P$-value</th>
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<tbody>
<tr>
<td>Site categories and localities</td>
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<td></td>
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<td>Between categories</td>
<td>2, 6</td>
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<td>Between localities within categories</td>
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<td>Between categories and year of study</td>
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<td>Between categories within years</td>
<td>2, 9</td>
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<td>0.0095</td>
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<tr>
<td>Between years within categories</td>
<td>9, 842</td>
<td>0.80</td>
<td>0.6178</td>
</tr>
</tbody>
</table>

Yauk, Fox, McCary and Quinn, 2000. Mutation Research 452:211-218
Mutation rate is negatively correlated with proximity to steel production:

Yauk, Fox, McCary and Quinn, 2000. Mutation Research 452:211-218
Germline Mutation Screening: Herring Gulls

- Germline minisatellite mutation rates elevated at sites with integrated steel mills
- Mutation rates negatively correlated with proximity to steel mills

(Yauk and Quinn 1996 PNAS, Yauk et al. 2000 Mutat Res.)
What is the Route of Mutagen Exposure?

Are Other Organisms at Risk?
Germ-line mutation induction in other species

- Elevated minisatellite mutation rates - humans living near Chernobyl and in Kazakhstan

- Elevated microsatellite mutation rates - barn swallows living near Chernobyl
Potential Difficulties in Interpreting Comparisons Among Natural Populations:

- Underlying Genetics
- Isolating Route of Exposure
- Lifestyle / Behaviour Differences
Development of mouse-specific repetitive DNA markers (ESTRs):

- Acute gamma irradiation (Dubrova et al. 1998, 2000)
- PCBs and diesel exhaust (Hedenskog et al. 1997)
- ESTR markers more sensitive and efficient than previous rodent tests (e.g., Specific locus test)
Challenge: An experimental approach to detect heritable mutations caused by pollution.

Sentinel Animals, ambient conditions + Sensitive ESTR markers in lab mouse studies
Part 2: Germline mutation screening in sentinel rodents
How Industrial By-Products Enter the Ecosystem in Hamilton Harbour:

Airborne Emissions

Fall Out

Contact Cooling
Pier 25 Exposure Site:

- Adjacent to steel industry and busy highway
- Close to herring gull nesting area
- Long term air quality data
Freelton Exposure Site:

- ~30km north of Pier 25
- Removed from known point sources of pollution
- Shed identical to one at Pier 25
Parents

1) Adults bred 6 weeks post-exposure

Offspring

2) Complete families sampled for comparative DNA Fingerprinting
Mutation Detection: Pedigree DNA Profiling

- ESTR locus Ms6-hm (GGGCA)_n
- Chromosome 4
- Highly unstable in germline

Mutations = changes in band size
Overall inherited mutation rates:

Ms6-hm  
Hm-2

MMS10

Somers, Yauk, White, Parfett and Quinn.  2002. PNAS  99: 15904-15907
* Elevation in Hamilton mice is due mostly to mutations inherited through the paternal germline

**Somers, Yauk, White, Parfett and Quinn. 2002. PNAS 99: 15904-15907**
Conclusions from Exp 1:

- Ambient air at Steel site induced heritable germ cell changes in exposed adult mice
- Male germline more susceptible than female
Next Steps?

- Replication of the findings
- Narrowing the field
Protecting sentinel mice from particulate matter: HEPA filtration

Removes:
- 99.99% > 0.3µm
- 99.97% > 0.1µm
Two-way ANOVA (Env. Exposure per family, Paternal and maternal single locus ESTR rates)

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
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<td>0.0090</td>
<td>3.68</td>
<td>0.0590</td>
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<td>HEPA-filtration</td>
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<td>0.0060</td>
<td>0.07</td>
<td>0.7948</td>
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<tr>
<td>Interaction</td>
<td>1, 69</td>
<td>13.79</td>
<td>0.0004</td>
<td>1.60</td>
<td>0.2098</td>
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Overall germline mutation rates:

Per-band mutation rate (±SE)

Ms6-hm
Hm-2

MMS10

Paternal and Maternal Mutation Rates

Paternal germline significantly affected

Pier 25

Freelton

Mean Daily TSP (g)

High-volume air sampling: 24 hour period
What might be causing mutations?

PAHs from coal combustion are a good candidate group

Exposure year 117.6 ng/m³
Air chemistry: PAH testing

*Pyrene
Benzo[a]fluorene
Benzo[b]fluorene
Benzo[b]naphtho[2,1-d]thiophene
Benzo[ghi]fluoranthenene
Benzo[c]phenanthrene
Benzo[b]naphtho[2,3-d]thiophene
Benz[a]anthracene
Cyclopenta[cd]pyrene
*Chrysene
Benzanthrone
Benz[a]anthracene-7,12 dione
Benzo[b]fluoranthene
Benzo[k]fluoranthenene
Benzo[j]fluoranthenene
*Benzo[e]pyrene
*Benzo[a]pyrene
*Perylene
Indeno[1,2,3-cd]pyrene
*Dibenz[a,c]anthracene
Picene
Benzo[ghi]perylene
*Coronene
Dibenzo[a,e]pyrene
Dibenzo[a,i]pyrene
Dibenzo[a,h]pyrene
Total PAHs: TSP

Weighted average: 33-fold difference between sites; Rural = 0.4ng/m^3, steel = 13.4ng/m^3
Implications?
Possible Human Health Risks to the unborn

- Genetic diseases influenced by tandem repeat DNA:
  - Cancers associated with *Hras* oncogene
  - Type I Diabetes
  - Type of Epilepsy
  - Huntington’s disease
  - Fragile X syndrome

- Possible Changes in “coding” genes (requires demonstration that ESTR assay reflects similar changes in genes)
Around your home
Around Your City

• Leaves filter particulate air pollution
• Protect forests, plant trees
• Limit road construction and urban sprawl
• Walk, bike, bus, or car pool
Around your city/province/country

Dofasco Stack Key (View from the northeast standing along Eastport Drive)

Stelco Stack Key (View from the northeast standing on the pier near the lift bridge)
Bad air a ‘genetic risk’

McMaster research shows genetic link to air pollution

By ERIC McGUIINNESS
Environment Reporter

McMaster University research first in the world to show urban air pollution causes damage which animals pass on to the next generation.

Ecologists Jim Quinn and colleagues have demonstrated male laboratory mice exposed to Hamilton steel-mill emissions transfer mutated genes to their young. And they say the same thing could be happening in humans.

The gene mutations may raise risk of cancer and defects, the McMaster researchers say they can’t make a direct comparison to humans.

At the same time, they say every reason to believe genes react the same way in mice as in people.
Dofasco CEO calls gene damage study ‘speculative’

By ERIC McGUIINNESS
Environment Reporter
The Hamilton Spectator

Dofasco Inc. is sharply attacking the findings of McMaster University scientists who blame steel mill emissions for gene damage in mice housed on the Hamilton Harbour, downwind of the city’s two major steel plants.

John Mayberry, board chair and chief executive officer of Dofasco, issued a statement late yesterday saying: “We dismiss this study as speculative and irresponsible. This is disappointing. There appeared to be no effort whatsoever to scientifically establish the actual cause of the gene damage among the test mice.”

He was referring to work by biologists Jim Quinn, an associate professor, and Chris Somers, a PhD student, published Monday in the U.S. journal Proceedings of the National Academy of Sciences.

As The Hamilton Spectator reported yesterday, Quinn, Somers and several Health Canada scientists say mice downwind of the steel mills for 10 weeks developed DNA mutations at 1.5 to two times the rate of a colony placed on a farm 30 kilometres upwind. They also report that mutations in the liver are higher among the mice living near the mills.

Take the link...

Canada has agreed to cut greenhouse gas emissions 20 to 30 per cent by 2012. The Kyoto Protocol was ratified yesterday. See page D1.

THE HAMILTON SPECTATOR
Manuscript underwent review

Researchers say steel company CEO should have read article

By Jim Quinn
and Chris Somers

It is not the normal role of university scientists to debate with industry CEOs in the press; however, a response from our regarding the attack on our study, Air pollution induces harmful DNA mutations, by John Mayberry of Dofasco Inc., is warranted.

For the moment, we are willing to ignore the fact that we were labelled “arbitrary and irresponsible” by Mayberry, and stick to a discussion of the science.

(The Hamilton Spectator reported Dec. 11 that the authors and several Health Canada scientists say mice downstream of the steel mills for 10 weeks developed DNA mutations at 1.5 to two times the rate of a colony placed on a farm 38 kilometres upstairs. They also report that mutations in the adult males were passed on to their young, the first evidence in any living organism that ambient air causes hereditary DNA mutations.)

The response to our publication would have been more appropriate had Mayberry actually read and referred to the published article rather than to the media reports. Below we present the comments made by Mayberry followed by our responses.

“There appeared to be no effort whatsoever to scientifically establish the actual cause of the genetic damage among the test mice.”

We state in the introduction of our paper that the mice exposures were researchers distinguishing steel mill emissions from other sources upstream of the test site. We saw no evidence of them distinguishing steel mill emissions from vehicle exhausts. We also believe there were adequate research controls in the study in regards to identifying or distinguishing the potential sources of emissions or their effects.

We do not deny that there are other sources of air pollution in our study area. In fact, we clearly identify the colonies had mutation rates that were significantly elevated above local colony, the colony located in Toronto, which happens a city than Hamilton with both industry and traffic (it all colony is on the Lakeshore Highway, the Gardiner Expressway and the Tonn Valley Parkway) but lacking steel mills, did not.

Furthermore, the distance of a place from the steel mill was not an adequate measure for determining the impact of air pollution, as the first time anybody has asked questions about air pollution and heritable mutation.
### Parental Investment in Gametes?

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**Timing?**

![Spermatogenesis Diagram](image1)

![Parental investment in Gametes](image2)
Future Research (collaboration with Carole Yauk and others)

• In utero exposures – maternal mutations
• Stability of mutations to $F_2$
• DNA expression in exposed individuals
  – protection of germ-line DNA
• Possible genomic instability
Exposure through pregnancy to birth

Score pedigree mutation to determine mutation rates in oocytes

Score mutation in sperm using SM-PCR

Non-exposed male
Conclusions:

• Urban/Industrial air pollution induces germline mutations in tandem repeat DNA

• Males more sensitive than females

• HEPA filtration reduces mutation induction

• Airborne particles play major role in mutation induction
Acknowledgements

- Dr. Carole Yauk
- Dr. Chris Somers

- ECOWISE (Tri-Council Green Plan)
- National Cancer Institute of Canada
- Wildlife Toxicology Fund
- Toxic Substance Research Initiative
- NSERC

- Environment Canada
- Health Canada

- Dr. Bradley White
- Dr. Brian McCarry

- Numerous Field and Laboratory Research Assistants

- Co-operative Landowners