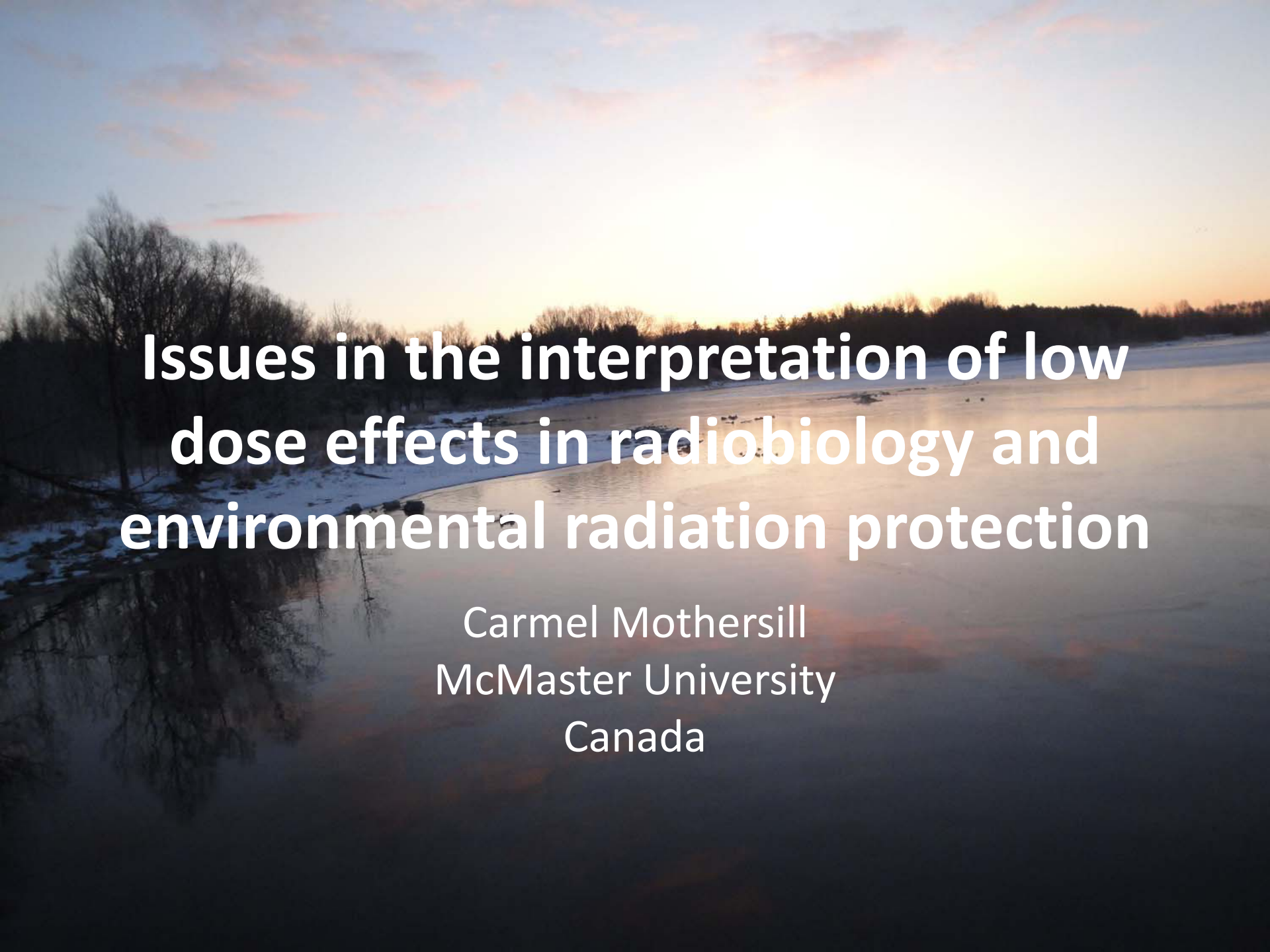


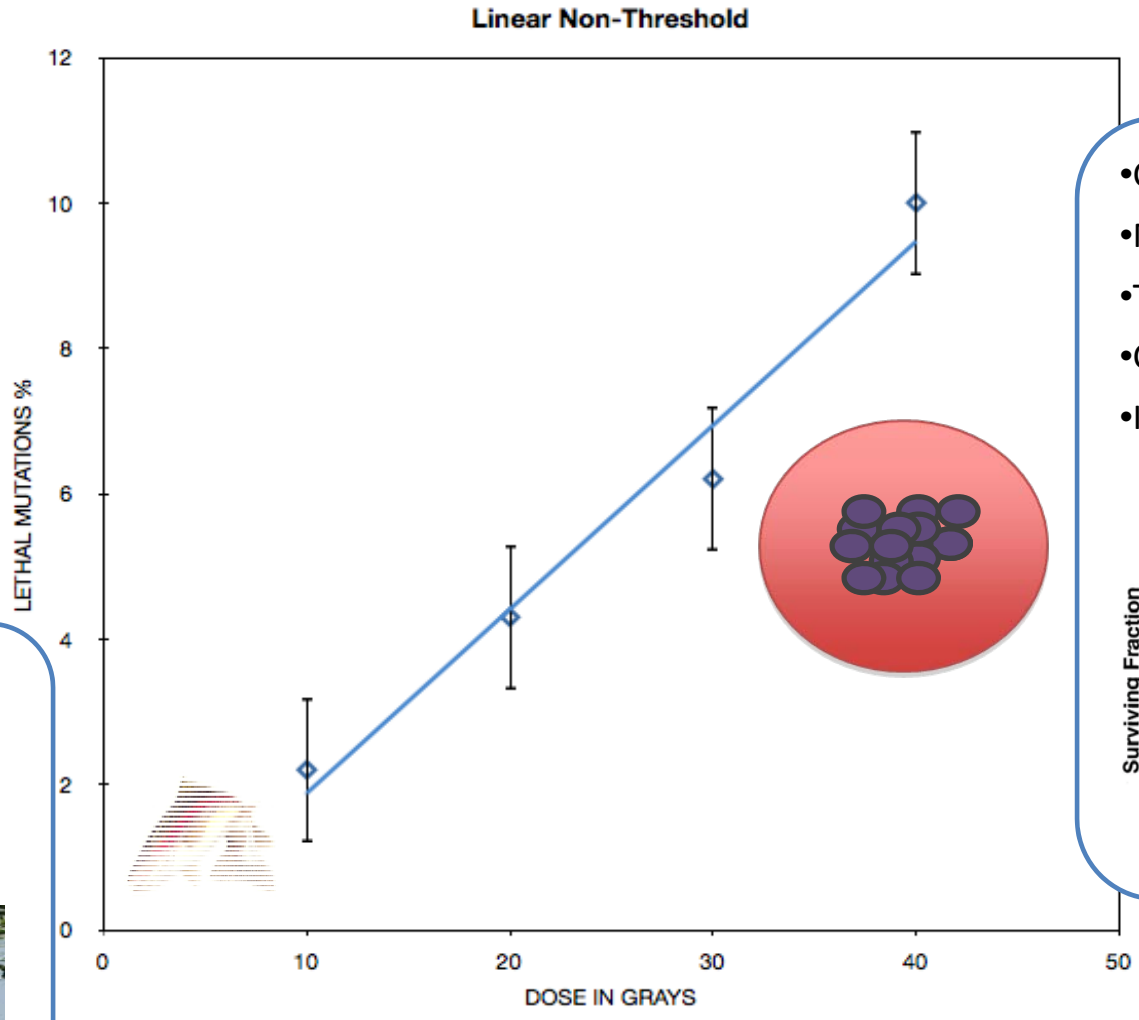
# Issues in the interpretation of low dose effects in radiobiology and environmental radiation protection

Carmel Mothersill
McMaster University
Canada

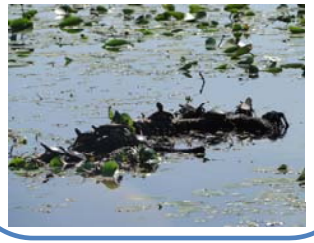
# Risk estimates are designed to address low dose risks

- Here is where non-targeted effects (NTE) predominate
- Effect is not linearly related to dose
- They may not be in any way related to dose but maybe triggered as a signaling cascade by energy deposition above a threshold
- Extrapolation cannot  therefore work
- Uncertainty must be accepted
- LNT must be dumped as a concept of value in the low dose region

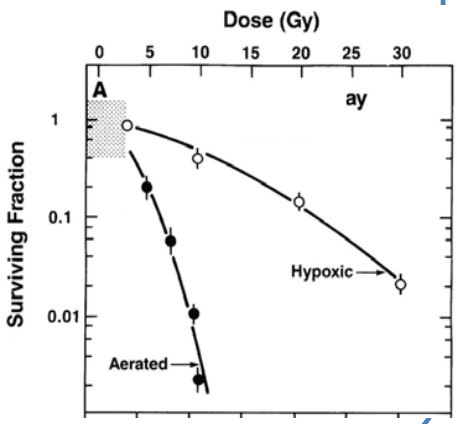
Environmentally relevant dose range is where NTE's dominated the effect




- Whole Organism
- Population
- Ecosystem
- Transgenerational



- Cellular Response
- Micro-environment
- Therapeutic Advantage
- Consistency
- Individual



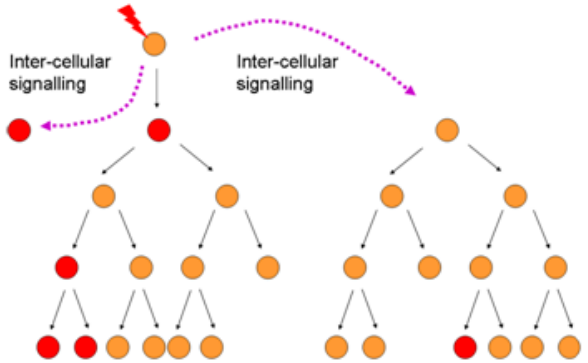
# 'Non-targeted' radiation effects

## Bystander effects

Effects in neighbouring cells

## Genomic Instability

Effects in unirradiated descendant cells

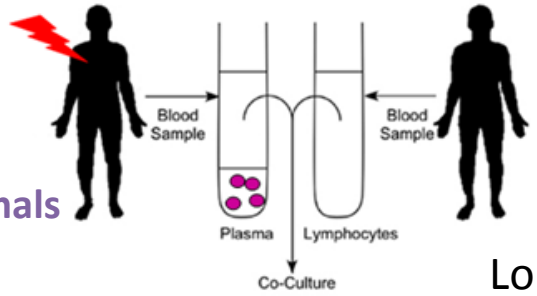



## Abscopal effects

Effects in neighbouring tissues

## Clastogenic factors

Ex vivo effects in cultured cells




## Inter-animal signaling

Effects in neighbouring animals

**Inflammatory Processes may provide mechanistic link**

Long-term effects on innate immune response function may occur

# Assumptions of LNT which is the current risk estimate tool

- Effect is proportional to dose
  - WRONG
- DNA is the only damage lesion of interest
  - WRONG
- Target theory provides the mechanism
  - WRONG
- Dose and dose rate are linked by a multiplier of 2
  - WRONG

# NTE’s and risk

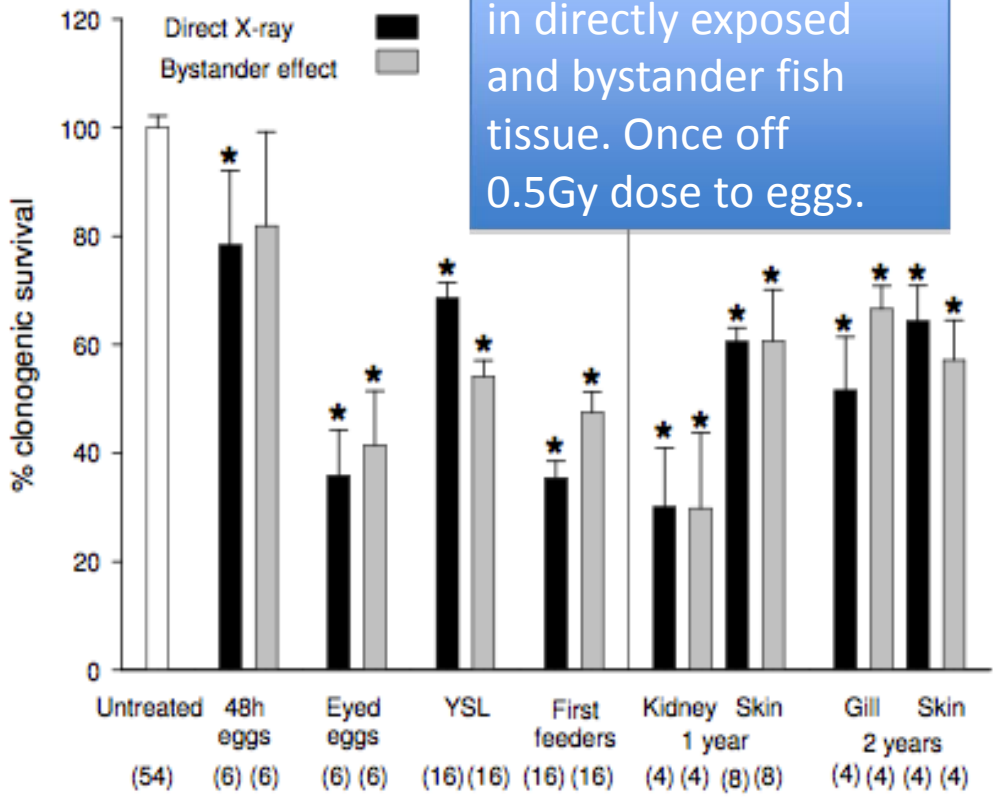
- Bystander effects and genomic instability may increase or decrease risk depending on lifestyle and genetic factors
- Adaptive responses may increase risk if they spare damaged cells or decrease risk if induce repair capacity

# Our Recent Research Highlights

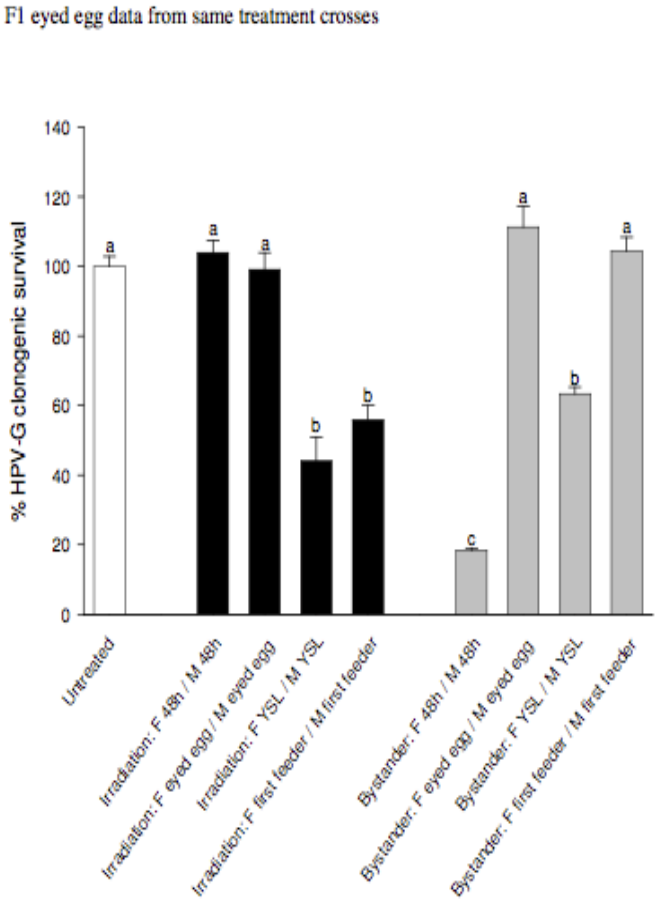
- Phenomenology
  - Major life time legacy study completed for trout given one acute 0.5Gy x-ray at early life stages.
  - G1 progeny of these fish show transgenerational effects
  - Adult fish show abnormal (excess) heavy metal uptake
  - Bystander fish unirradiated but swimming in water with irradiated fish show effects which are also transgenerational but appear protective
  - Neutrons do not induce these effects in vivo or in vitro
  - 4mGy gamma or x-ray exposure is enough to trigger these effects
  - Ra-226 accumulation (and therefore “dose”) is not necessarily proportional to ingested activity
  - mBq/g daily feeds can cause biological effects

# Legacy data

Signal secreted for life in directly exposed and bystander fish tissue. Once off 0.5Gy dose to eggs.




Progeny continue to show the effect



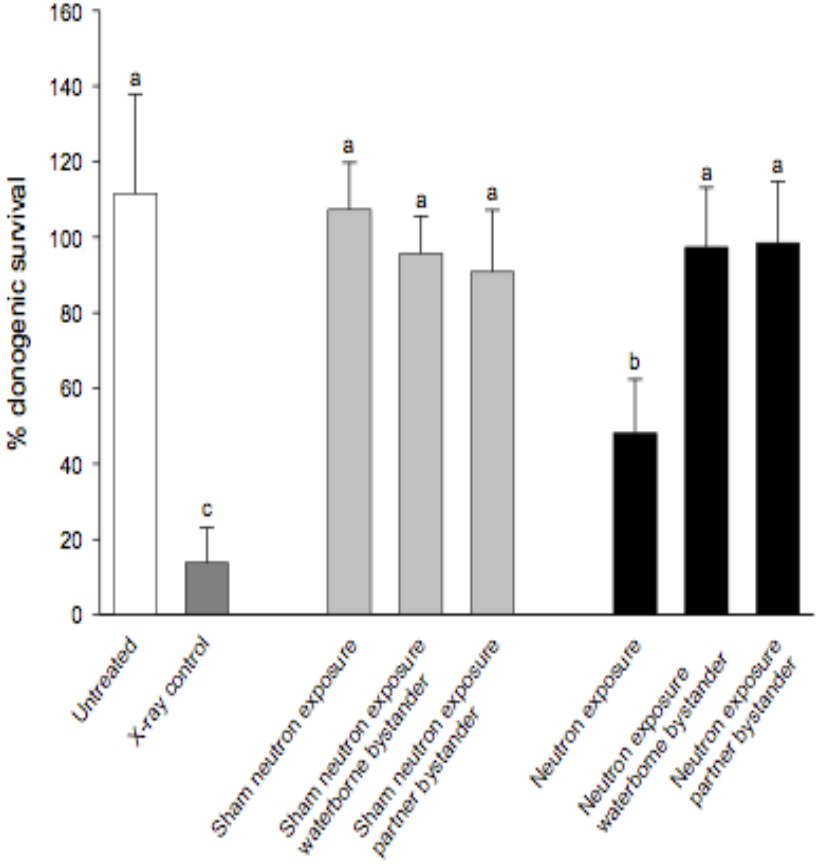

Singh, H et al Radiation-induced bystander effects in mice given low doses of radiation in vivo, Dose response, in press.
Irradiation of rainbow trout at early life stages results in legacy effects ir adults C. Mothersill, R. W. Smith, R. Saroya, J. Denbeigh, B. Rowe, L. Banevicius, R. Timmins, R. D. Moccia and C. B. Seymour, IJRB 2010

# Neutron data

**TABLE 2**
**Numbers of Colonies Formed and Survival Percentages for the Neutron-Irradiated and Bystander Cells**

| Neutron dose (mGy) | γ-ray dose component (mGy) | Survival (%) | |
|---|---|---|---|
| | | Irradiation | Bystander |
| 1.3 ± 0.1 | 0.040 ± 0.004 | 97 ± 8 | 101 ± 8 |
| 2.6 ± 0.3 | 0.080 ± 0.008 | 90 ± 6 | 107 ± 4 |
| 3.8 ± 0.4 | 0.11 ± 0.01 | 97 ± 13 | 105 ± 5 |
| 7 ± 1 | 0.20 ± 0.02 | 100 ± 8 | 85 ± 7 |
| 27 ± 3 | 0.80 ± 0.08 | 115 ± 21 | 96 ± 12 |
| 33 ± 3 | 1.0 ± 0.1 | 100 ± 6 | 105 ± 6 |
| 1000 ± 300[a] | 30 ± 9[a] | 8 ± 2 | 111 ± 11 |

[a] This neutron high-dose radiation experiment was a very preliminary trial, and doses were estimated based on the beam current setting of the accelerator.




No neutron bystander effect in vitro or vivo


Z. Liu, C Mothersill, F McNeill, SH Byun, C Seymour W Prestwich, A dose threshold for a medium transfer bystander effect in a skin cell line, Radiat. Res.(2006) 166, 19-23

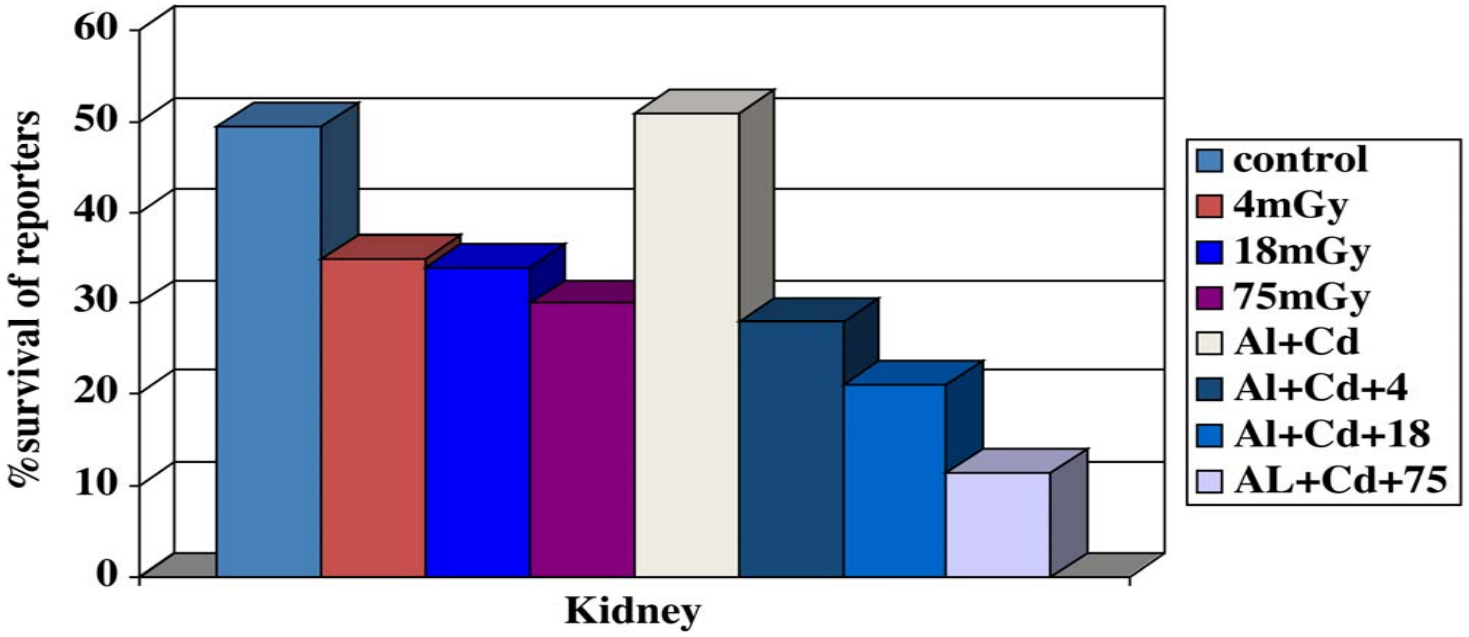
Z. Liu, S.H. Byun, W.V. Prestwich, C.E. Mothersill, C.B. Seymour, F.E. McNeill, Fluence and dose measurements for an accelerator neutron beam, Nucl. Instr. Meth. B., Vol. 263, (2007), pp. 326-328.

C. Wang, SH Byun, R Smith F McNeill, W Prestwich, C Mothersill and CB Seymour, Neutrons do not produce a bystander effect in zebrafish irradiated in vivo IJRB accepted for publication

# Low dose multiple stressors

4mGy induces effects in vivo in salmonids and metals change the response

Norwegian collaborator

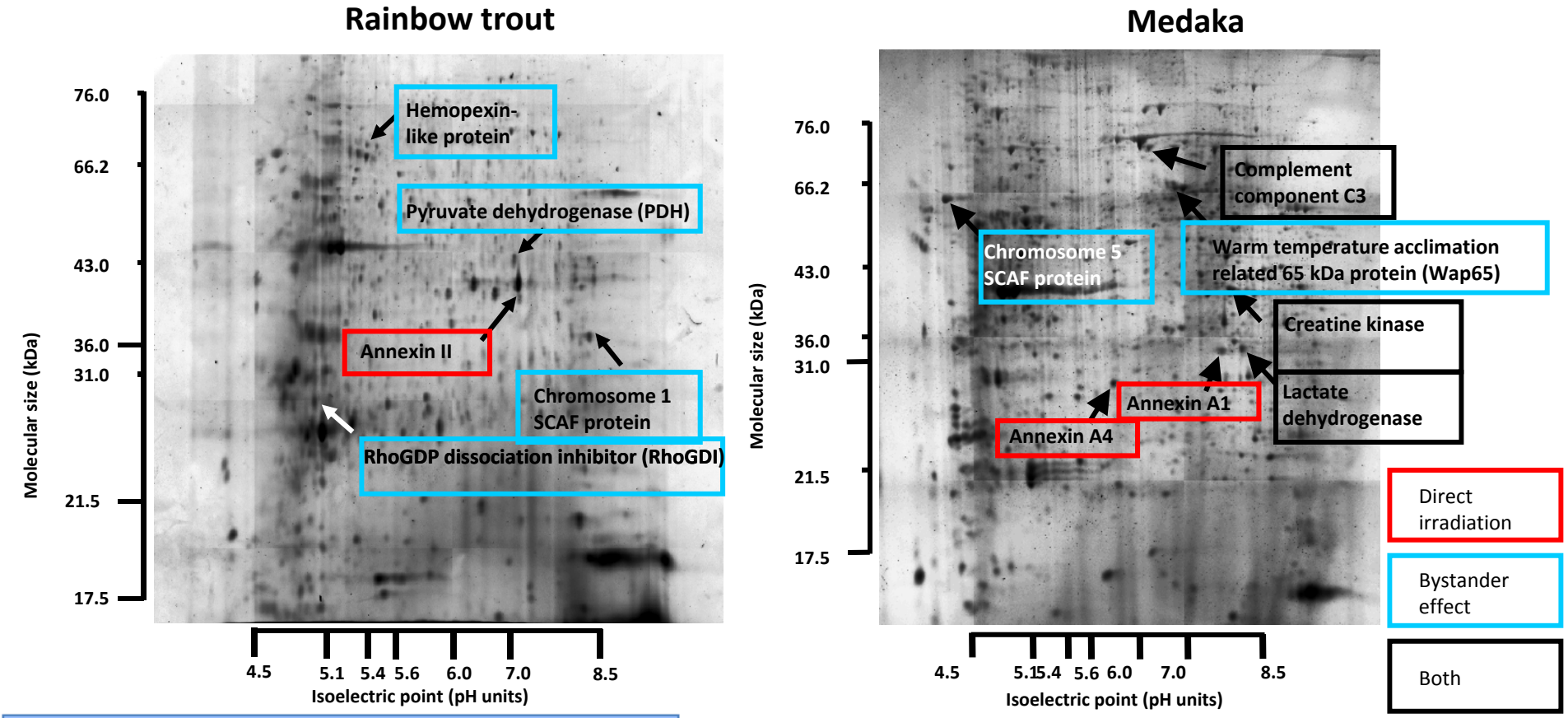



Salbu et al 2008, Environmentally relevant mixed exposures to radiation and heavy metals induce measurable stress responses in Atlantic salmon

# Research Highlights

- Mechanistic
  - Other species of fish show similar patterns of communication and similar proteomic responses
  - DNA repair deficient mutants have damage inducing bystander communicated signals
  - TGFb and p53 shown to be involved in signalling response but not generation of the signal
  - Serotonin shown to be involved in signal generation

# Gill proteomics in two species



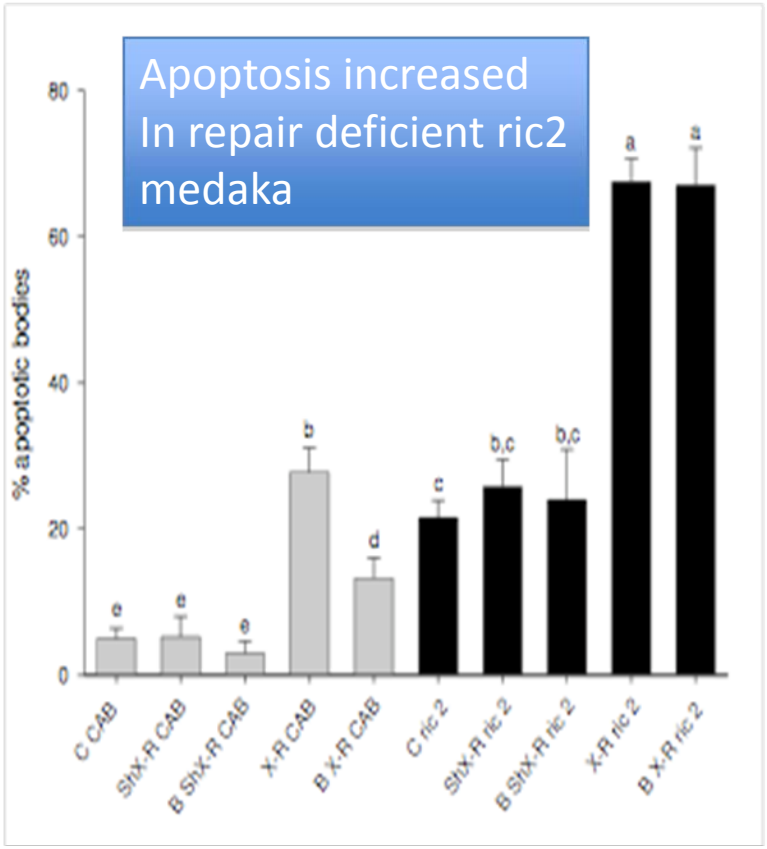

Trout bystander proteome protective

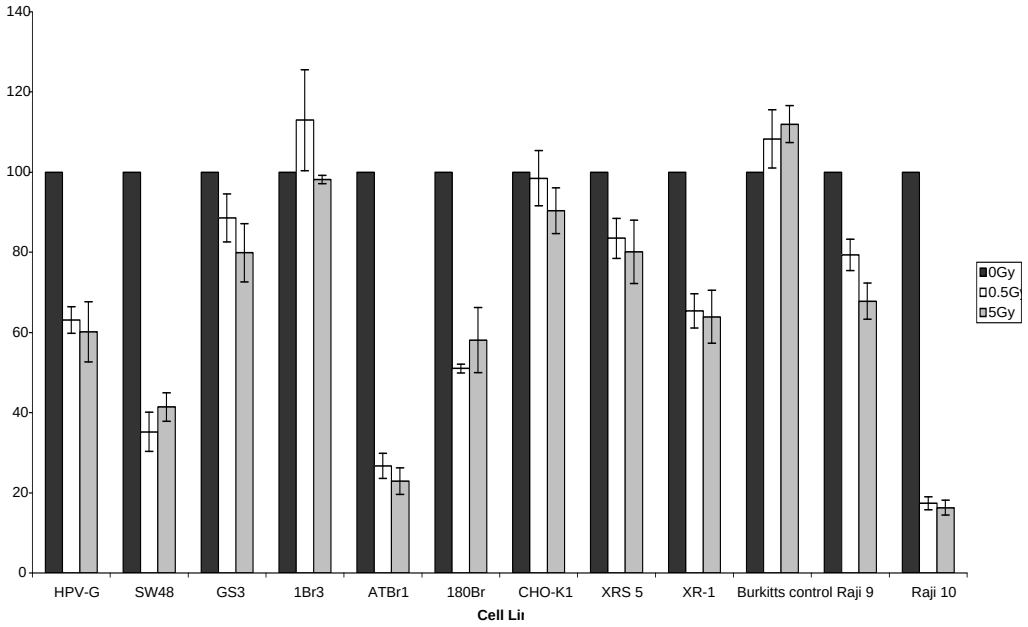
Smith RW, et al 2007 Evidence for a protective bystander response in rainbow trout gills exposed to x-irradiation. Proteomics. 7(22):4171-80.

.Smith RW et al Proteomic changes in the gills of DNA repair proficient and DNA repair deficient medaka following exposure to direct irradiation and to X-ray induced bystander BBA 2011 signals

Medaka bystander proteome may indicate protective and adaptive response

# DNA repair important



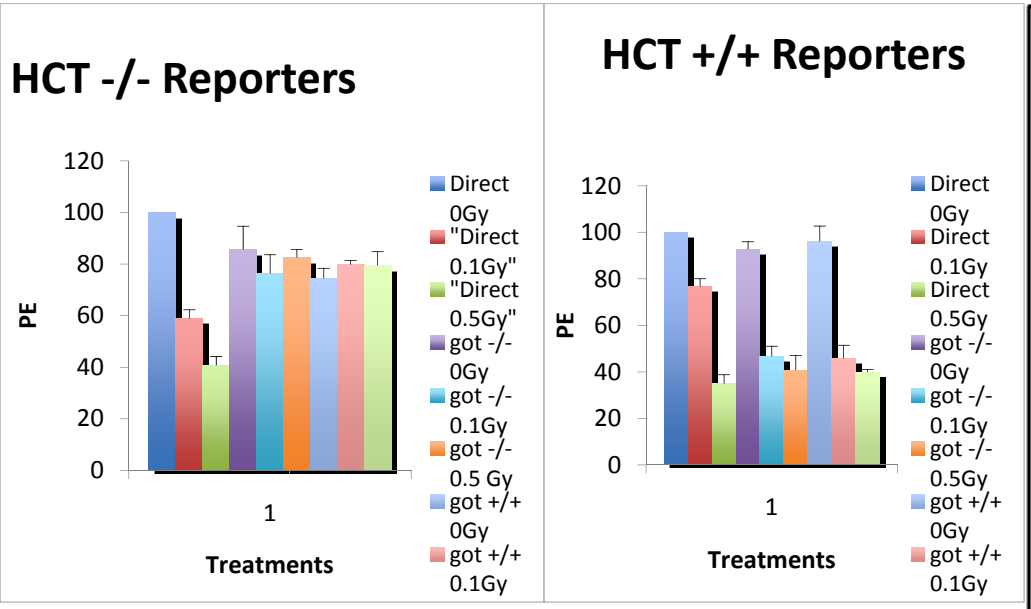

Repair deficient cell lines (SW48, ATBr1, 180Br, xrs5, xr1 , raj9 and raj10) have more toxic BSE than their parent lines

Proteomic changes in the gills of DNA repair proficient and DNA repair deficient medaka following exposure to direct irradiation and to X-ray induced bystander signals. R Smith et al BBA being revised after review

Increased radiosensitivity in human cell lines treated with medium from repair deficient cells Radiat Res. 165(1):26-34. Mothersill C, Seymour RJ, Seymour CB. 2006.

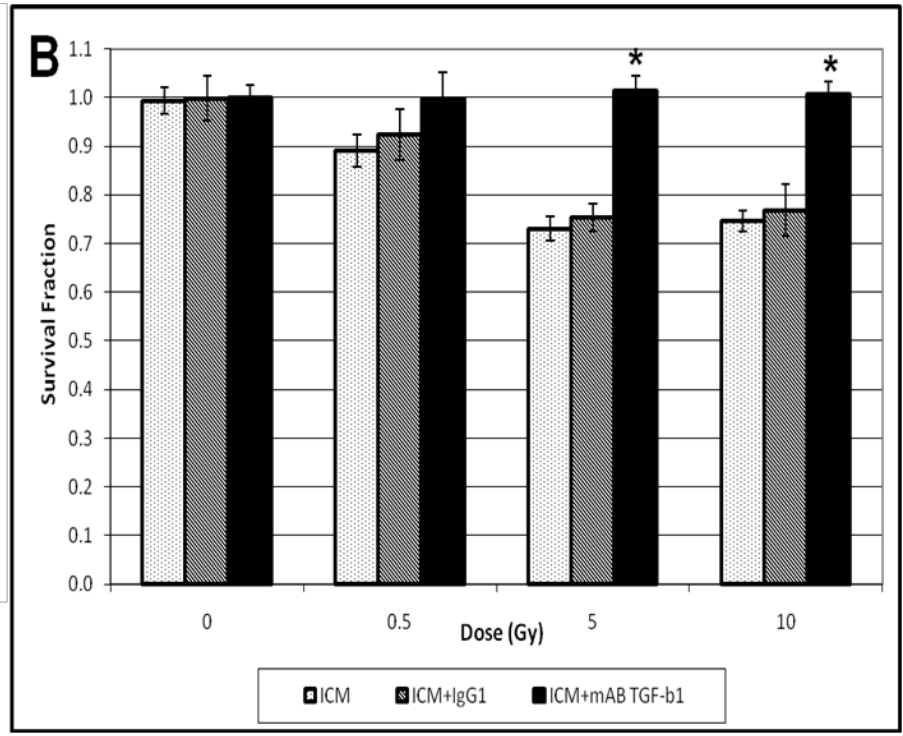
Communication of Radiation-Induced Signals in Vivo between DNA Repair Deficient and Proficient Medaka (*Oryzias latipes*). Environ. Sci. Technol. 43 (9):3335–3342 . Mothersill C, Smith RW, Hinton TG, Aizawa K, Seymour CB. 2009

# P53 and TGFβ important in response







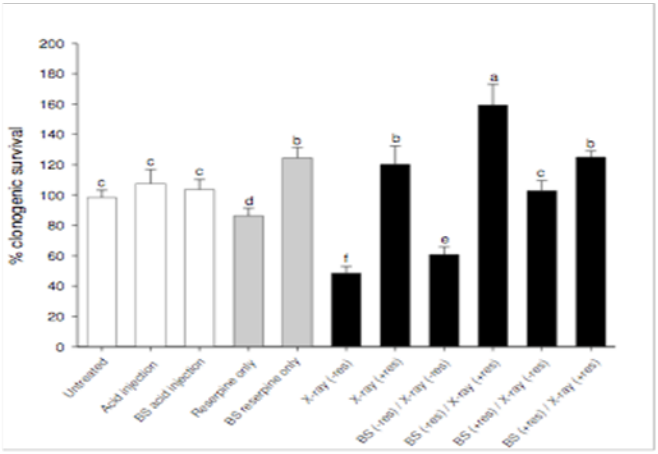



P53 knockouts generate bystander signals but do not respond so the -/- reporters give no BSE even if +/+ medium is applied. +/+ reporters respond even to -/- medium

TGF-β1 antibody completely abolished bystander cell death .

M D Gow et al Induction of Bystander Cell Death in Human Glioma by High Energy Electrons: A Role for TGF-beta1, Radiation Research, in press

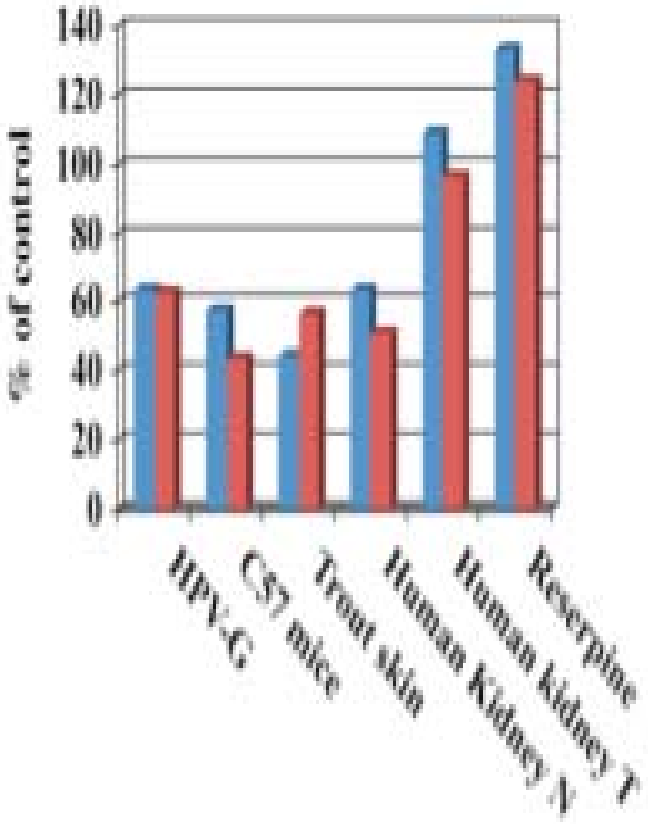
# Serotonin important in signal generation




| Sham treatment | % clonogenic survival |
|---|---|
| Sham X-ray (-reserpine injection) | $103.8 \pm 8.1^{*,\ddagger,\dagger}$ |
| Sham X-ray (+reserpine injection) | $111.7 \pm 8.6^{\ddagger}$ |
| Bystander (-reserpine) / sham X-ray (-reserpine) | $102.0 \pm 4.8^{*,\dagger}$ |
| Bystander (-reserpine) / sham X-ray (+reserpine) | $137.0 \pm 6.8^{*,\ddagger}$ |
| Bystander (+reserpine) / sham X-ray (-reserpine) | $93.5 \pm 3.6^{\dagger}$ |
| Bystander (+reserpine) / sham X-ray (+reserpine) | $130.6 \pm 4.3^{\ddagger}$ |

* significantly different to equivalent X-ray treatment
‡ significantly different to untreated and reserpine injected fish.
† significantly different to reserpine injection only induced bystander effect.

Fish injected with reserpine do not communicate the bystander signal



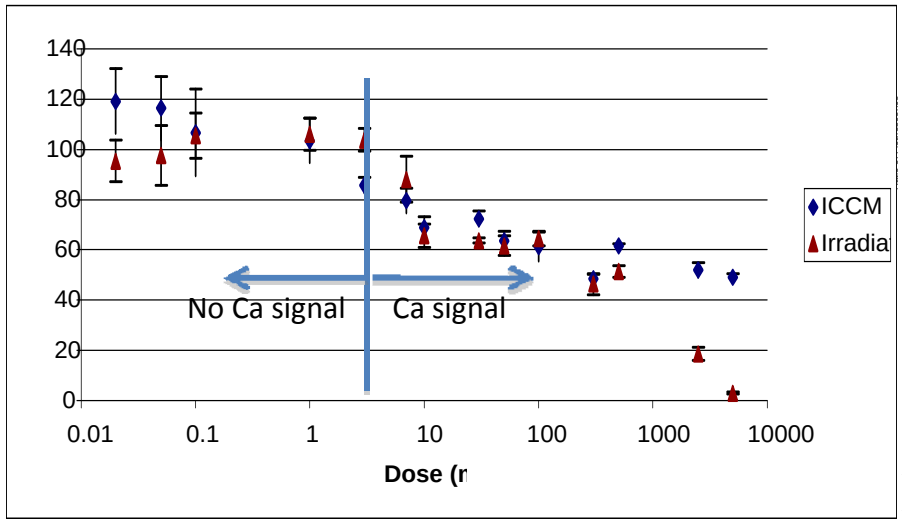

Reserpine inhibits serotonin binding and prevents the bystander effect in vitro and in vivo

Saroya, R et al, Injection of reserpine into zebrafish prevents fish to fish communication of radiation-induced bystander signals; confirmation in vivo of a role for serotonin in the mechanism, Dose response, in press

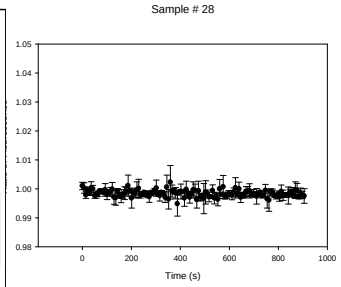
Poon RC et al 2007. Bystander effects of ionizing radiation can be modulated by signaling amines. Environ Res. 105(2):200-211.

# Calcium pulse is the first sign

Dose response in vitro



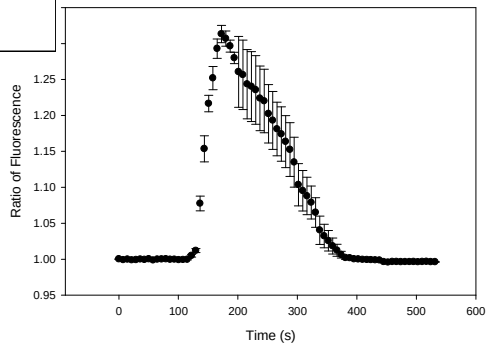

Sham



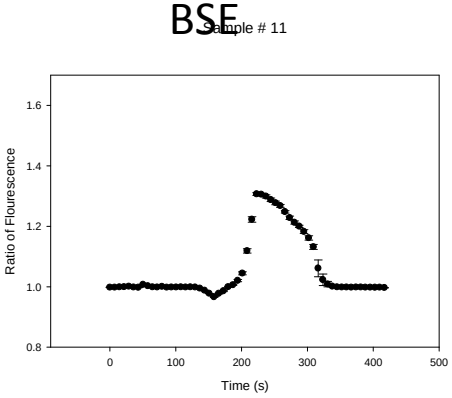

Transgenerational memory of calcium signal in vivo. Traces are from eyed egg progeny where parents x-rayed as eyed eggs 3 years ago

Calcium signal and bystander effect only triggered after dose exceeds 2mGy gamma rays

0.5Gy to parents




BSE

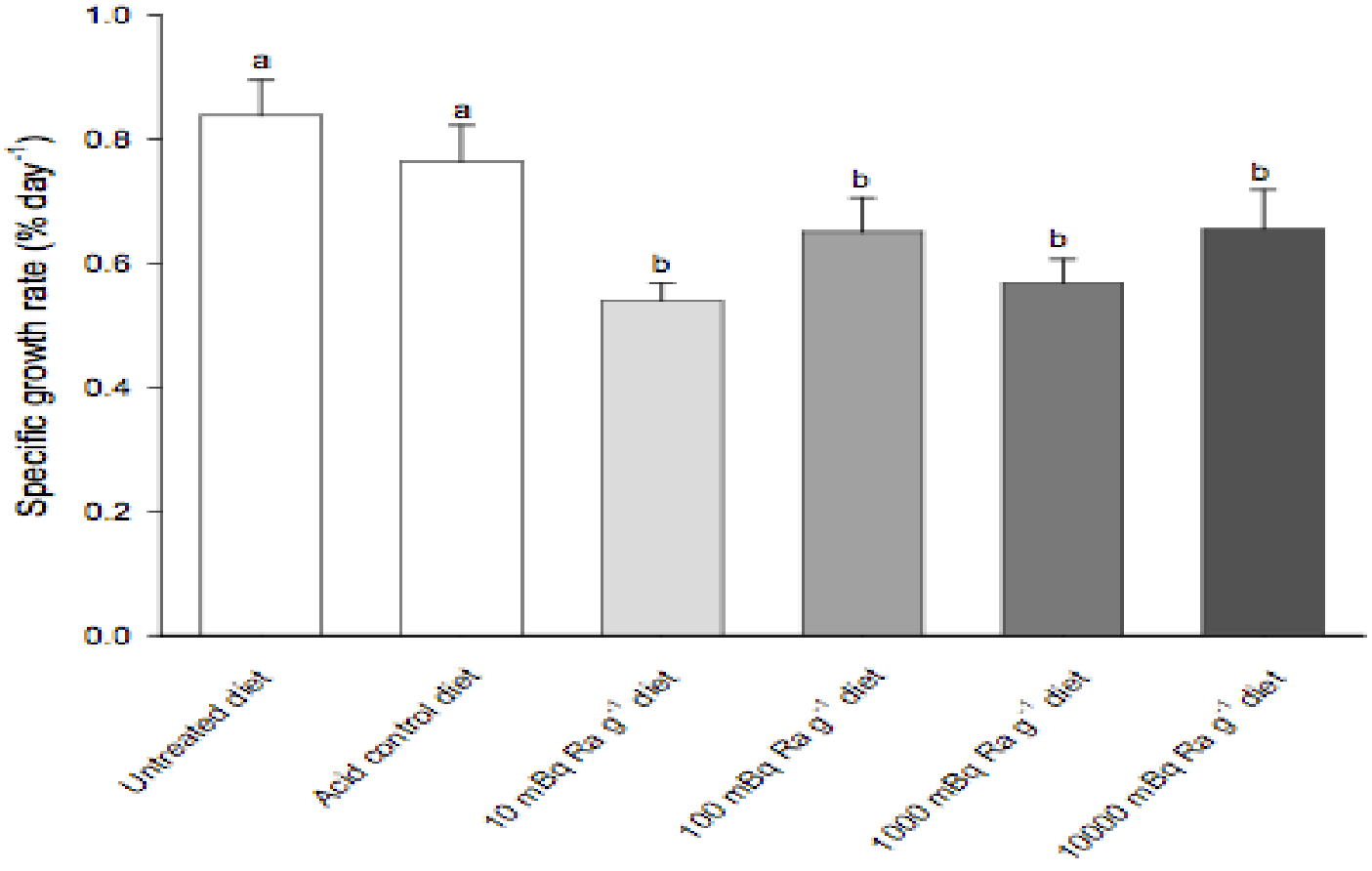



Z Liu, C Mothersill, F McNeill, SH Byun, C Seymour W Prestwich,
A dose threshold for a medium transfer bystander effect in a skin cell line,
Radiat. Res.(2006) 166, 19-23

# Radium -226 bioaccumulation

| Sample number | ID | Activity (Bq $kg^{-1}$ wet) | Annual dose (mGy $y^{-1}$) |
|---|---|---|---|
| 1.1 | Control Fish | 36 ± 22 | 0,9 ± 0,5 |
| 2.1 | Control Fish | 28 ± 28 | 0,7 ± 0,7 |
| 9.1 | Fed 10 mBq $g^{-1}$ | 39 ± 15 | 1,0 ± 0,7 |
| 10.1 | Fed 10 mBq $g^{-1}$ | 23 ± 8 | 0,6 ± 0,2 |
| 11.1 | Fed 100 mBq $g^{-1}$ | 11 ± 12 | 0,2 ± 0,2 |
| 12.1 | Fed 100 mBq $g^{-1}$ | 9 ± 12 | 0,2 ± 0,3 |
| 13.1 | Fed 1 Bq $g^{-1}$ | 26 ± 11 | 0,7 ± 0,3 |
| 14.1 | Fed 1 Bq $g^{-1}$ | 33 ± 13 | 0,8 ± 0,3 |
| 15.1 | Fed 10 Bq $g^{-1}$ | 100 ± 18 | 2,5 ± 0,4 |
| 16.1 | Fed 10 Bq $g^{-1}$ | 124 ± 16 | 3,0 ± 0,4 |

# Specific growth rates (sampling after 6 months on diet)



# Research Highlights- Conclusions

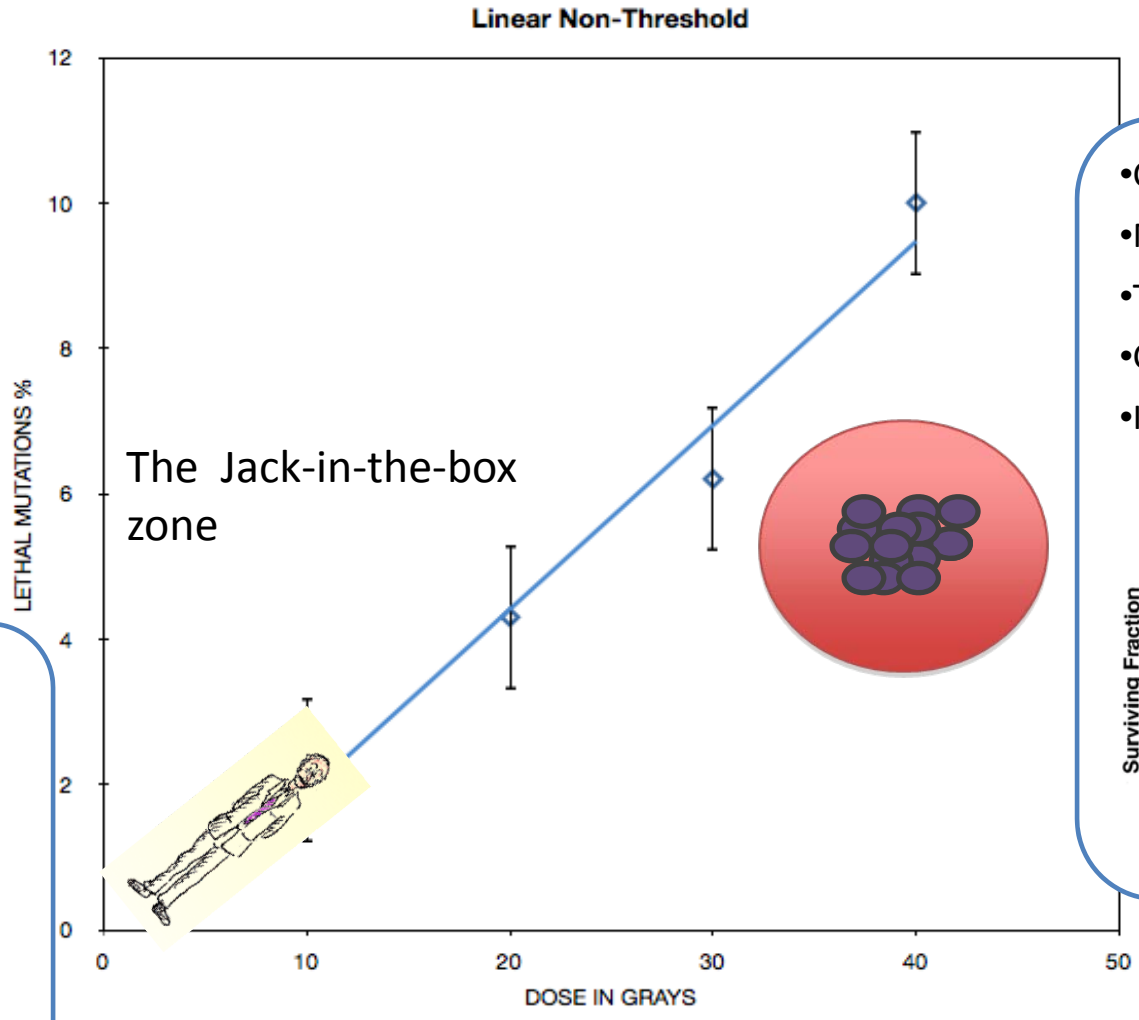
- Low dose radiation exposure of fish to acute or chronic low LET radiation induces a “stress response” in irradiated individuals. Chronic alpha caused reduced growth rate and stress signal production.
- This leads to an inter-fish signaling mechanism which causes recipient fish to exhibit protective responses
- Effects are transgenerational (acute low LET)
- Mechanisms involve serotonin and calcium for signal generation and cytokine pathways for response

# So given the new data - does ICRP Over- Or Underestimate Risk?

# Does ICRP Over- Or Underestimate Risk?


Good reasons to use LNT BUT lack of accord means there is NO RIGHT ANSWER

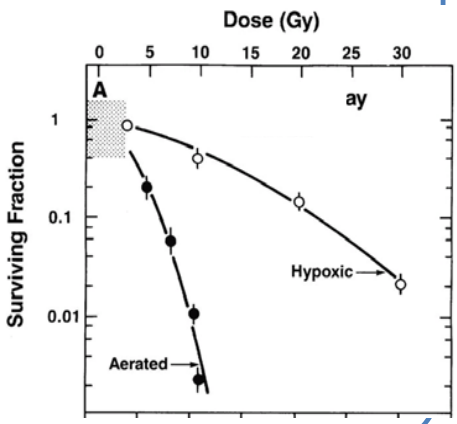
What we have is the zone of surprise!



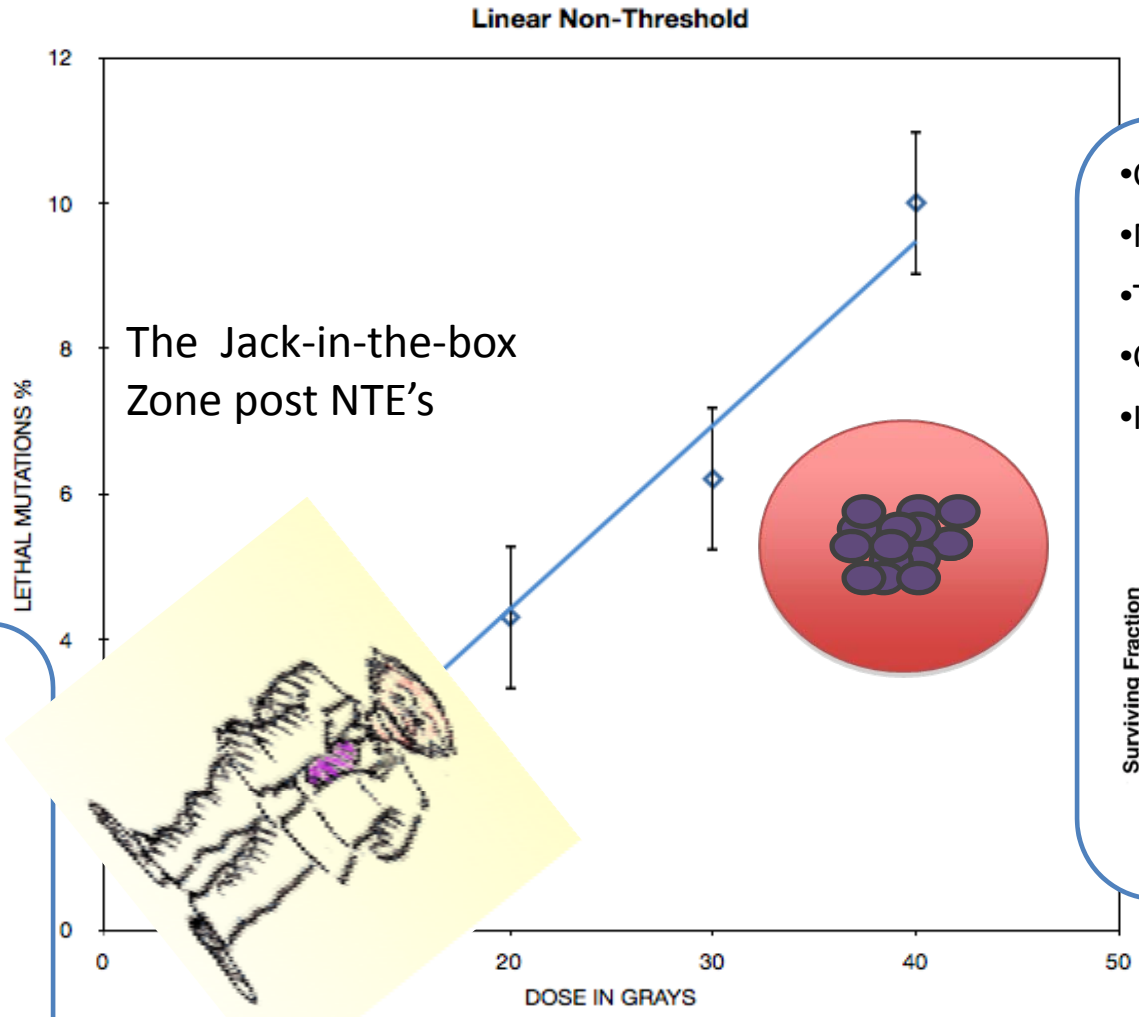

The Jack-in-the-box zone

- Whole Organism
- Population
- Ecosystem
- Transgenerational

- Cellular Response
- Micro-environment
- Therapeutic Advantage
- Consistency
- Individual



ICRP after NTE's!




The Jack-in-the-box Zone post NTE's





- Whole Organism
- Population
- Ecosystem
- Transgenerational

- Cellular Response
- Micro-environment
- Therapeutic Advantage
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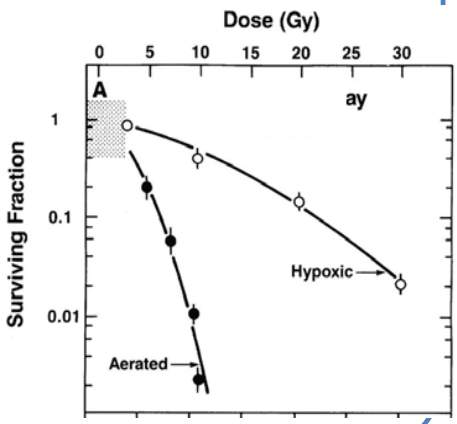
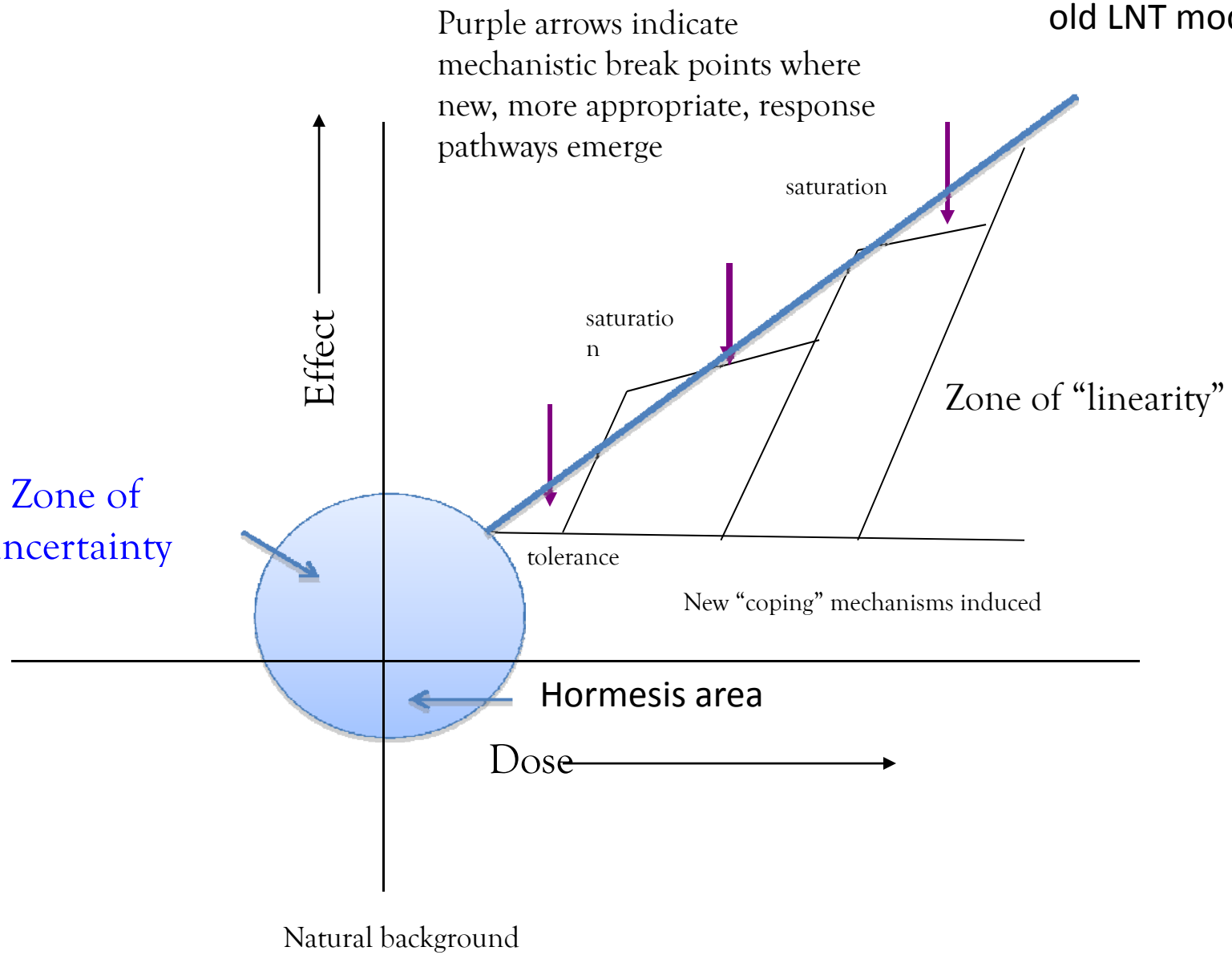
- Individual

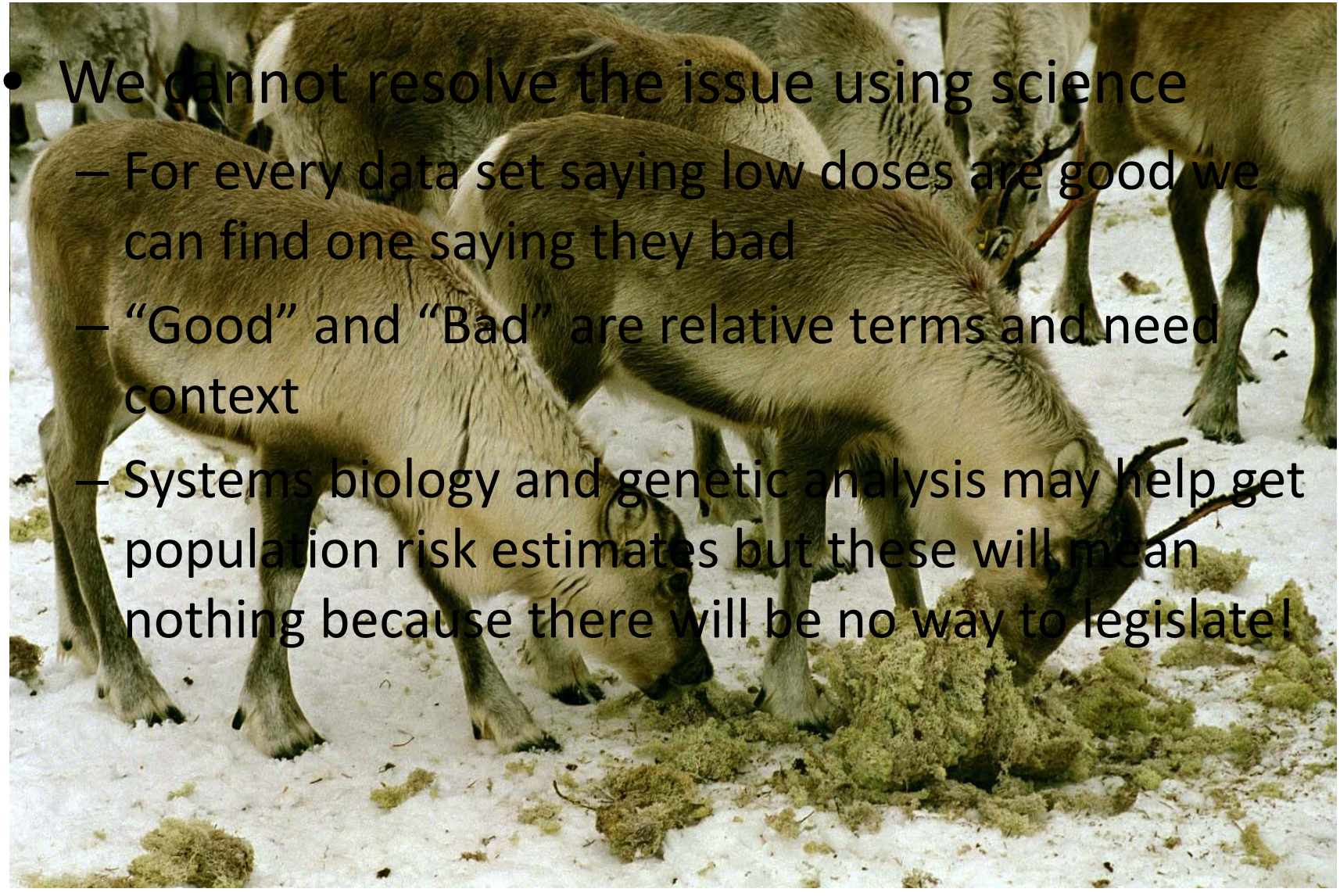


# Proposed dose response relationship for radiation in the context of non-targeted effects

Blue line represents old LNT model

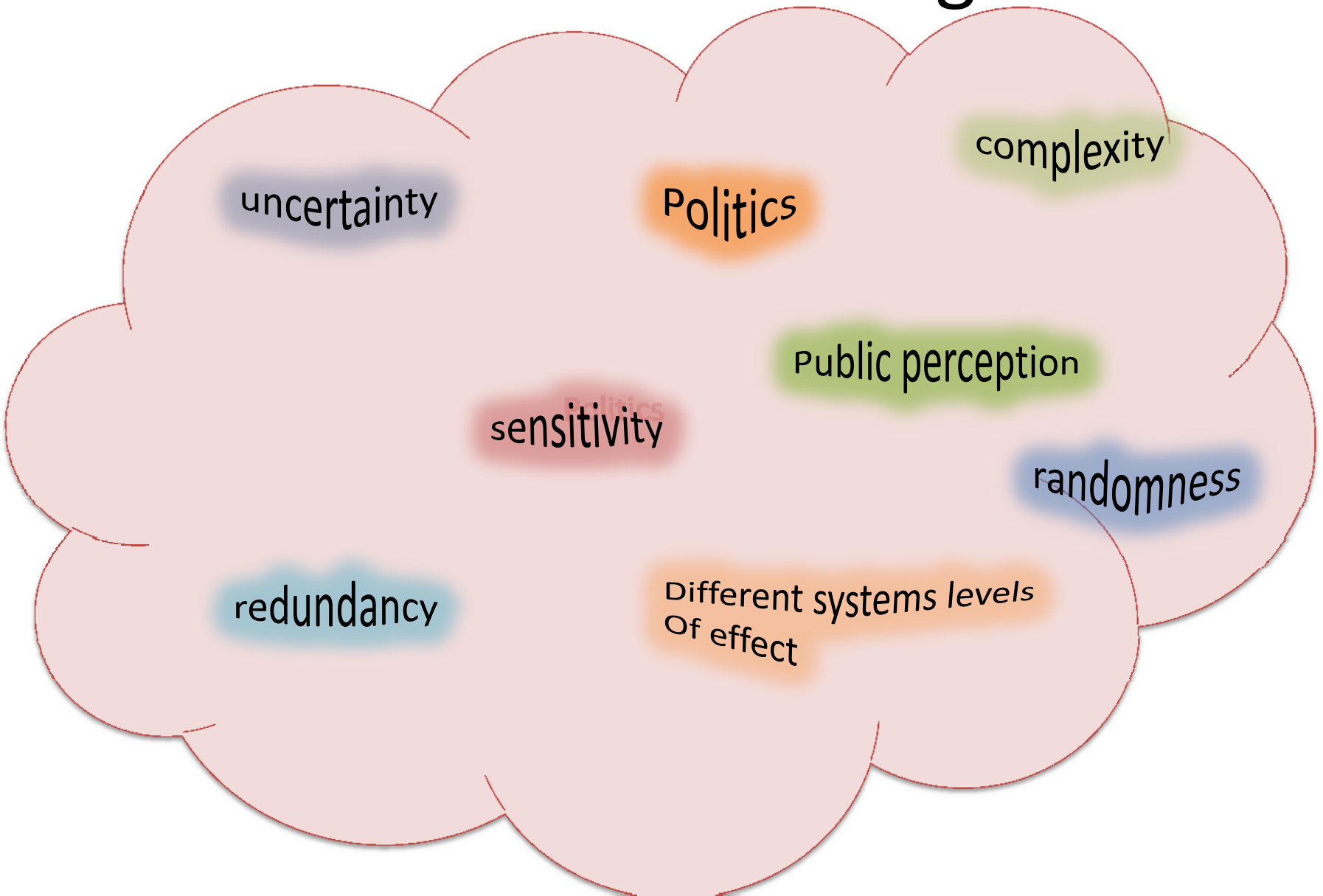


# Talk science ‘til the cows (reindeer) come home BUT



- We cannot resolve the issue using science
  - For every data set saying low doses are good we can find one saying they bad
  - “Good” and “Bad” are relative terms and need context
  - Systems biology and genetic analysis may help get population risk estimates but these will mean nothing because there will be no way to legislate!

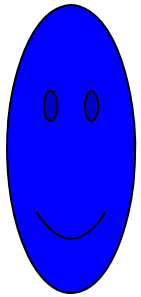
# So what are we dealing with?



# NTE's allow multiple outcomes – facilitate adaptive responses and evolution

- Genomic Instability (GI) opens up the chance for change and adaptive or mal-adaptive evolution
- Bystander effects (BSE) signal between hierarchical levels to coordinate responses at different organisational levels
- GI + BSE allow spatial and temporal system control but…
- in any system change only favours a few

# The unifying theory!



Original phenotype

A biologists view!

Environmental stress

Genetic background

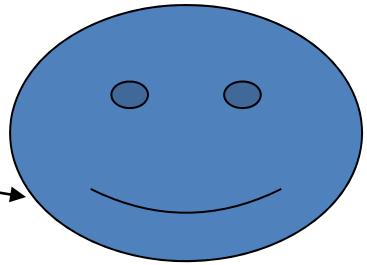
Unstable cell

Hypermutable

Selection pressure

Chance to change

Adaptation



New phenotype

CM 1996

Non targeted effects exist

They manifest at high frequency in many ways

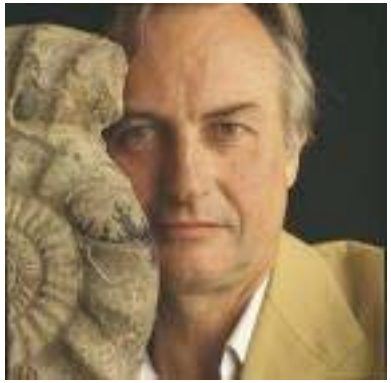
They cause “stress-like” symptoms

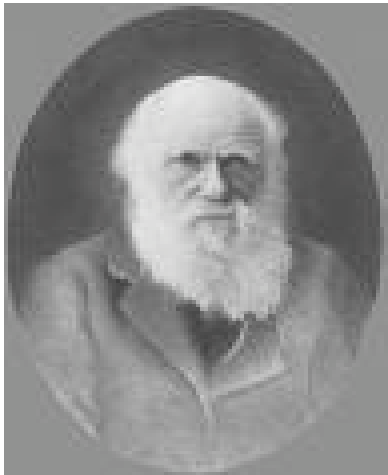
We know nothing about how they impact low dose risk  except that they mean it is not a simple relationship

We know a lot about the mechanisms but little about the reasons why they are tolerated

The underlying debate about purpose or chance is as old as Plato and Aristotle

# SUMMARY





Bottom Line: NTE’s mean the current approach to risk estimates is fatally flawed


THANK YOU