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Genotoxic and epigenetic effects of different low dose rates of gamma radiation in three mouse strains

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Norwegian Institute of Public Health

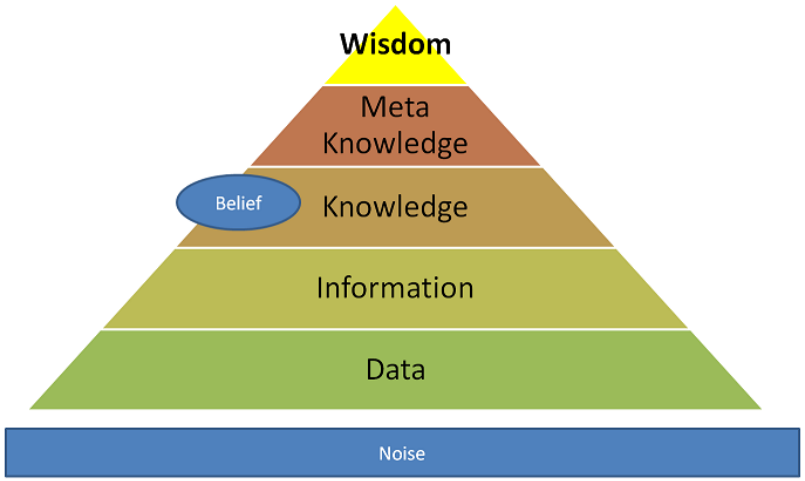
Outline

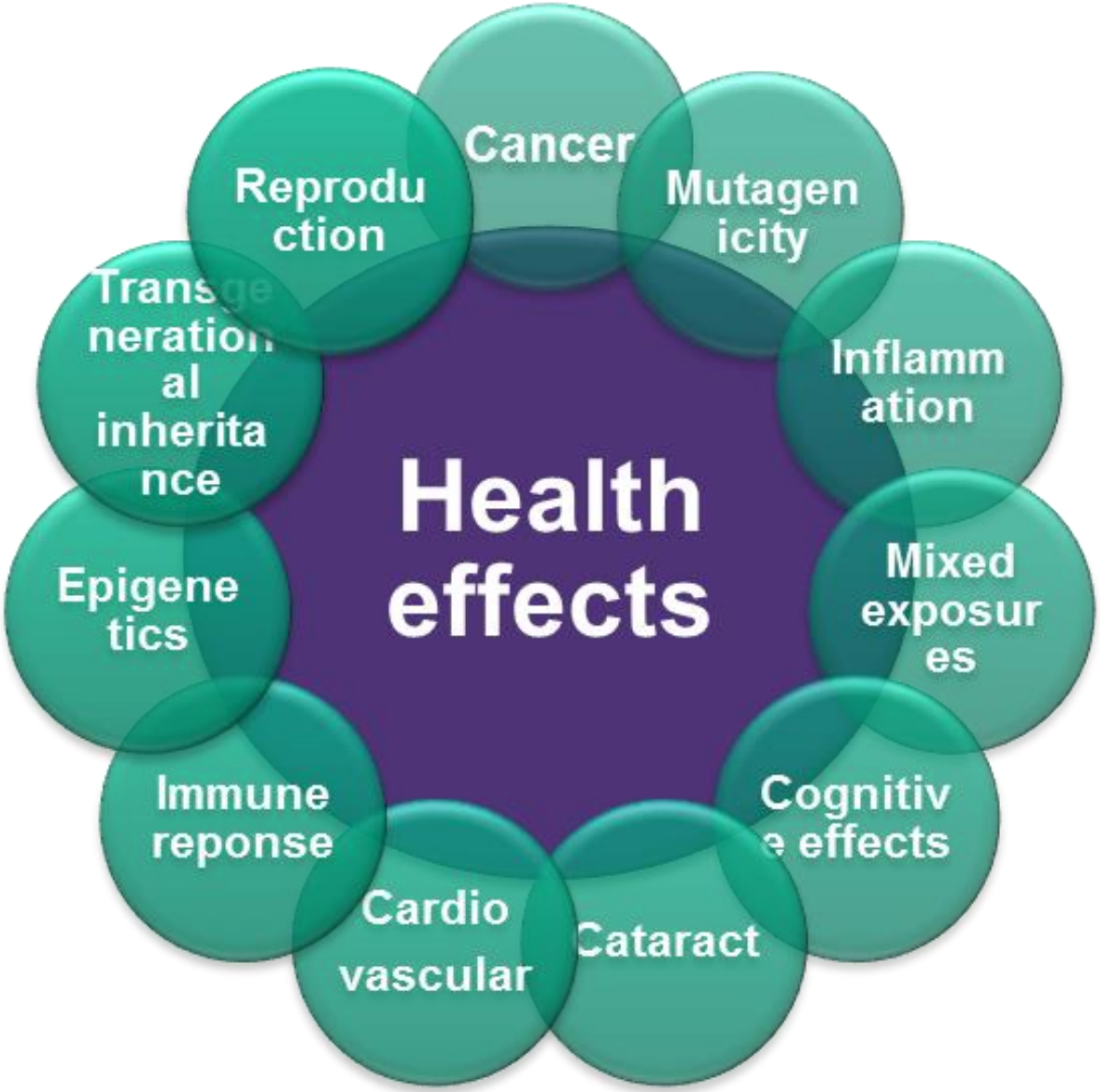
1. Background
2. Aims
3. Study design
4. Results
5. Conclusion

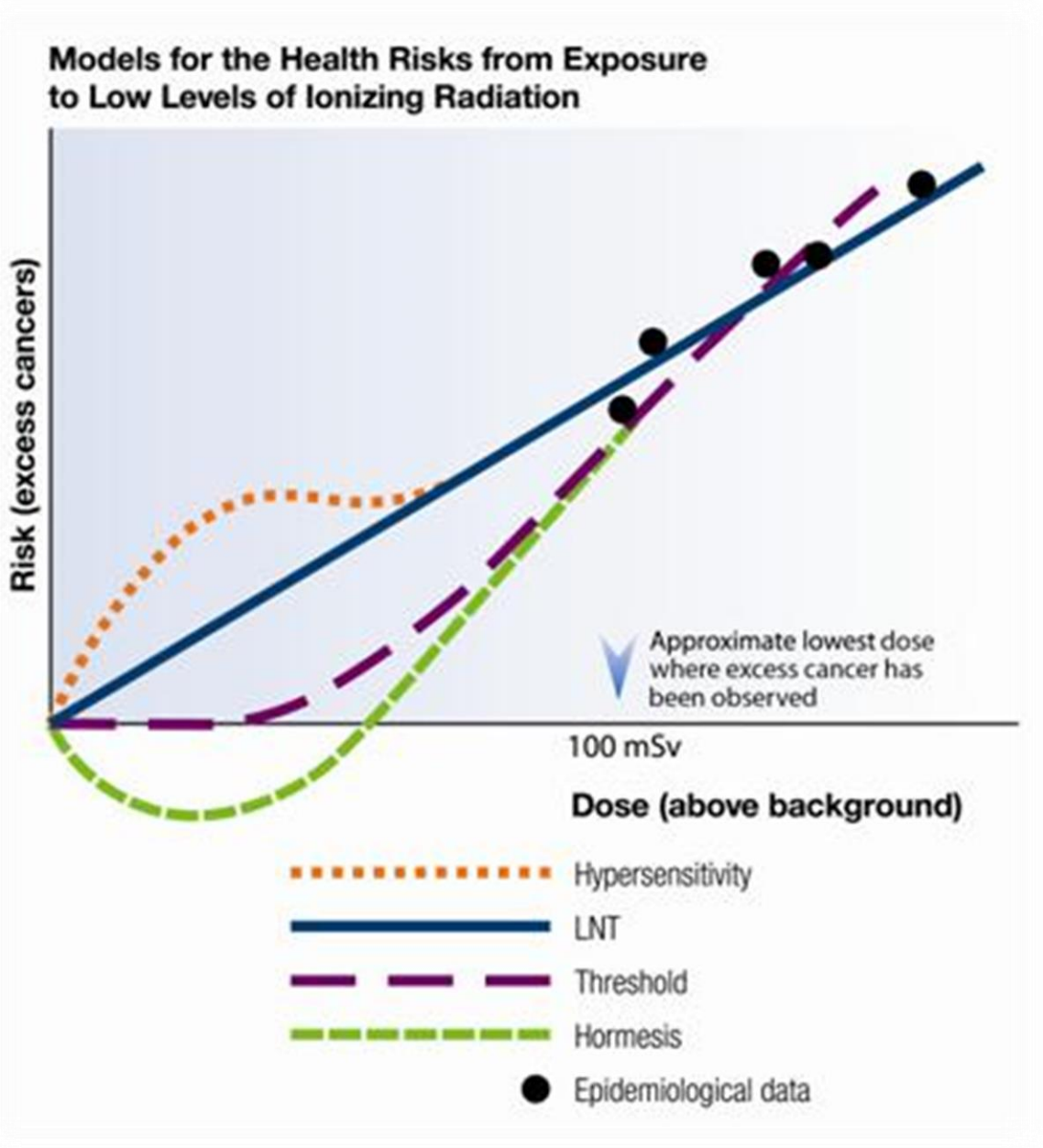
Background

Concern

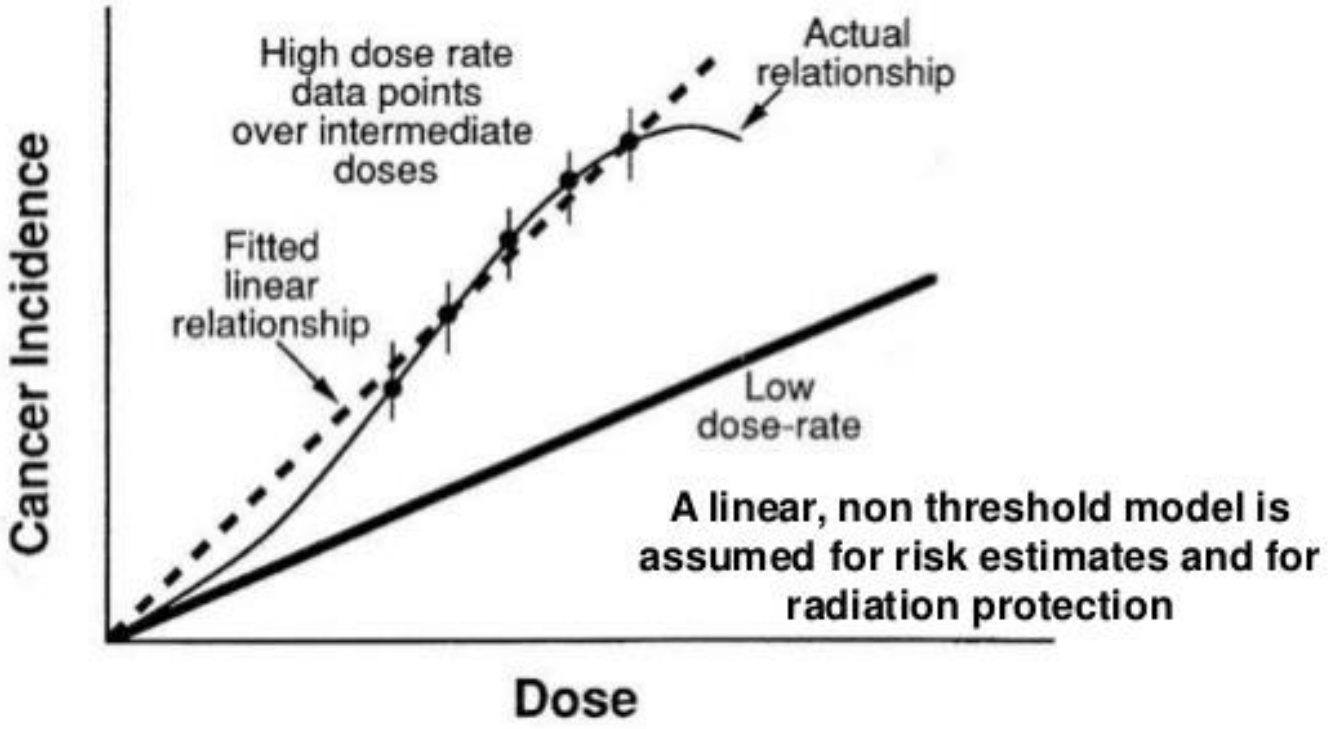
- Humans – risk assessment uncertainties
- Contribute to knowledge based decisions




Health effects
Cancer
Mutagenicity
Inflammation
Mixed exposures
Cognitive effects
Cataract
Cardio vascular
Immune reponse
Epigenetics
Transgenerational inheritance
Reproduction


Models for the Health Risks from Exposure to Low Levels of Ionizing Radiation
Risk (excess cancers)
Approximate lowest dose where excess cancer has been observed
100 mSv
Dose (above background)
Hypersensitivity
LNT
Threshold
Hormesis
Epidemiological data

Shape of dose response curve



Dose Rate Effectiveness Factor (DREF) = Ratio of cancer risk at high compared to low dose or low dose rate.

The *Figaro* facility

- Gamma radiation source (^{60}Co)
- 12 Ci (2012)
- Climate-controlled experimental hall (temperature, light and humidity)
- GMO approved
- Ca. 6 m x 4 m x 20 m
- Dose rate range: 2.9 Gy/h – 400 µGy/h

Background

	1. Project – 2013
Exposure	Gamma radiation
Dose rate	1.4 mGy/h
Duration	45 days
Total dose	1.5 Gy
Mouse strain/model	C57BL/6 background *Ogg1*$^{+/-}$ and *Ogg1*$^{-/-}$
Main findings	LDR is genotoxic (chromosomal damage, increased DNA damage) Graupner *et al.* 2016 *Scientific Reports*

Low dose rate: <6 mGy/h
(UNSCEAR, 2010)

Background

	1. Project – 2013	2. Project – 2015
Exposure	Gamma radiation	Gamma radiation
Dose rate	1.4 mGy/h	2.1 mGy/h
Duration	45 days	32 and 63 days
Total dose	1.5 Gy	1.7 Gy and 3.1 Gy
Mouse strain/model	C57BL/6 background *Ogg1*$^{+/-}$ and *Ogg1*$^{-/-}$	CBAB6 F1 background *Apc*$^{+/+}$ and *Apc*$^{Min/+}$
Main findings	LDR is genotoxic (chromosomal damage, increased DNA damage) *Graupner et al. 2016 Scientific Reports*	Chromosomal damage (independent of total dose) *Graupner et al., in review*

Low dose rate: <6 mGy/h
(UNSCEAR, 2010)

Background

	1. Project – 2013	2. Project – 2015	3. Project – 2016
Exposure	Gamma radiation	Gamma radiation	**Gamma radiation**
Dose rate	1.4 mGy/h	2.1 mGy/h	**2.5, 10, 100 mGy/h**
Duration	45 days	32 and 63 days	**30 h, 14 days, 54 days**
Total dose	1.5 Gy	1.7 Gy and 3.1 Gy	**3 Gy**
Mouse strain/model	C57BL/6 background *Ogg1*$^{+/-}$ and *Ogg1*$^{-/-}$	CBAB6 F1 background *Apc*$^{+/+}$ and *Apc*$^{Min/+}$	**C57BL/6N** **CBA/Ca** **CBA Spm/+** **(acute myeloid leukemia)**
Main findings	LDR is genotoxic (chromosomal damage, increased DNA damage) *Graupner et al. 2016 Scientific Reports*	Chromosomal damage (independent of total dose) *Graupner et al., in review*	

Low dose rate: <6 mGy/h
(UNSCEAR, 2010)

Aims

Aims

1) Main aim: reduce uncertainties in risk assessment
   1) Establish dose-rate-response relationships by employing several dose rates (low and high)
   2) Assess the general applicability of results and verify previous findings by using three different mouse strains
      (two inbred and one mutant strain acquiring radiation relevant cancer)
   3) Identify potential adaptation due to continuous radiation
   4) Determine if the radiation has inflicted epigenetic changes in the genome

Study design

Study design

www.jax.org

C57BL/6N, CBA/Ca and CBA Spm/+
8 weeks ♂

Group I
100 mGy/h for 30 hours

Group II
10 mGy/h for 14 days

Group III + Group IV
2.5 mGy/h for 55 days

3 Gy total dose

Group IV
7 days w/out IR

Acute x-ray:
1.5 Gy/min (total dose 3 Gy)

Study design

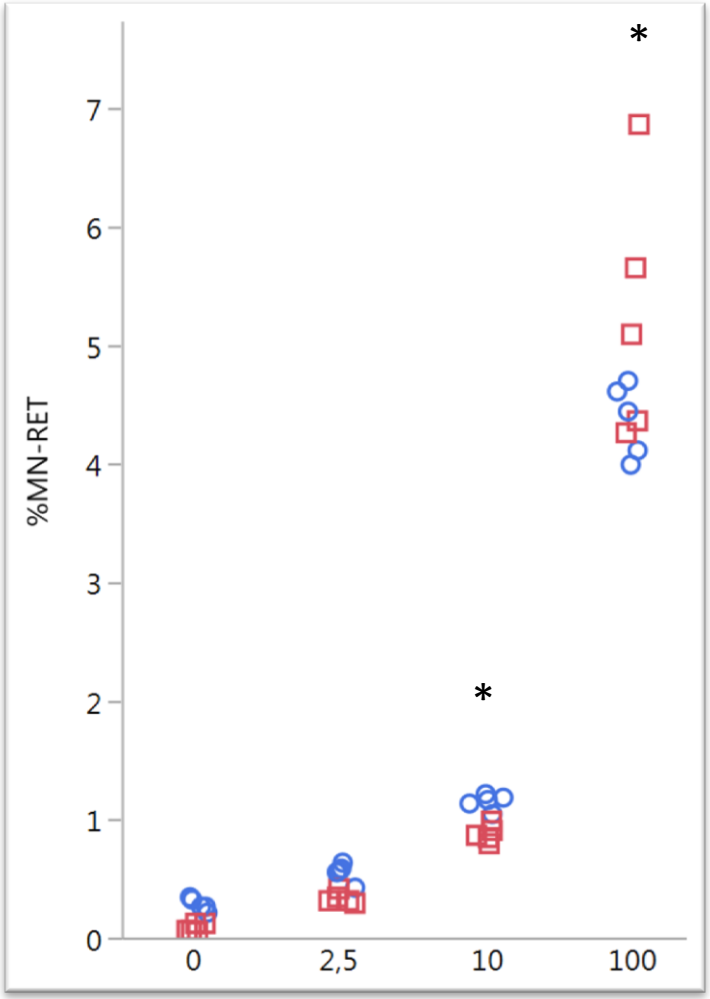
a. Genotoxic endpoints
   i. Induction of **micronuclei (flow cytometer)***
   ii. Induction of mutations using the *Pig-a* gene mutation assay (flow cytometer)
   iii. DNA damage analyses using the single cell gel electrophoreses (SCGE or Comet assay) with emphasis on oxidized DNA (inclusion of Fpg)
b. Epigenetic endpoints
   i. Global **DNA methylation (HPLC-MS/MS)***
   ii. miRNA-analyses
   iii. Histone modifications

***herein presented preliminary results**

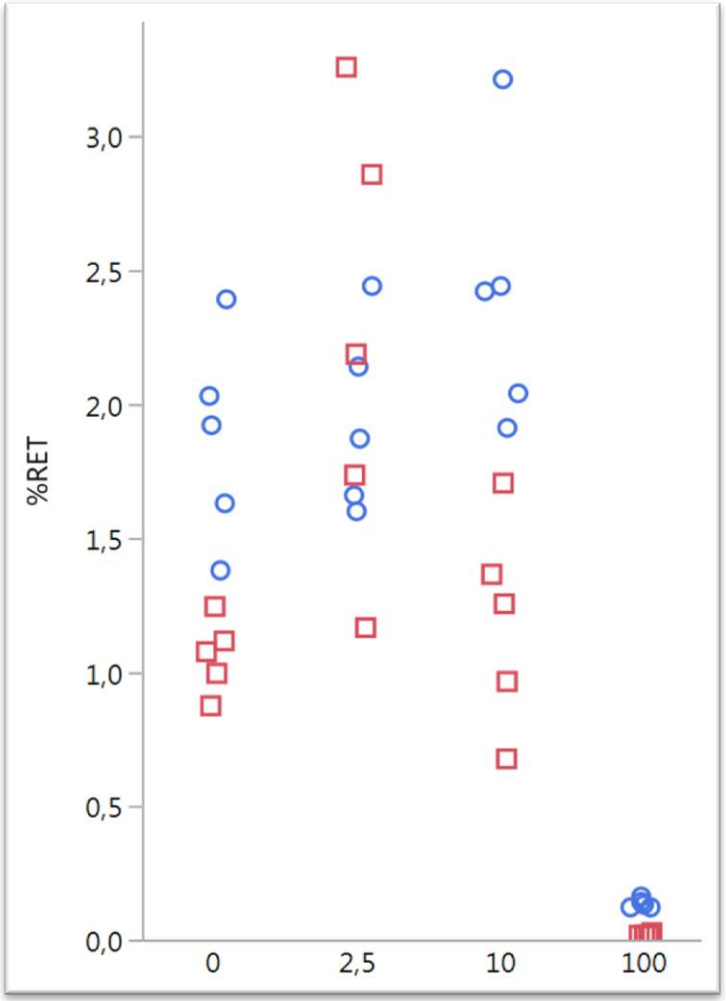
Results

Micronucleus assessment

(state of the art method, flow cytometry based)



mGy/h gamma radiation



mGy/h gamma radiation

* p<0.001

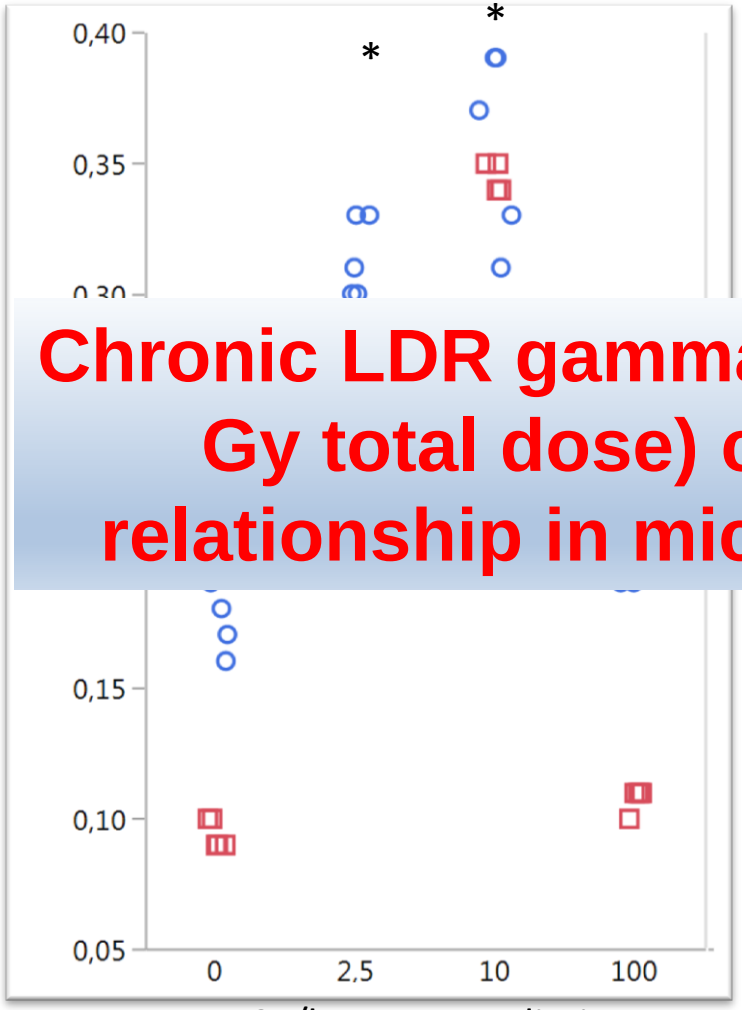
CBA/CaOla

C57BL/6N

Norwegian Institute of Public Health

Micronucleus assessment

(state of the art method, flow cytometry based)



mGy/h gamma radiation

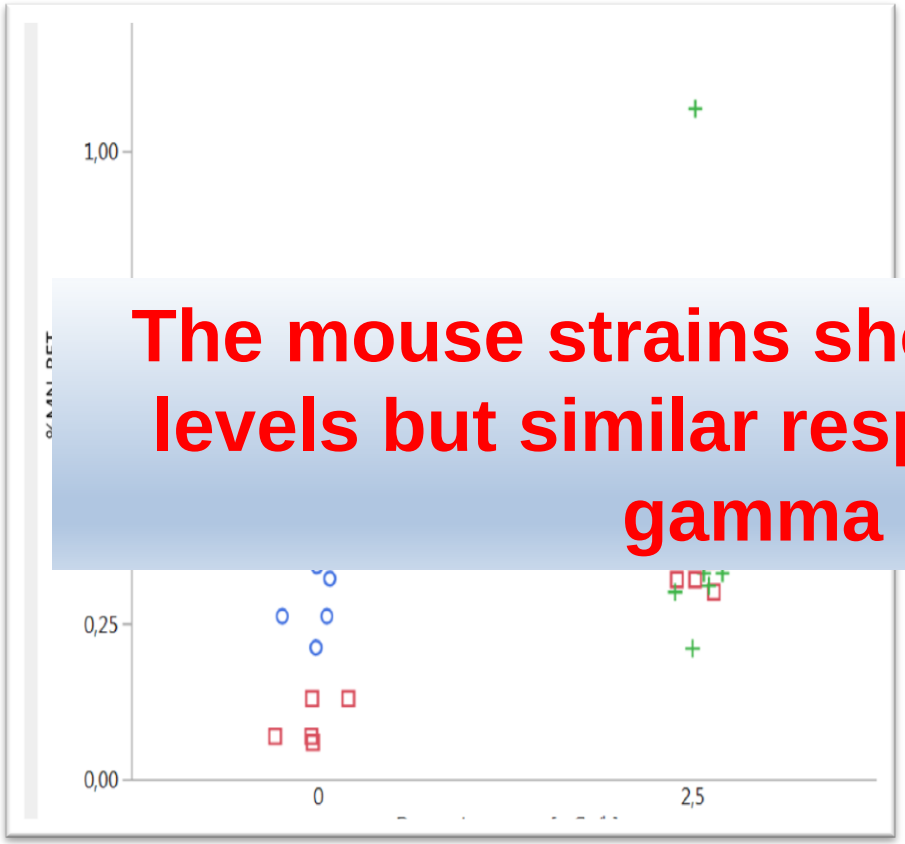
* p<0.001

Chronic LDR gamma radiation (2.5-100 mGy/h, 3 Gy total dose) causes a dose response relationship in micronucleated reticulocytes.

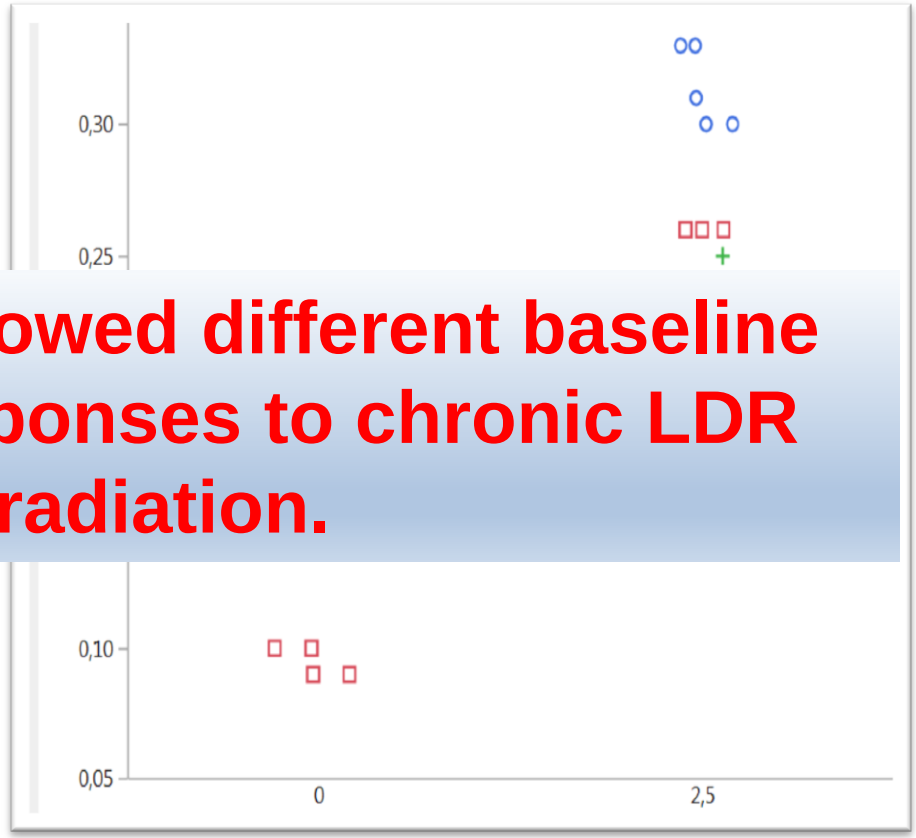
CBA/CaOla

C57BL/6N

Generality of response, 3 mouse strains



mGy/h gamma radiation



mGy/h gamma radiation

The mouse strains showed different baseline levels but similar responses to chronic LDR gamma radiation.

+ CBA Spm/+

□ CBA/CaOla

○ C57BL/6N

Study design

www.jax.org

C57BL/6N, CBA/Ca and CBA Spm/+
8 weeks ♂

Group I
100 mGy/h for 30 hours

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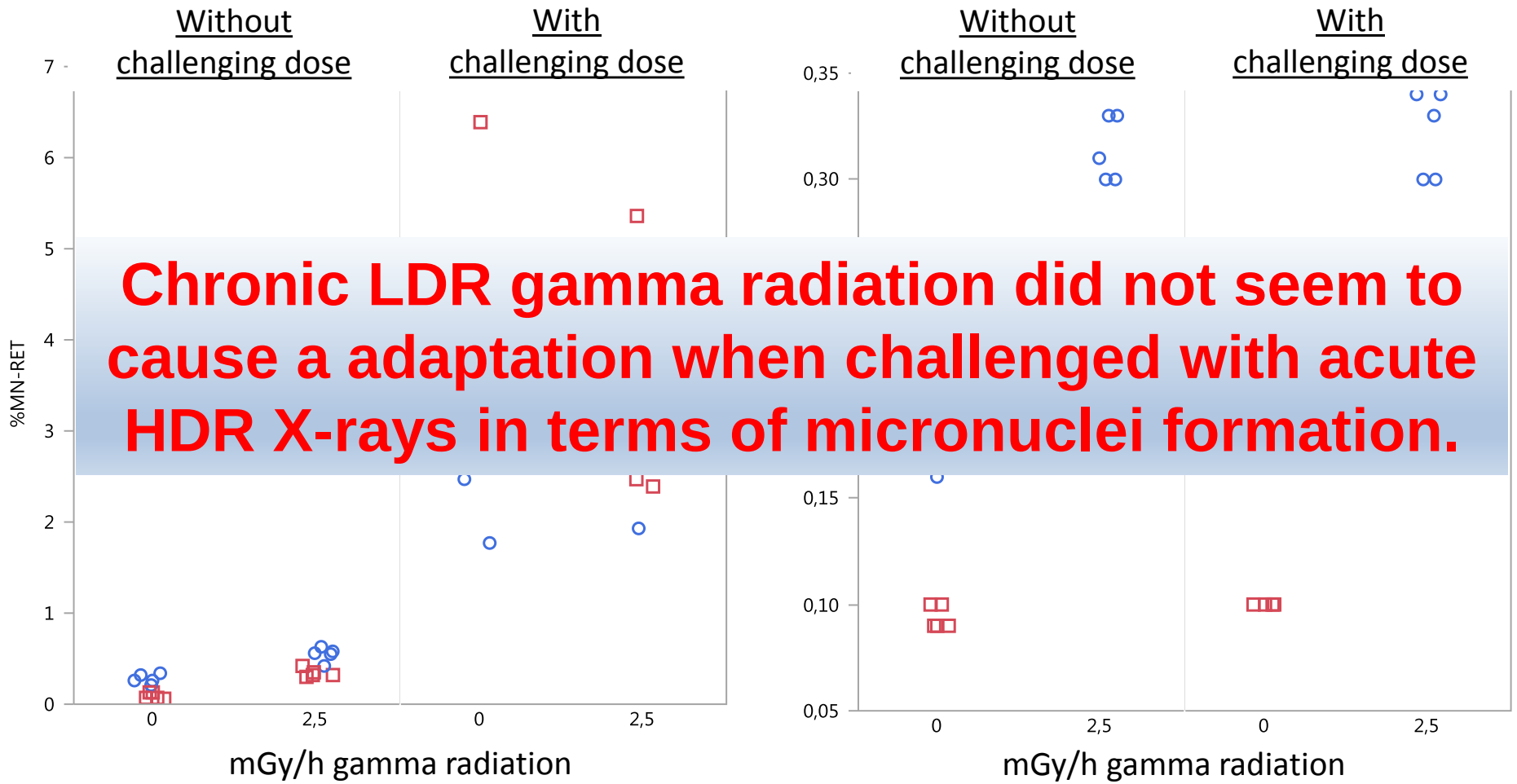
3 Gy total dose

Group III + Group IV
2.5 mGy/h for 55 days

Group IV
7 days w/out IR

Acute x-ray:
1.5 Gy/min (total dose 3 Gy)

Micronucleus assay (flow cytometry)



Chronic LDR gamma radiation did not seem to cause a adaptation when challenged with acute HDR X-rays in terms of micronuclei formation.

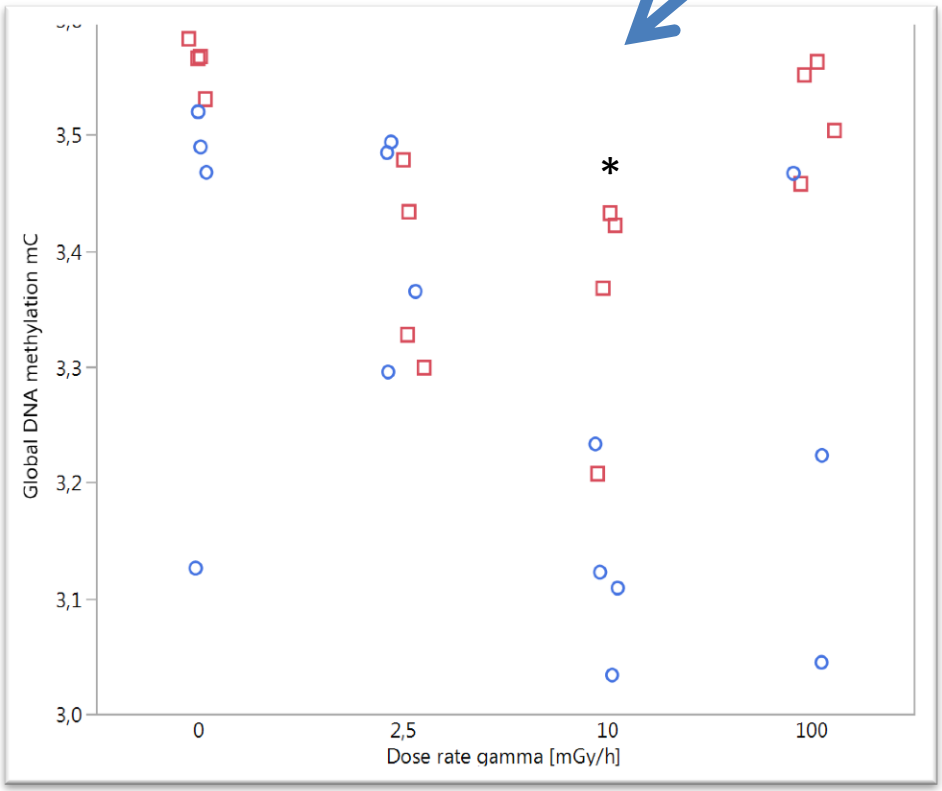
CBA/CaOla

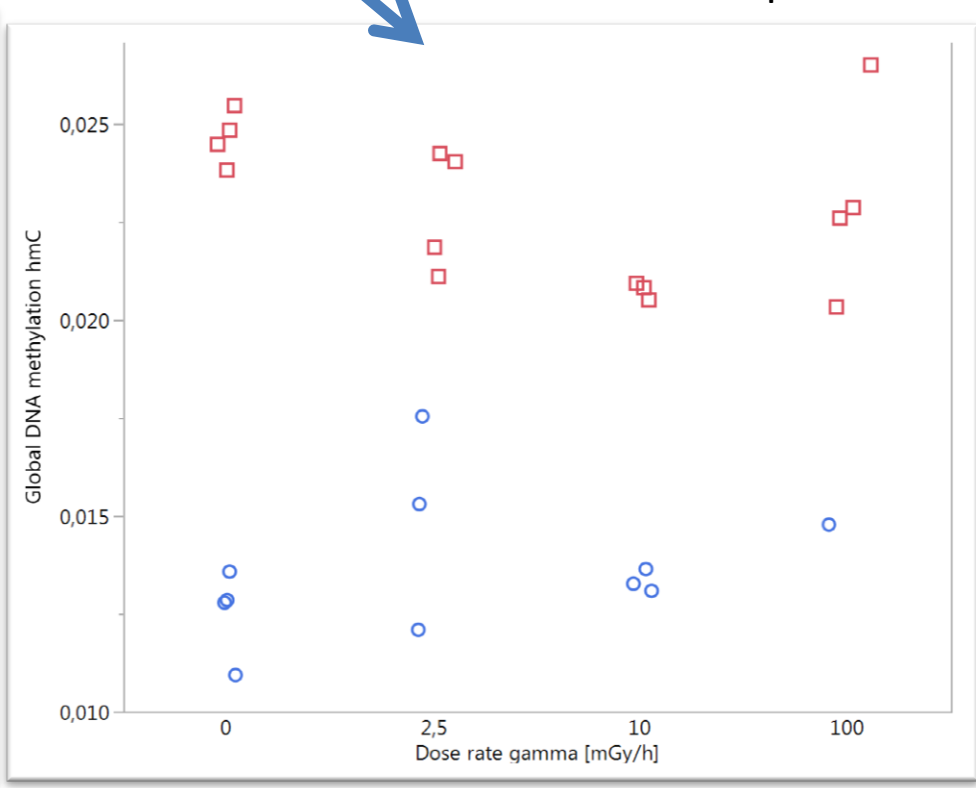
C57BL/6N

Epigenetic changes: Global DNA methylation

Cytosin → 5-methyl-cytosin → 5-hydroxymethyl-cytosin

* p<0.001



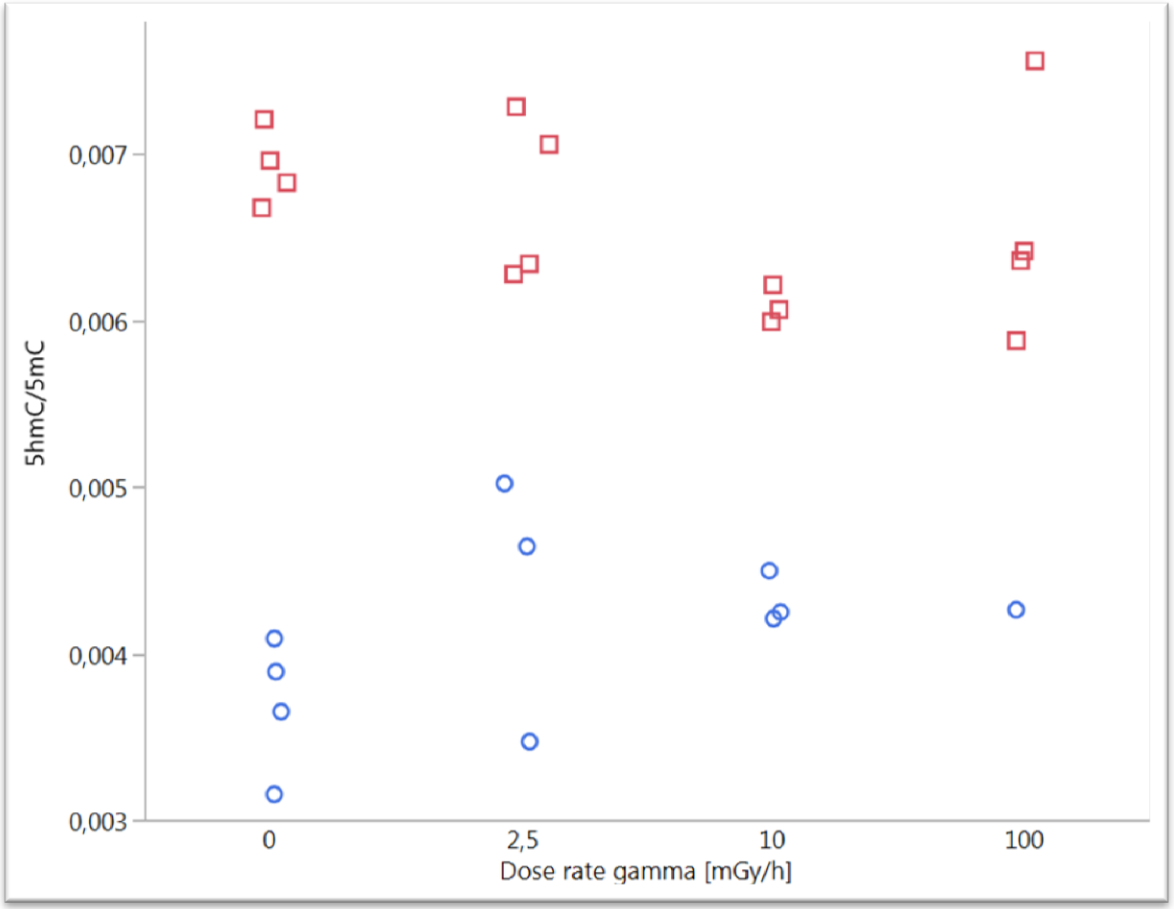


CBA/CaOla

C57BL/6N

Global DNA methylation, ratio 5hmC/5mC

Cytosin → 5-methyl-cytosin → 5-hydroxymethyl-cytosin



5-hydroxymethyl-cytosin/
5-methyl-cytosin

CBA/CaOla

C57BL/6N

Conclusions

- Chronic LDR gamma radiation (2.5, 10, 100 mGy/h) causes a dose response relationship in micronucleated reticulocytes (total dose 3 Gy).

- The mouse strains showed different baseline levels but similar responses to chronic LDR gamma radiation.

- A chronic LDR gamma radiation did not seem to cause a adaptation when challenged with acute HDR X-rays in terms of micronuclei formation.

Norwegian Institute of Public Health

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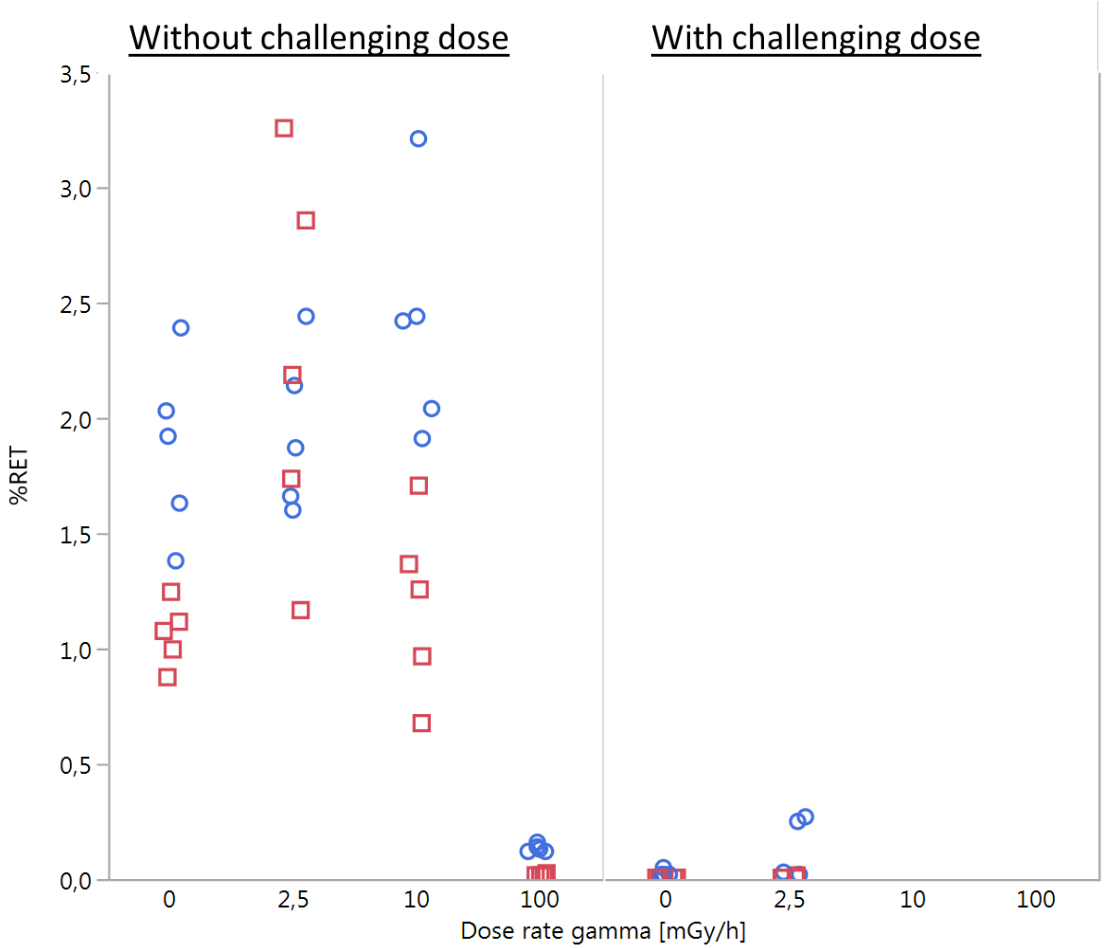
Micronucleus assay (flow cytometry)



CBA/CaOla

C57BL/6N