Relative Biological Effectiveness of Thermal Neutrons

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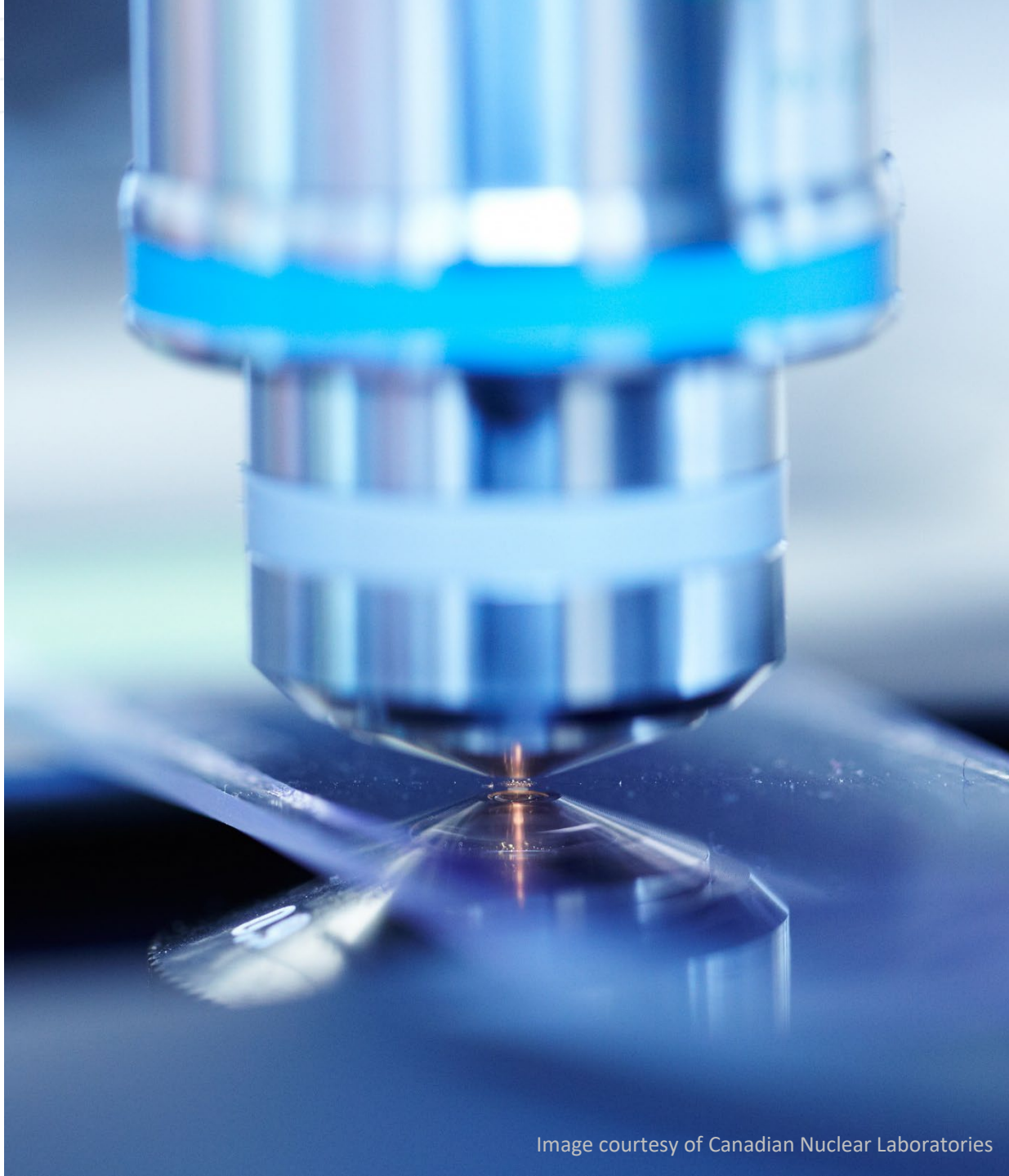
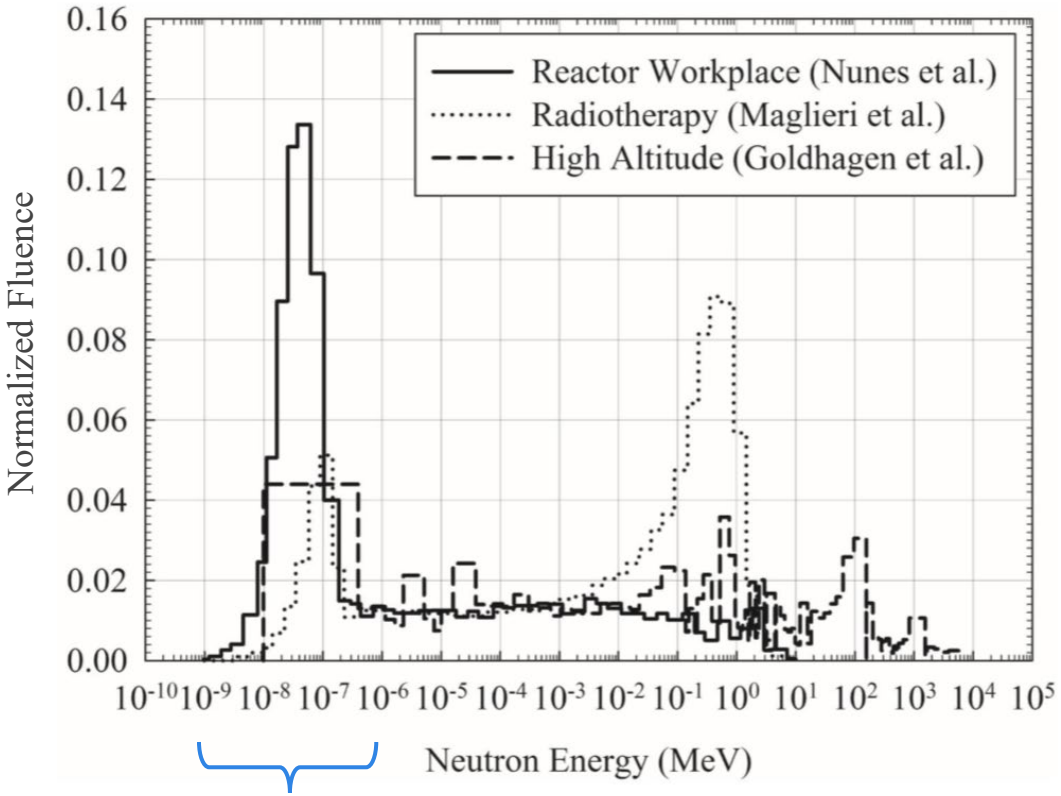


Image courtesy of Canadian Nuclear Laboratories

Background

- Secondary neutrons are a by-product of proton and high-energy photon radiotherapy treatments. These neutrons cannot be shielded, resulting in out-of-field patient exposures. There is concern that these neutrons may drive iatrogenic cancer development in patients where life expectancy is anticipated to exceed the latency period for secondary malignancy development.
- A wide range of neutron energies can be found in photon radiotherapy treatment bunkers, inside high altitude aircraft, and in nuclear reactor workplaces (shown on graph). It is important to know the neutron energy spectrum because neutron-induced carcinogenesis is energy-dependent.
- Previous theoretical microdosimetry by Ali et al. (2018) indicated the mean quality factor of **low-energy** thermal neutrons was nearly 10 times larger than the International Commission on Radiological Protection (ICRP) radiation weighting factor (w_R) of 2.5. Quality factors and w_R factors are used to describe the biological damage imparted by a particular radiation, as compared to gamma or x-ray radiation (which have a w_R of 1).

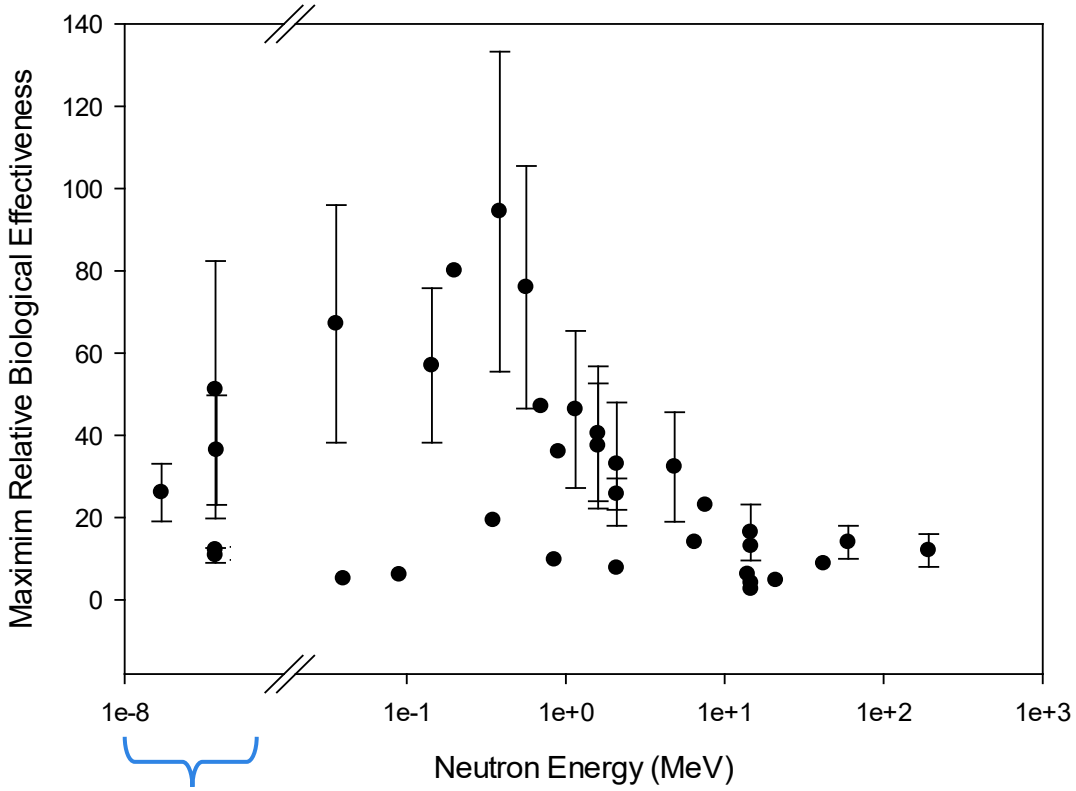


Thermal neutron energy range

Image from: Ali, F., Atanackovic, J., Boyer, C., Festarini, A., Kildea, J., Paterson, L.C., Rogge, R., Richardson, R.B. *Dosimetric and microdosimetric analyses for blood exposed to reactor-derived thermal neutrons.* Journal of Radiological Protection, 38:1037-1052 (2018)

Background

- **Goal:** The goal of this study was to experimentally examine the relative biological effectiveness (RBE) of 64 meV thermal neutrons in human peripheral blood lymphocytes (as compared to gamma radiation), to determine whether experimental data aligned with the ICRP w_R factor. Clustered damage, often considered a hallmark of densely ionizing radiation, was also evaluated.
- **Three different endpoints were used in this study:** dicentric chromosome, micronuclei, and apoptosis. There are many previously published dicentric chromosome assay (DCA) RBE studies in human lymphocytes for comparison. Less data exists for other endpoints. For the DCA data (graph on right), there are large discrepancies between laboratories (especially at lower energies), and large variances for many of the data points.
- **Hypothesis (null):** There is no difference between the thermal neutron RBE and the ICRP w_R of 2.5.
- **Hypothesis (alternative):** There is a significant difference between the thermal neutron RBE and the ICRP w_R of 2.5.



Thermal neutron energy range

Graph data compiled by L. Paterson

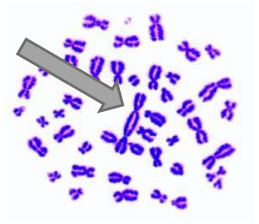
Methods

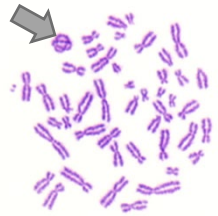
- Quartz test tubes containing human blood were irradiated with 64 meV thermal neutrons at Canadian Nuclear Laboratories in Chalk River, ON in the NRU reactor facility using the Canadian Neutron Beam Centre's E3 spectrometer. The 64 meV energy was chosen due to availability. The details of the beam-line configuration and the associated physical modelling has been described previously by Ali *et al.* (2018).
- The $^{14}N(n.p)^{14}C$ capture reaction contributed nearly 71% of the total absorbed dose, and tertiary electrons from the $^{1}H(n,\gamma)^{2}H$ capture reaction were responsible for the remaining absorbed dose.
- Cell cultures were established 18 hours post-exposure. This time interval had to be accepted to ensure sufficient radionuclide decay prior to sample handling.
- For the dicentric chromosome assay (DCA), complete metaphase spreads in the first cell cycle containing 46 centromeres were evaluated by microscopy. The presence of dicentric and ring chromosomes were enumerated as damages.
- For the micronucleus assay, micronuclei were quantified via microscopy in cytochalasin-blocked bi-nucleated cells.
- Apoptosis was defined as Annexin V+/7AAD- and Annexin V+/7AAD+ cells. Image data was collected using an Amnis Image Stream Mark II imaging flow cytometer.
- Maximum RBE (RBE_M) was calculated as the ratio of the dose-response regression equation α-coefficient values from thermal neutron exposures and reference photon exposures.
- Clustering of damage was evaluated by testing for compliance with the Poisson distribution by calculating the dispersion index (variance, σ^2 / mean, y) and the u test statistic, where statistic values above 1.96 indicated non-Poissonian over-dispersion (clustering) at the 5% significance level.

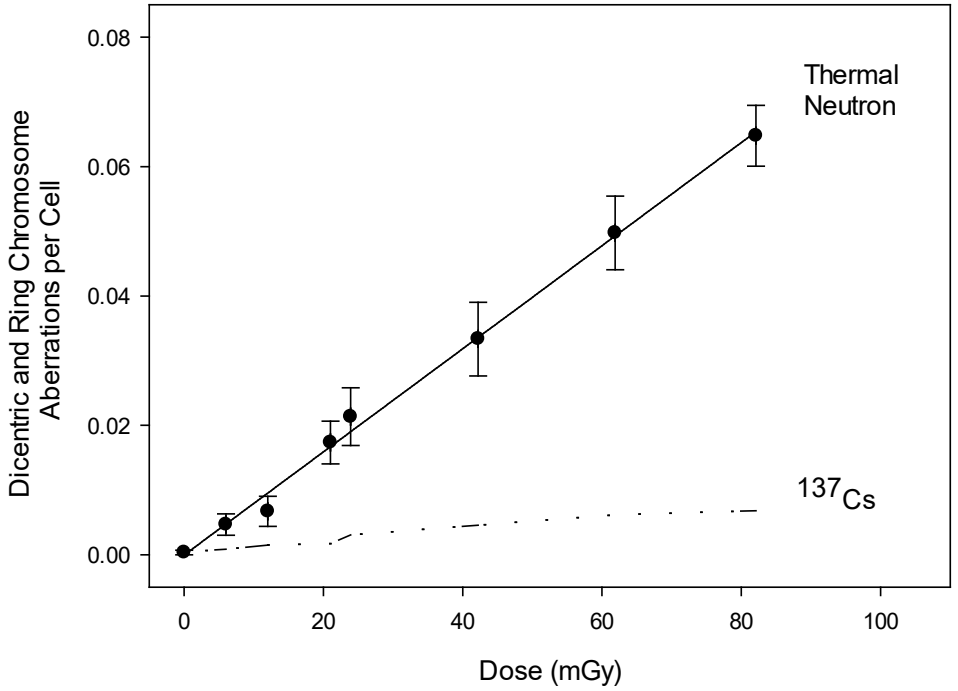
Results

DICENTRIC CHROMOSOME ASSAY (DCA)

- RBE_M was calculated using previously published ^{137}Cs DCA dose response curve by Flegal et al. (2012) in which the first author participated.
- This resulted in an **$RBE_M = 11.3 \pm 1.6$**
- Clustered damage was seen at 5 of 7 dose points. However, as a result of the very low doses examined here, only a single additional cell containing two or more aberrations was required to achieve over-dispersion at both dose points.
- **Key finding:** Thermal neutron DCA RBE_M is much higher than w_R of 2.5 prescribed by ICRP.





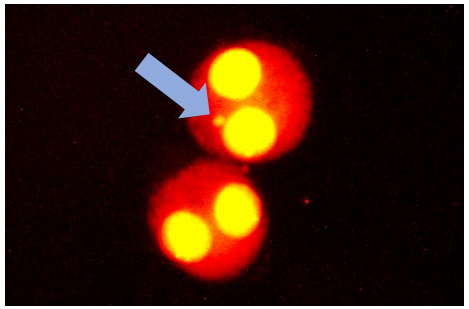


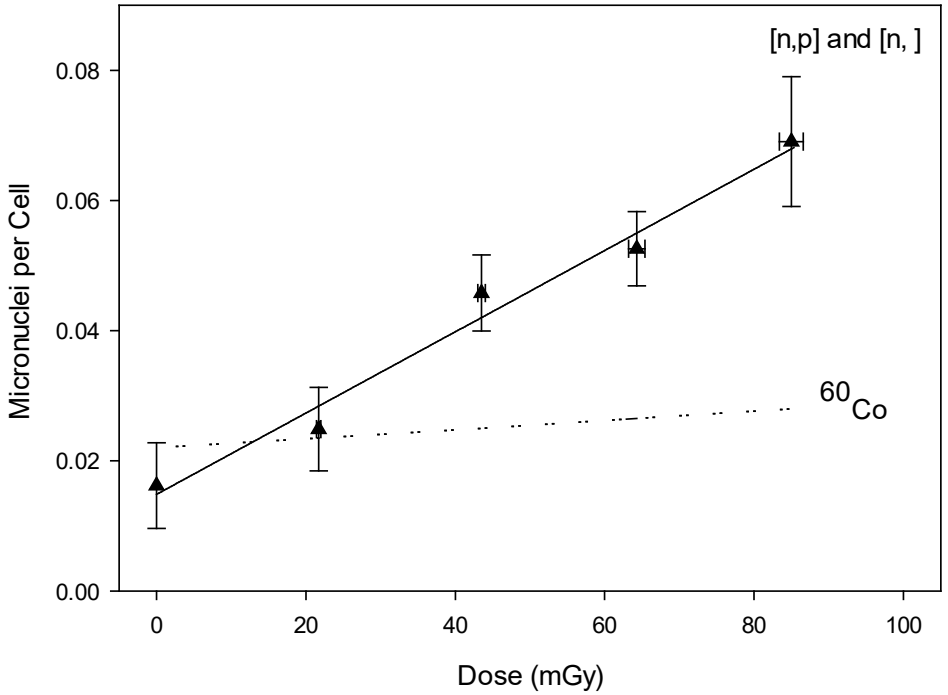
Total Dose ± SE (mGy)	Cells Scored	Total Aberr.	Distribution of Aberr. 0	1	2	3	Disp. Index (σ^2/y)	u value
0 ± 0	2,800	1	2,799	1	0	0	1.00	-
6.0 ± 0.2	1,500	7	1,494	5	1	0	1.28	**8.34**
12.0 ± 0.3	1,414	11	1,404	11	0	0	0.99	-0.20
21.0 ± 0.4	1,500	26	1,478	18	4	0	1.29	**8.13**
23.9 ± 0.6	1,500	32	1,469	30	1	0	1.04	1.16
42.2 ± 0.6	1,500	50	1,456	39	4	1	1.25	**6.84**
61.9 ± 0.7	2,030	101	1,940	80	9	1	1.19	**6.03**
82.1 ± 1.8	1,575	102	1,487	75	12	1	1.23	**6.49**

Results

MICRONUCLEUS ASSAY

- RBE_M was calculated using a previously published ^{60}Co micronucleus dose response curve by McNamee et al. (2009) in which the first author participated.
- This resulted in an **$RBE_M = 9.1 \pm 1.1$**
- Clustered damage was found at all micronucleus dose points, an unsurprising finding for the micronucleus assay due to the assay tendency towards over-dispersion.
- **Key finding:** Thermal neutron DCA RBE_M is much higher than w_R of 2.5 prescribed by ICRP.



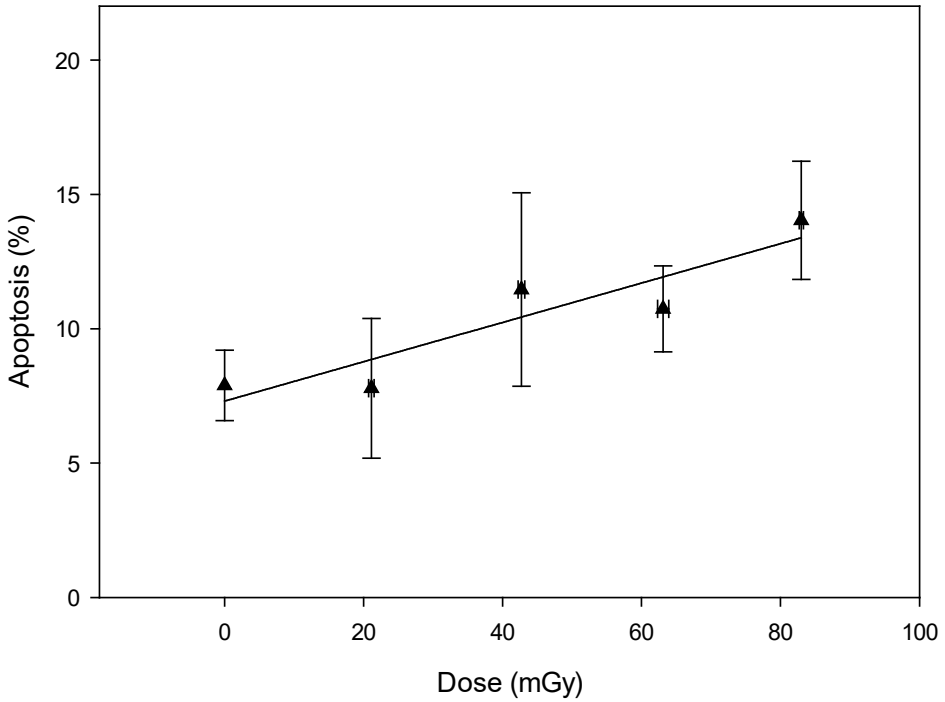


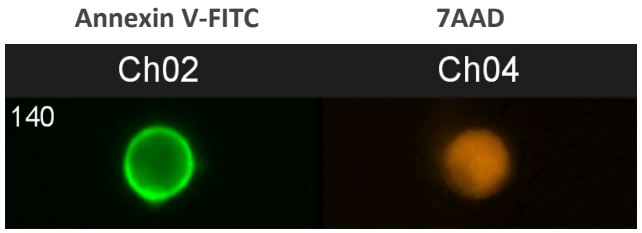
Total Dose ± SE (mGy)	Cells Scored	Total Aberr.	Distribution of Aberr. 0	1	2	3	Disp. Index (σ^2/y)	u value
0 ± 0	15,000	243	14,757	216	12	1	1.11	**9.31**
21.7 ± 0.3	15,000	373	14,627	281	37	6	1.27	**23.42**
43.5 ± 0.5	15,000	687	14,313	499	82	8	1.26	**22.76**
64.3 ± 1.1	15,000	789	14,211	578	83	15	1.27	**23.53**
85.0 ± 1.6	15,000	1,036	13,964	759	122	11	1.23	**19.89**

Results

APOPTOSIS ASSAY

- Apoptosis defined as Annexin V+/7AAD- (early apoptosis) and Annexin V+/7AAD+ cells (late apoptosis).
- RBE_M was calculated using a previously published ^{60}Co apoptosis curve (not shown) created under similar experimental conditions.
- This resulted in an **$RBE_M = 3.6 \pm 1.3$**
- **Key finding:** Thermal neutron RBE_M is similar to w_R of 2.5 prescribed by ICRP.

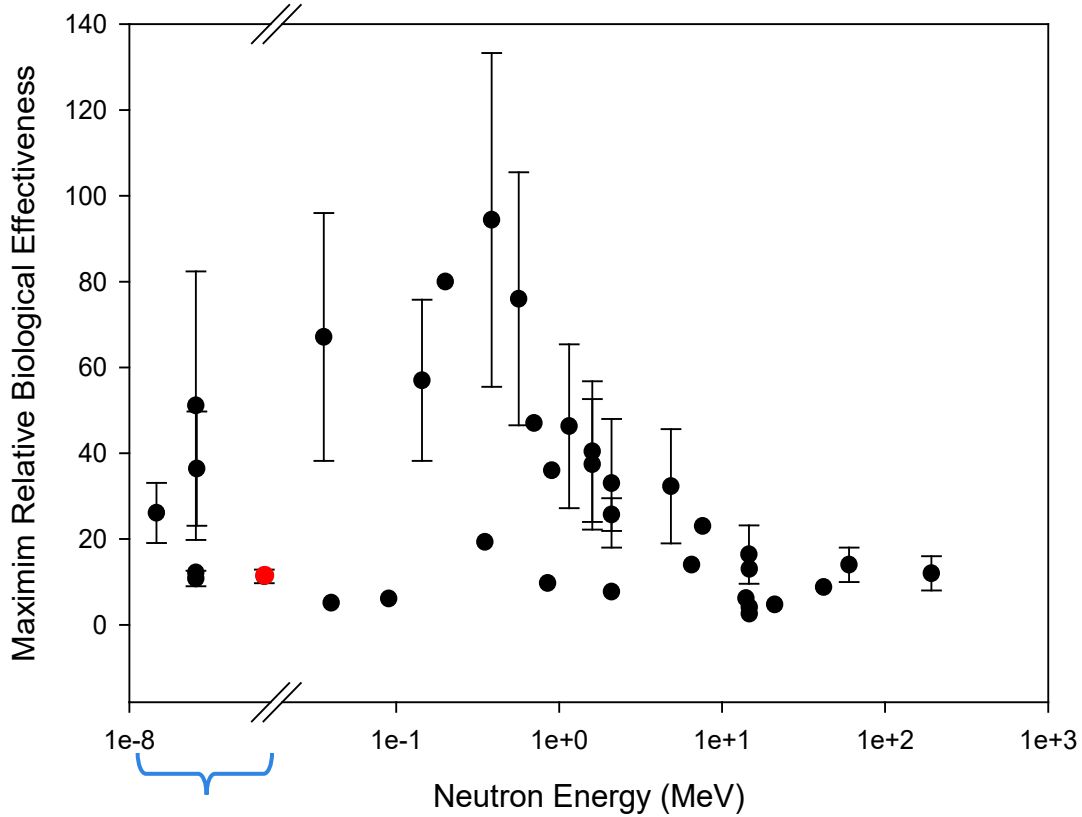




Dose (mGy)	Early Apoptosis %	Early Apoptosis Std Err	Late Apoptosis %	Late Apoptosis Std Err	Live Damaged %	Live Damaged Std Err	Live Cells %	Live Cells Std Err
0 ± 0	3.9	1.2	4.0	0.6	5.6	5.1	86.4	2.0
21.1 ± 0.4	4.2	2.1	3.5	1.6	6.9	4.1	84.4	2.4
42.7 ± 0.5	6.4	2.8	5.1	2.2	5.6	4.4	82.6	0.5
63.1 ± 0.8	5.1	1.4	5.6	0.8	7.8	4.0	81.4	2.6
83.0 ± 0.3	6.3	1.3	7.7	1.7	5.3	3.4	80.7	4.6

Discussion

1. We found DNA damage RBE_M values of around 10 (from the DCA and micronucleus assay), therefore significantly higher than ICRP w_R of 2.5, confirming our alternative hypothesis.
2. The apoptosis RBE_M value was similar to the ICRP w_R, confirming our null hypothesis.
3. While RBE values are known to differ with endpoint, we were surprised to find a difference between the RBE for DNA damage and apoptosis. Further work is needed to elucidate the reason for this discrepancy.
4. Many previously published thermal neutron DCA RBE values had large variability (graph on right). The RBE_M values generated in our study had comparatively low error and better certainty (new DCA value in red).

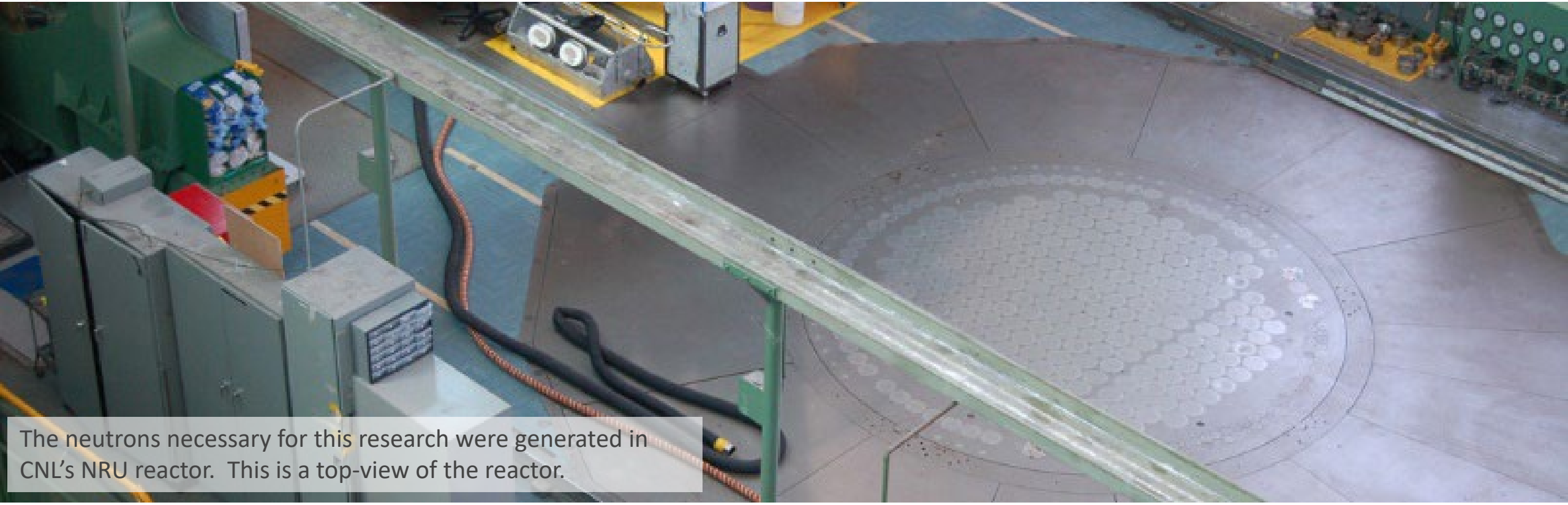


Thermal neutron energy range

Graph data compiled by L. Paterson

Conclusion

This work furthers the understanding of the biological consequences of low-energy thermal neutrons and accurately confirms that extraneous neutrons can cause higher than currently accepted DNA damage in healthy tissues, which could theoretically result in mutation-induced carcinogenesis.



The neutrons necessary for this research were generated in CNL's NRU reactor. This is a top-view of the reactor.

Image courtesy of Canadian Nuclear Laboratories