Evidence for Radiation Hormesis in Human Lymphocytes

Kanokporn Noy Rithidech, Ph.D.
Pathology Department, Stony Brook University,
Stony Brook, NY 11794-8691,
E-mail: krithidech@notes.cc.sunysb.edu

Bobby R. Scott, Ph.D.
Lovelace Respiratory Research Institute,
Albuquerque, NM 87108,
E-mail: bscott@lrri.org

Victor P. Bond, M.D., Ph.D. (1919-2007)

OBJECTIVE

To Determine Hormetic Effects of Low Doses of Radiation with Different Qualities.

Mono-energetic fast neutrons (Radiological Research Accelerator Facility orRARAF of Columbia University, NY)

0.22, 0.44, 1.5, 5.9 and 13.7 MeV

Photons (Brookhaven National laboratory, NY):

X rays (70 and 250 kVp) and Gamma rays

Radiation Dose: 0, 10, 50, and 100 mGy

Chromosome aberrations were selected as a biological endpoint because.....

- Their prevalence is an important indicator of radiation exposure level.

- Their prevalence is a proven biomarker for carcinogenic activity of suspect agents.

Metaphase chromosome aberration assay

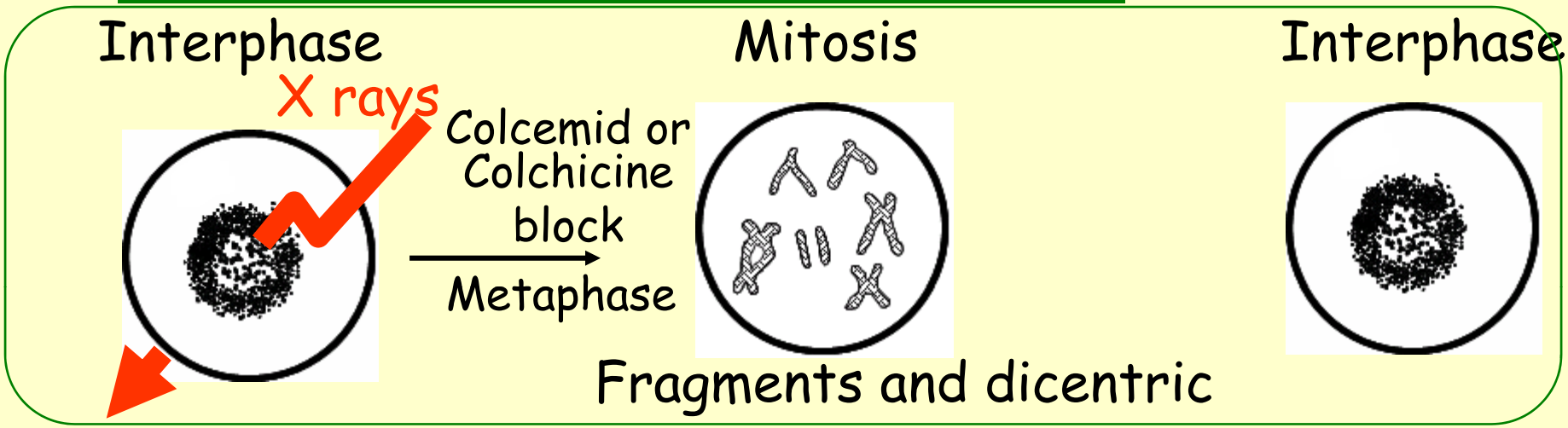
and

Micronucleus assay

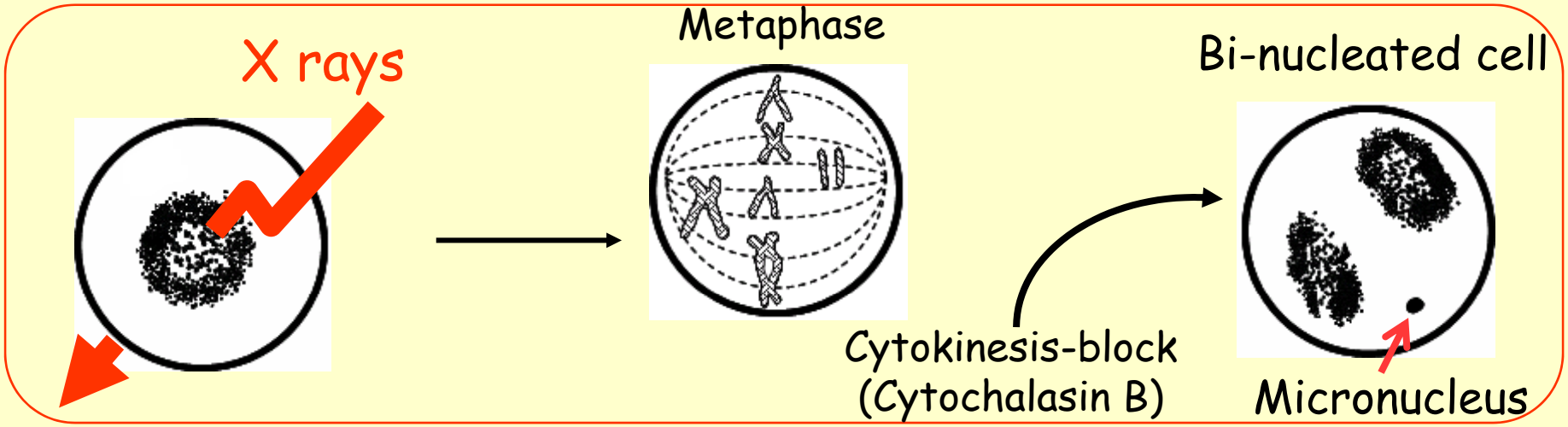
Comparison of

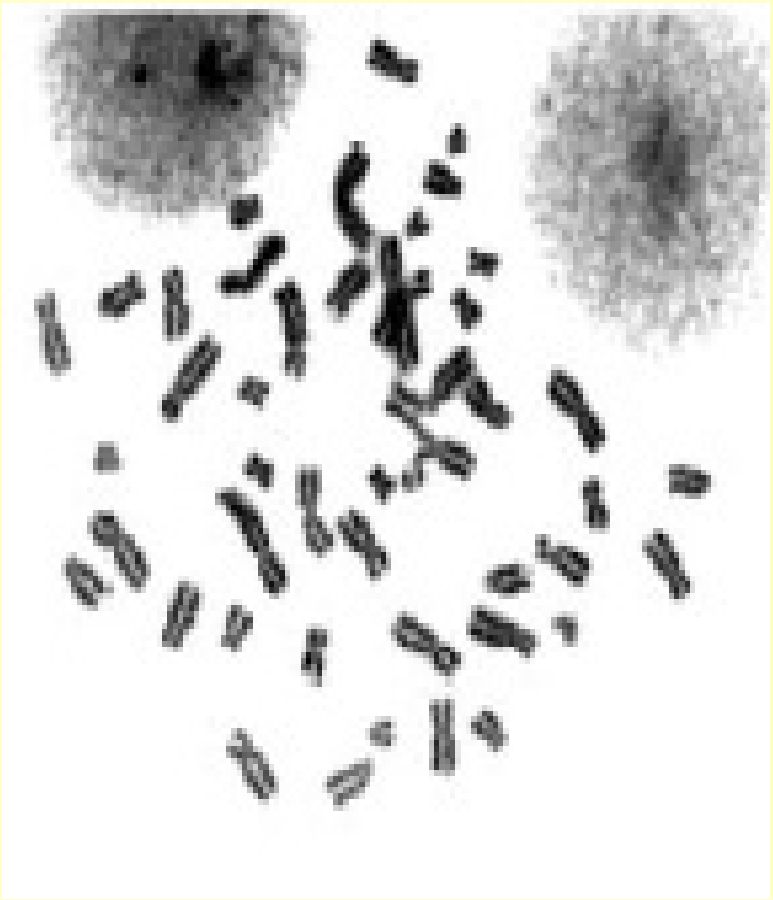
Metaphase Chromosome aberrations and Micronuclei

Metaphase Chromosome aberrations:



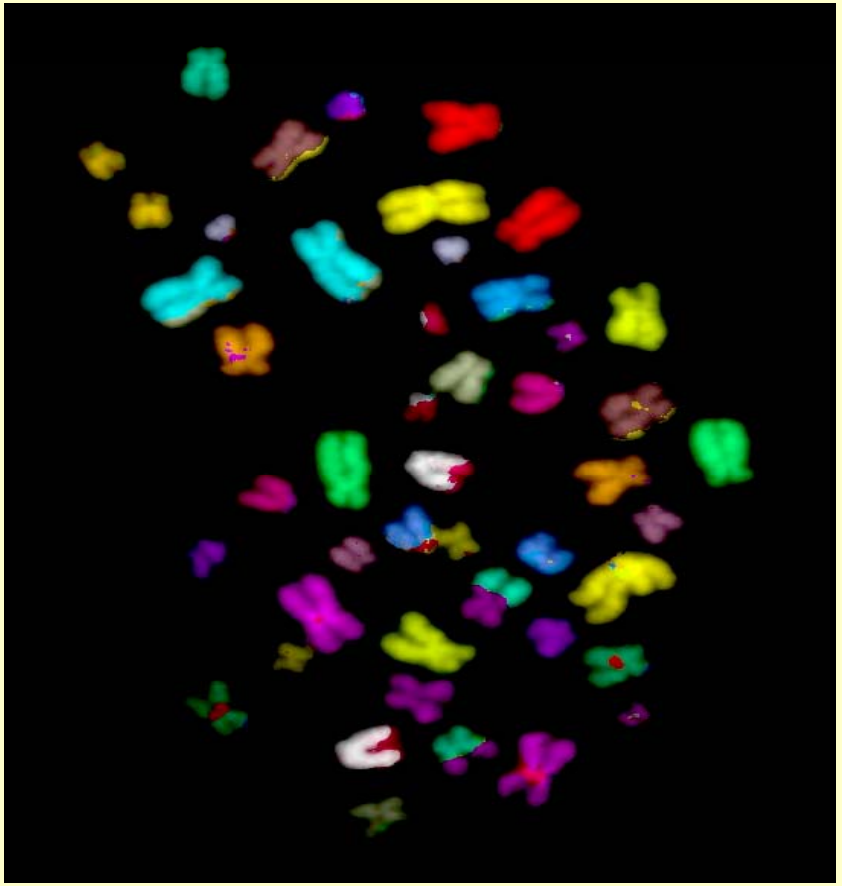
Micronuclei:





Metaphase chromosomes

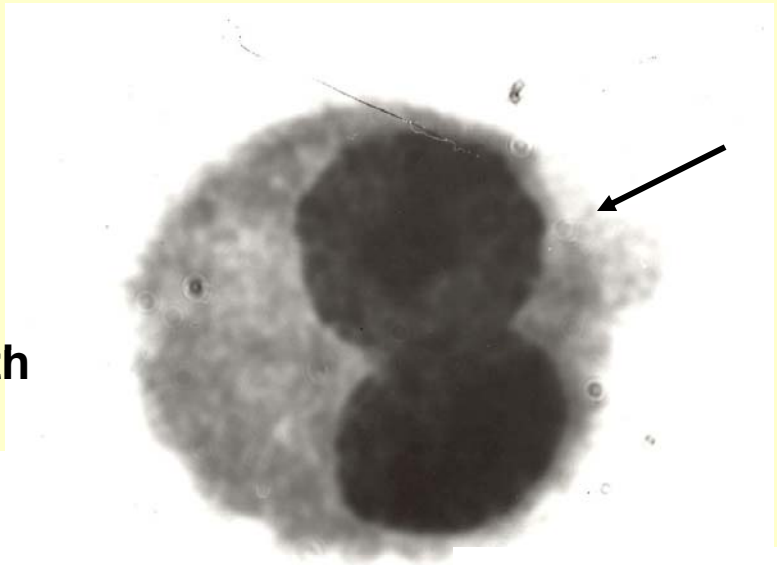
(conventional Giemsa staining method)



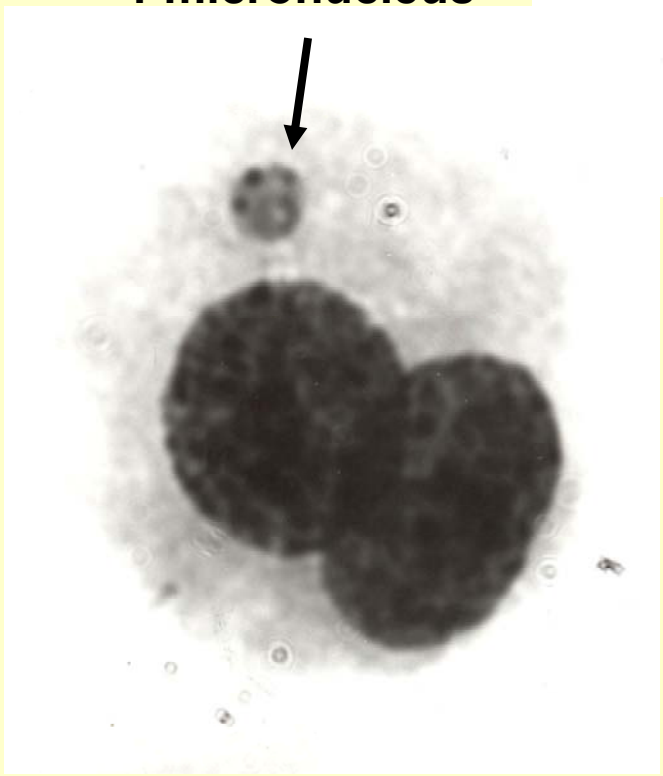
Metaphase chromosomes

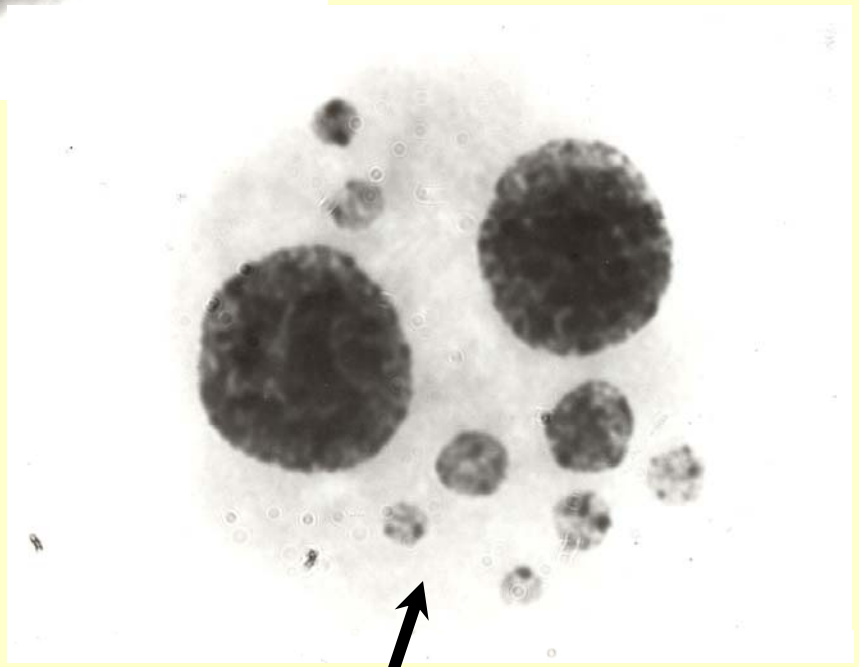
(whole genome multi-color flourescence *in situ* hybridization)

A normal binucleated cell



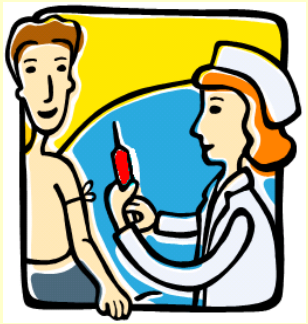
A binucleated cell with 1 micronucleus





A binucleated cell with 8 micronuclei

Cytokinesis Block MN (CBMN) assay (Fenech and Morley, 1985)



Human Lymphocytes
(Whole Blood)

↓

IRRADIATION

↓

Cell cultures
(Stimulated by Phytohemagglutinin)

↓ 44 hours*

Cytochalasin B Treatment
(3-6 µg/mL)

↓ 72 hours*

Cell Harvest

↓

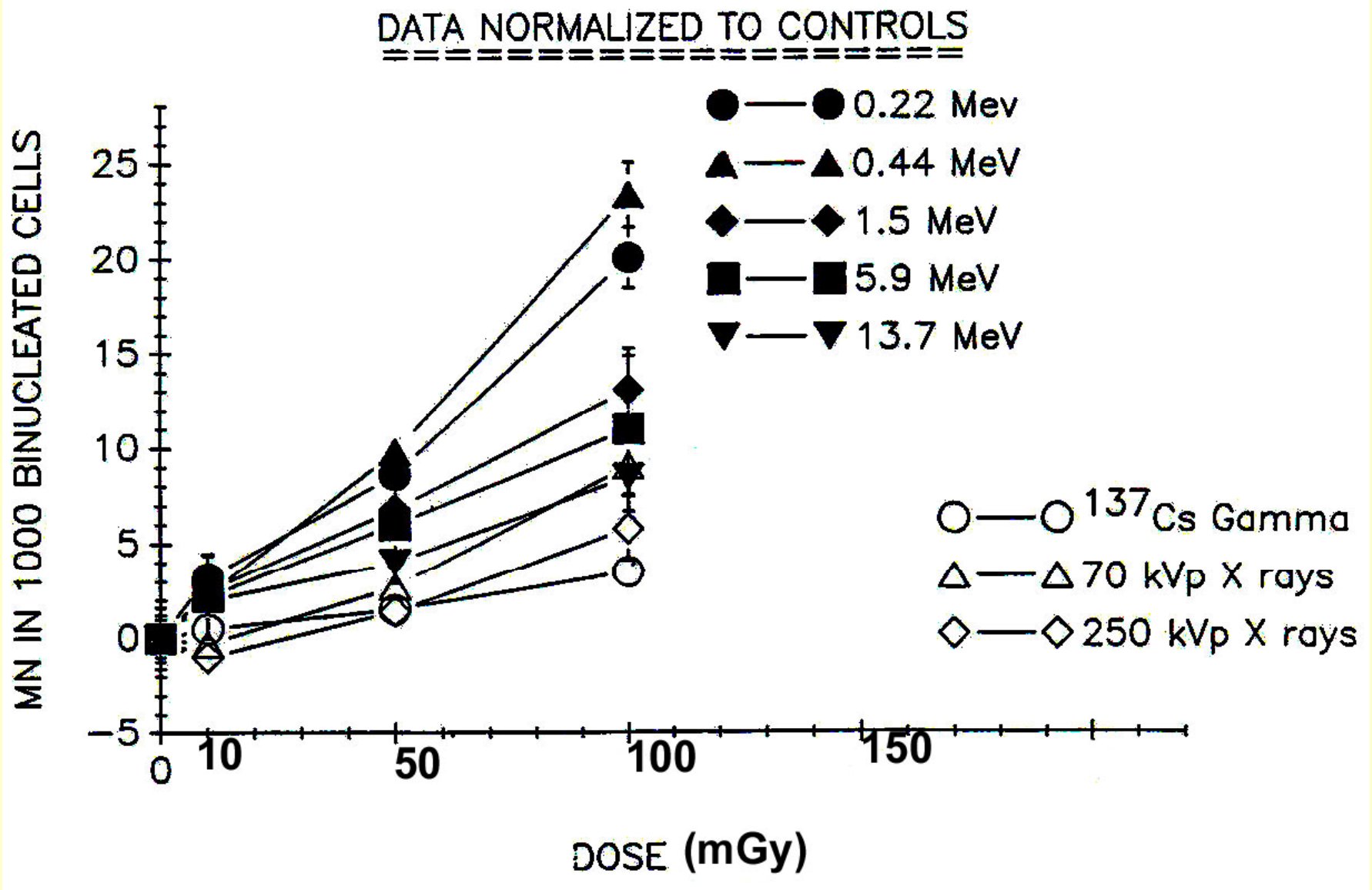
MICRONUCLEUS ANALYSIS
IN BINUCLEATED CELLS

For each dose of each type of radiation:

- **5 non-smoking healthy male volunteers (age ranging from 40-45 years old)**
- **These volunteers had no known history of previous exposure to other clastogenic agents.**

Note: * Hours after the initiation of tissue cultures

Radiation	Energy	Dose (Gy)	**Total cells scored**	Total micronuclei frequency	Mean micronuclei per 1000 cells ±S.E.	**Micronuclei distribution /cell**				
						0	**1**	**2**	**3**	**>3***
Neutron	.22 MeV	0.0	**12,019**	153	13.52 ± 4.53	**11,876**	**131**	**11**	**0**	**1(4)**
		0.01	**8,882**	150	16.27 ± 4.57	**8,757**	**107**	**16**	**1**	**1(7)**
		.05	**8,658**	185	23.09 ± 5.29	**8,491**	**149**	**15**	**2**	**1(4)**
		0.10	**11,744**	386	30.75 ± 6.63	**11,564**	**275**	**56**	**3**	**0**
Neutron	0.44 MeV	0.0	**13,680**	74	6.25 ± 1.44	**13,606**	**74**	**0**	**0**	**0**
		0.01	**12,027**	93	9.01 ± 4.02	**11,838**	**82**	**4**	**1**	**0**
		0.05	**11,964**	182	16.75 ± 2.84	**11,802**	**160**	**2**	**0**	**0**
		0.10	**9,625**	278	33.50 ± 5.95	**9,376**	**227**	**21**	**3**	**0**
Neutron	1.5 MeV	0.0	**9,060**	42	4.45 ± 1.20	**9,018**	**42**	**0**	**0**	**0**
		0.01	**9,769**	64	6.24 ± 1.05	**9,709**	**56**	**4**	**0**	**0**
		0.05	**9,827**	116	11.21 ± 2.11	**9,727**	**84**	**15**	**0**	**0**
		0.10	**9,873**	176	16.71 ± 2.43	**9,717**	**149**	**12**	**1**	**0**
Neutron	5.9 MeV	0.0	**10,942**	48	4.35 ± 2.16	**10,896**	**44**	**2**	**0**	**0**
		0.01	**13,730**	105	6.24 ± 2.36	**13,631**	**93**	**6**	**0**	**0**
		0.05	**12,787**	151	9.64 ± 2.57	**12,649**	**125**	**13**	**0**	**1(8)**
		0.10	**10,526**	178	13.80 ± 3.15	**10,364**	**144**	**18**	**0**	**0**
Neutron	13.7 MeV	0	**11,025**	26	1.18 ± 0.76	**11,001**	**22**	**2**	**0**	**0**
		0.01	**9,833**	40	3.98 ± 0.97	**9,794**	**38**	**1**	**0**	**0**
		0.05	**8,150**	44	5.53 ± 0.75	**8,110**	**36**	**4**	**0**	**0**
		0.10	**9,997**	102	10.31 ± 1.18	**9,904**	**84**	**9**	**0**	**0**
X rays	70 kVp	0.0	**9,256**	57	5.51 ± 1.97	**9,201**	**53**	**2**	**0**	**0**
		0.01	**7,549**	44	5.57 ± 1.74	**7,505**	**44**	**0**	**0**	**0**
		0.05	**8,826**	85	9.17 ± 1.24	**8,747**	**72**	**5**	**0**	**0**
		0.10	**6,683**	102	12.75 ± 0.39	**6,585**	**94**	**4**	**0**	**0**
X rays	250 kVp	0.0	**6,069**	57	9.35 ± 0.06	**6,046**	**44**	**5**	**1**	**0**
		0.01	**10,263**	86	8.43 ± 0.20	**10,184**	**72**	**7**	**0**	**0**
		0.05	**7,915**	85	10.72 ± 1.24	**7,837**	**74**	**1**	**3**	**0**
		0.10	**5,080**	77	15.09 ± 0.44	**5,007**	**70**	**2**	**1**	**0**
γ rays		0.0	**7,899**	19	2.39 ± 0.17	**7,881**	**17**	**1**	**0**	**0**
		0.01	**7,716**	23	2.96 ± 0.55	**7,695**	**19**	**2**	**0**	**0**
		0.05	**6,047**	28	4.22 ± 1.18	**6,020**	**26**	**1**	**0**	**0**
		0.10	**6,705**	42	6.25 ± 0.11	**6,664**	**40**	**1**	**0**	**0**



From: Brooks *et al*, 2003, Adv Space Res, 31(6):1505-1512

New Approaches to Evaluate the Induction of Micronuclei by Low Doses of radiation

1. Hazard Function

2. Microdosimetry

Both methods show similar hormetic effects of low-dose radiation.

Our study is the first to determine hormetic effects of neutrons.

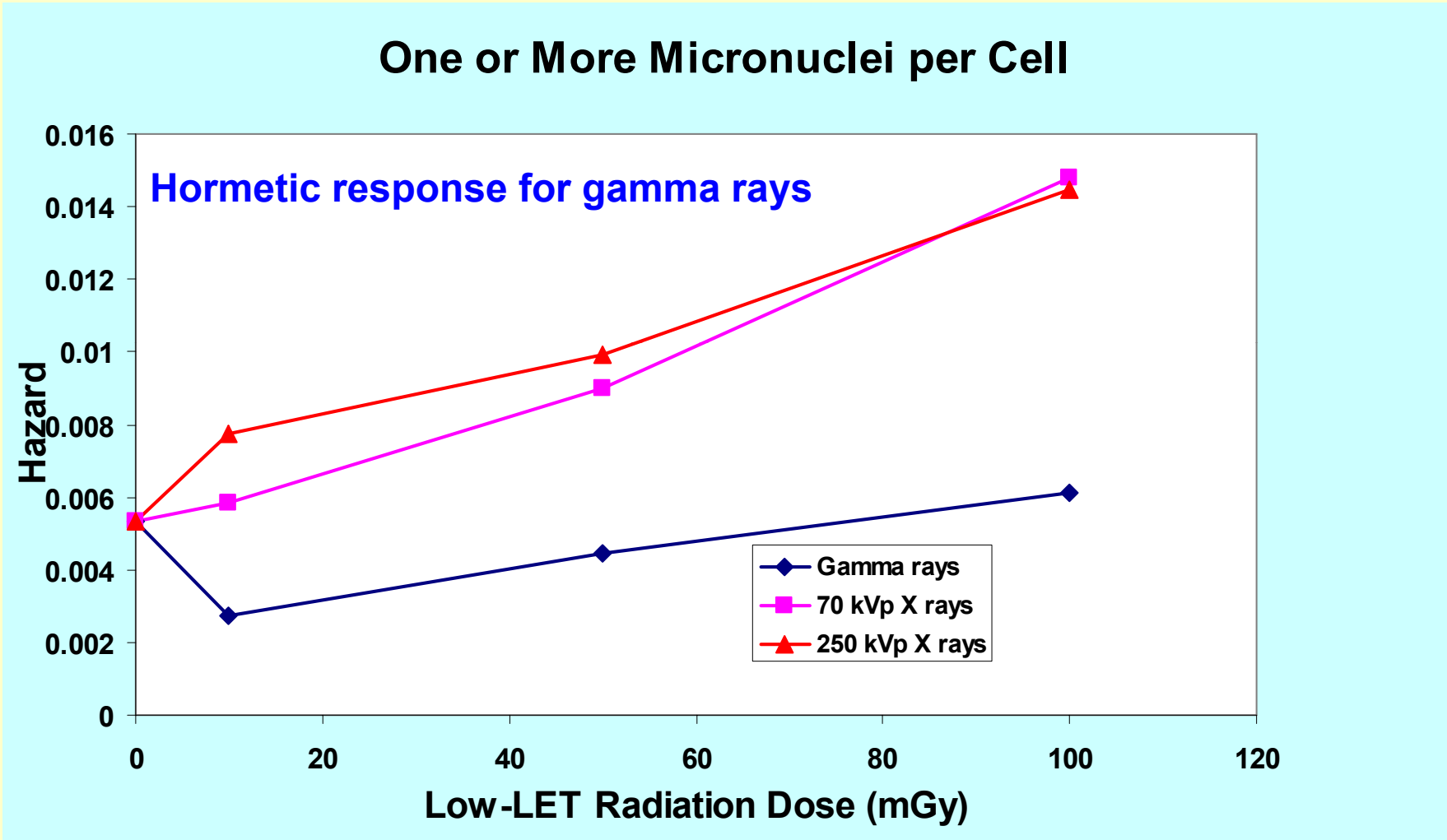
Micronuclei Hazard Function (h)

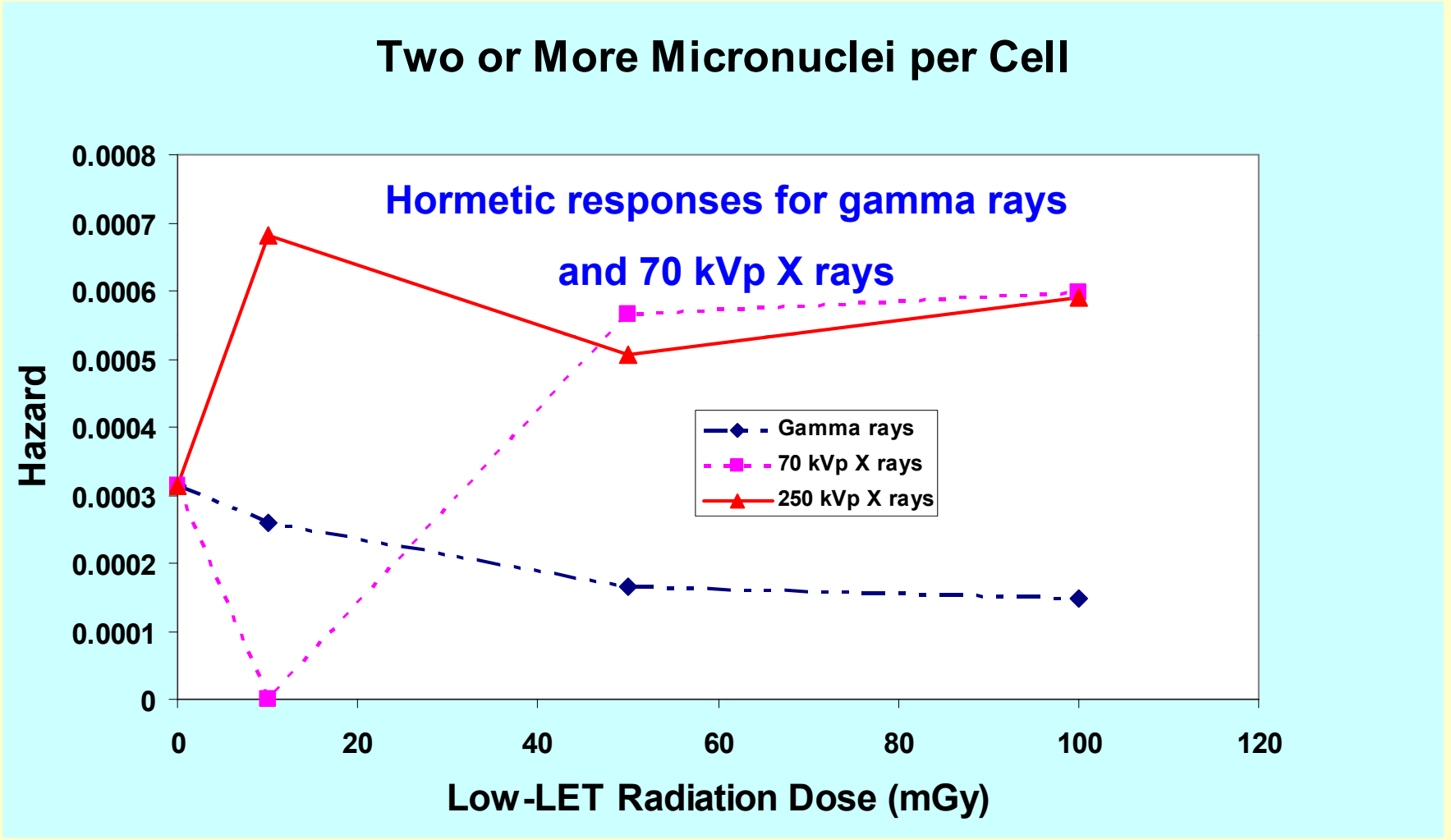
Bobby R. Scott introduced h as a measure of DNA damage to cells in 1991 (Scott BR, Radiat Prot Dosimetry, 37(2):103-109.

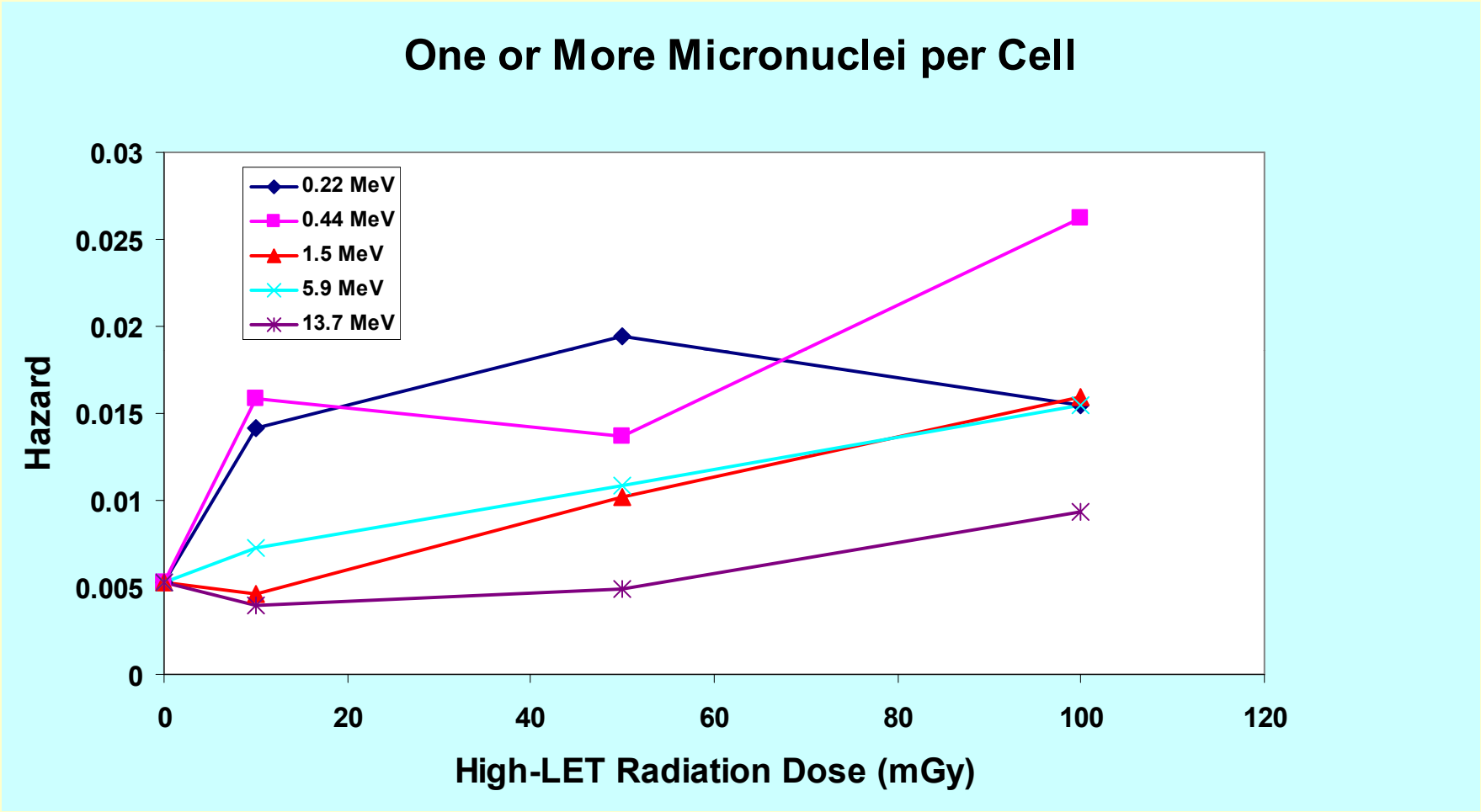
- **The micronuclei hazard *h* is given by:**

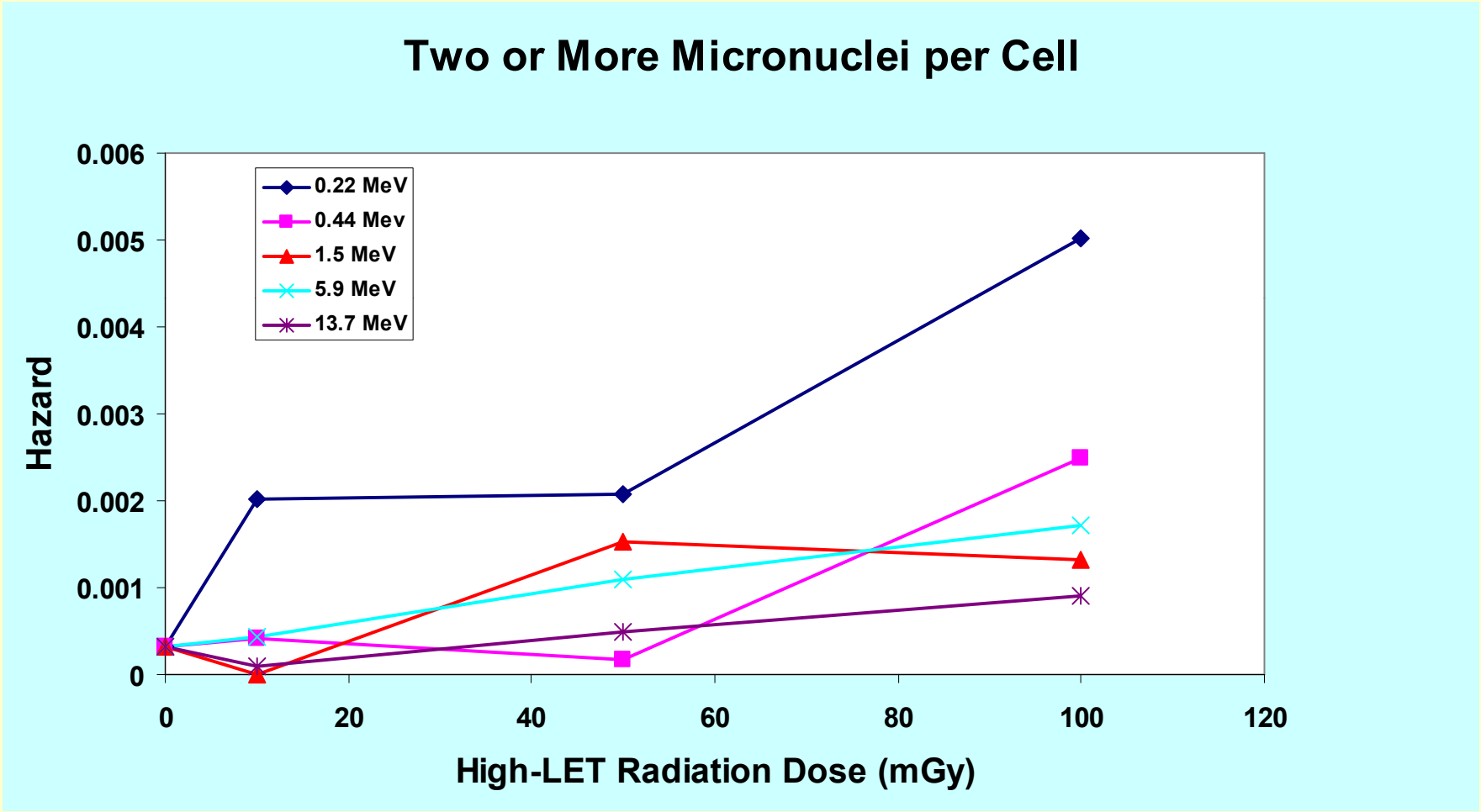
  $$h = -\ln(1-p)$$

- **Let "1-p" represent the proportion of surviving irradiated cells that do not have micronuclei.**
- **We then plot the "micronuclei hazard' as a function of radiation dose.**


One or More Micronuclei per Cell
Hormetic response for gamma rays
Hazard
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
0
20
40
60
80
100
120
Low-LET Radiation Dose (mGy)
Gamma rays
70 kVp X rays
250 kVp X rays


Two or More Micronuclei per Cell
Hormetic responses for gamma rays
and 70 kVp X rays
Hazard
0.0008
0.0007
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
0
0
20
40
60
80
100
120
Low-LET Radiation Dose (mGy)
Gamma rays
70 kVp X rays
250 kVp X rays


One or More Micronuclei per Cell
0.03
0.025
0.02
0.015
0.01
0.005
0
Hazard
0.22 MeV
0.44 MeV
1.5 MeV
5.9 MeV
13.7 MeV
0
20
40
60
80
100
120
High-LET Radiation Dose (mGy)


Two or More Micronuclei per Cell
0.006
0.005
0.004
0.003
0.002
0.001
0
Hazard
0.22 MeV
0.44 Mev
1.5 MeV
5.9 MeV
13.7 MeV
0
20
40
60
80
100
120
High-LET Radiation Dose (mGy)

Microdosimetric approach based upon average 1-hit microdose $\langle z_1 \rangle$ to nuclear size volumes

1. $\langle z_1 \rangle$ was scaled by LET values.

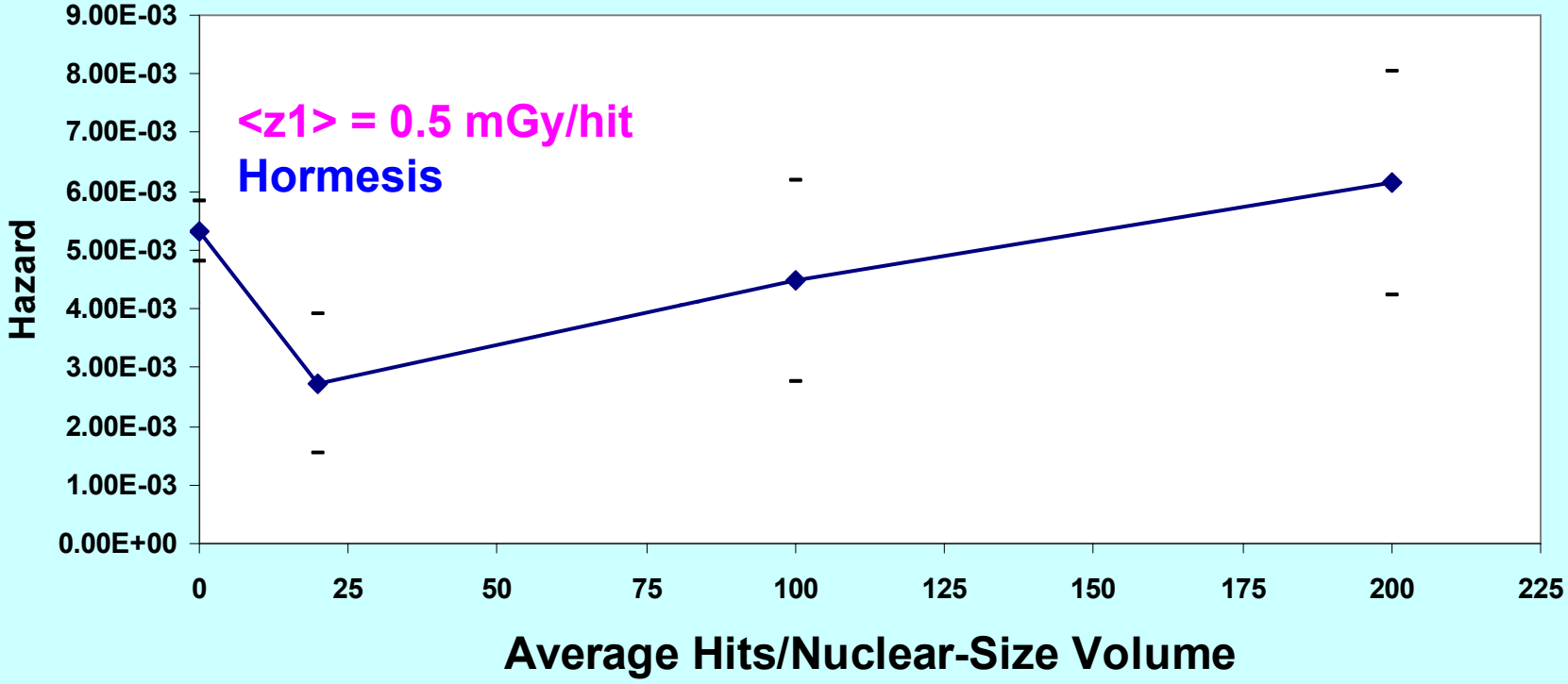
2. Average hits per nuclear-size volume (NSV) within the cells were calculated by:

total radiation dose/$\langle z_1 \rangle$.

3. Dose-response curves were generated for: a. Micronuclei hazard for 1 or more micronuclei being present

b. Micronuclei hazard for 2 or more micronuclei being present, as a function of average hits per NSV

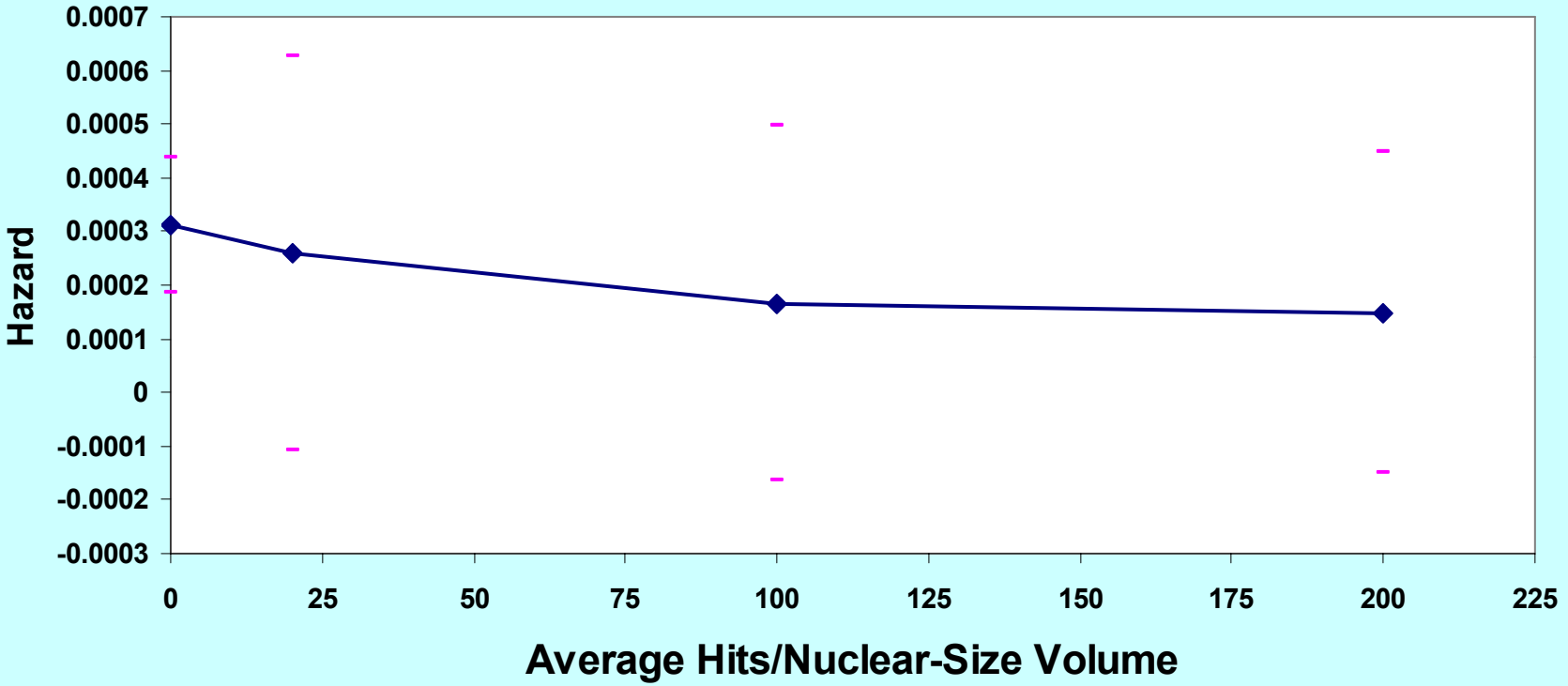
One or More Micronuclei per Cell: Gamma Rays



Significant hormetic effect for an average of 20 hits per nuclear-size volume (NSV) from gamma rays, $p < 0.0001$.

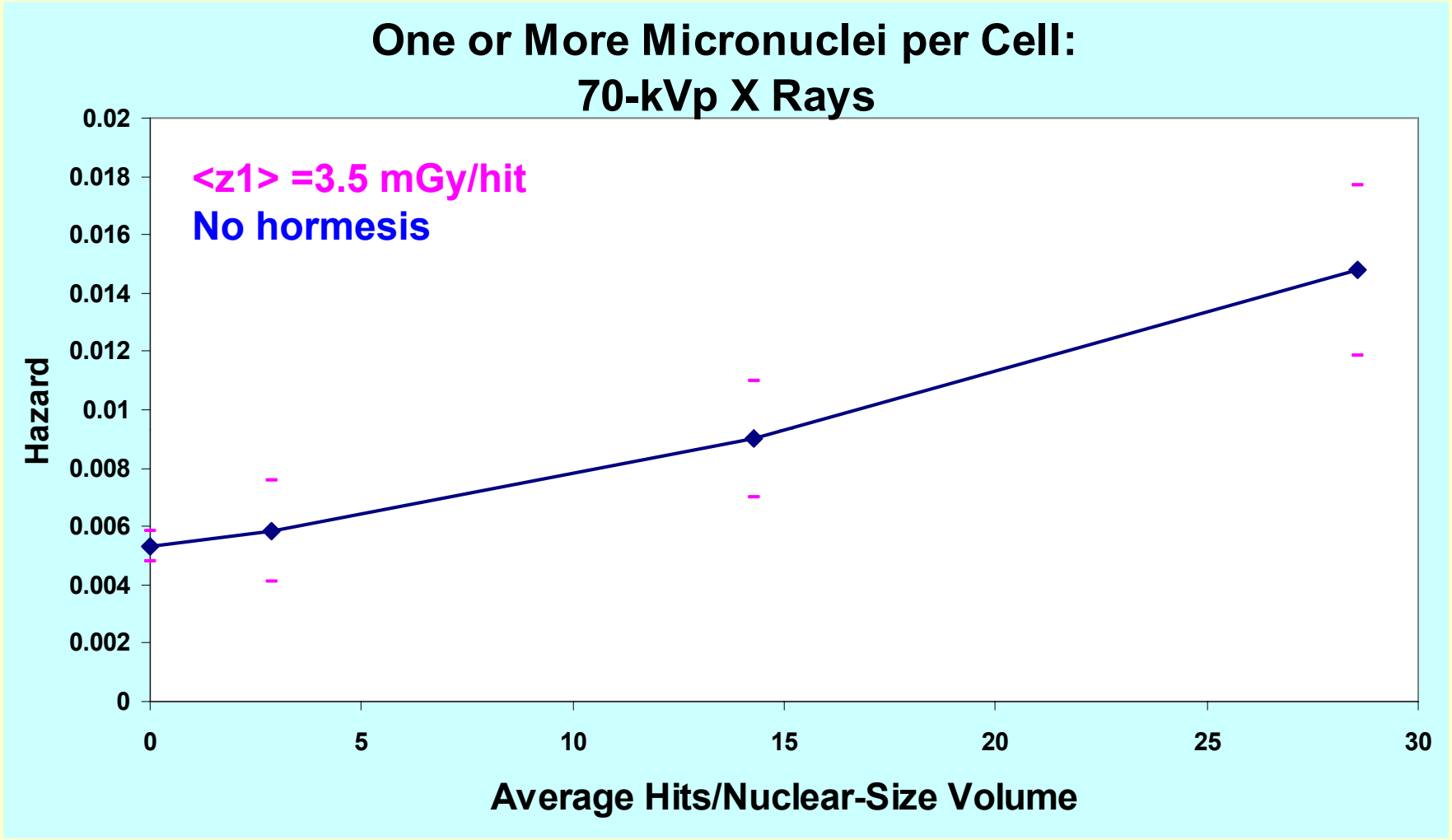
No significant difference from controls for higher number of average hits ($p > 0.05$).

Two or More Micronuclei per Cell: Gamma Rays



No single data point significantly less than controls. However, suggested mild hormetic trend for micronuclei hazard. No evidence for increased hazard over the indicated range of average hits.

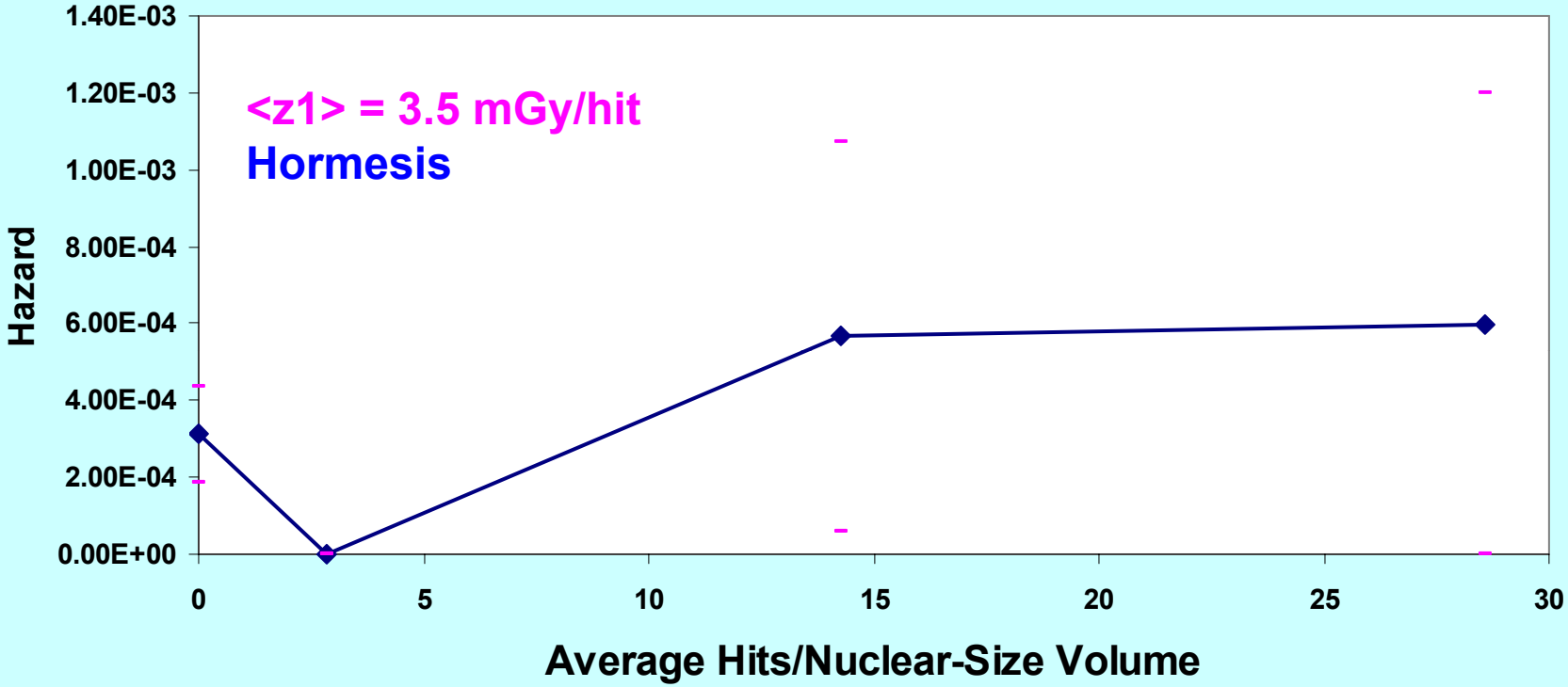
The data suggest that hormesis-related protective processes may be maintaining a steady-state level in the number of cells with two or more micronuclei.



Significant increase in hazard relative to controls.

No evidence for protective processes

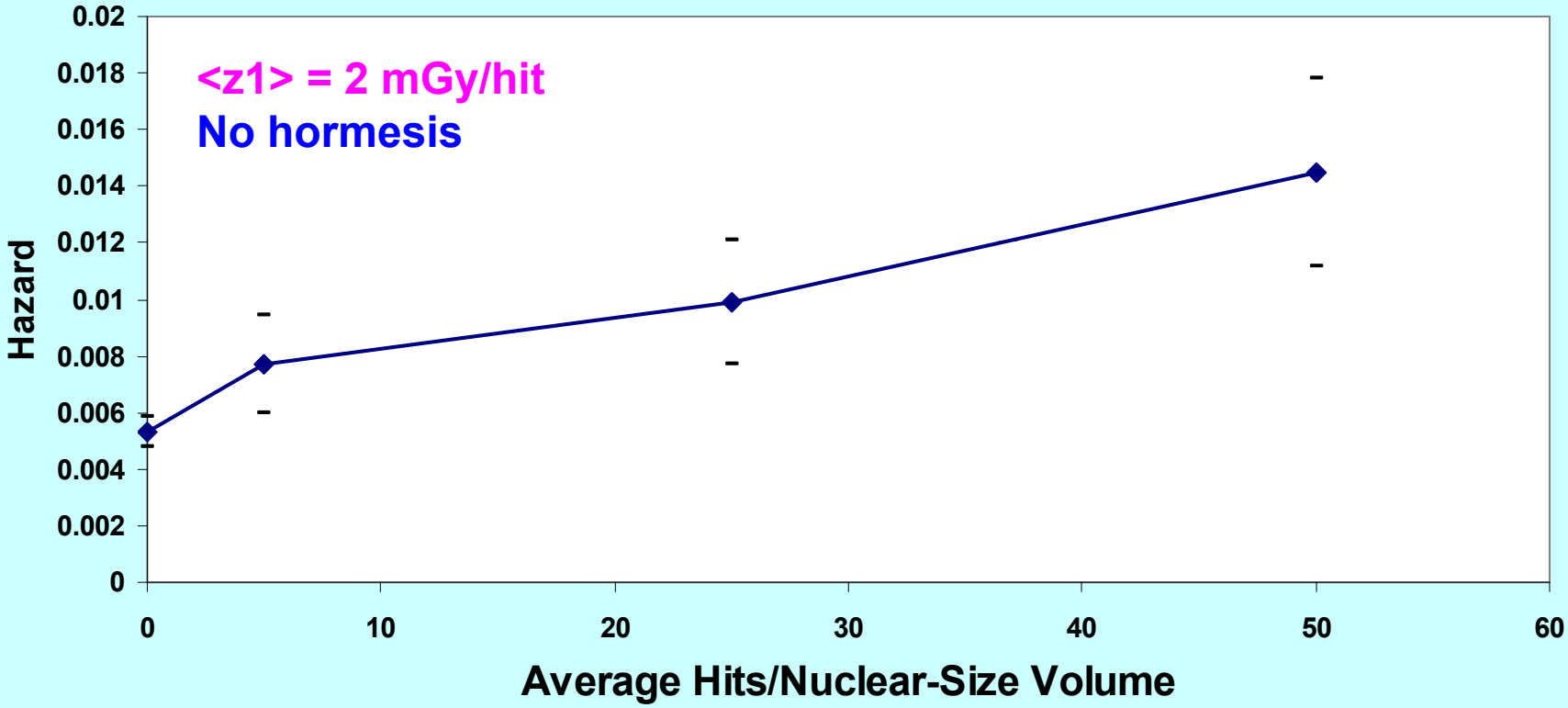
Two or More Micronuclei per Cell: 70-kVp X Rays



Significant hormetic effect seen for an average of 3 hits/NSV, p<0.0001. For higher number of average hits, micronuclei hazard not significantly different from controls, p > 0.05.

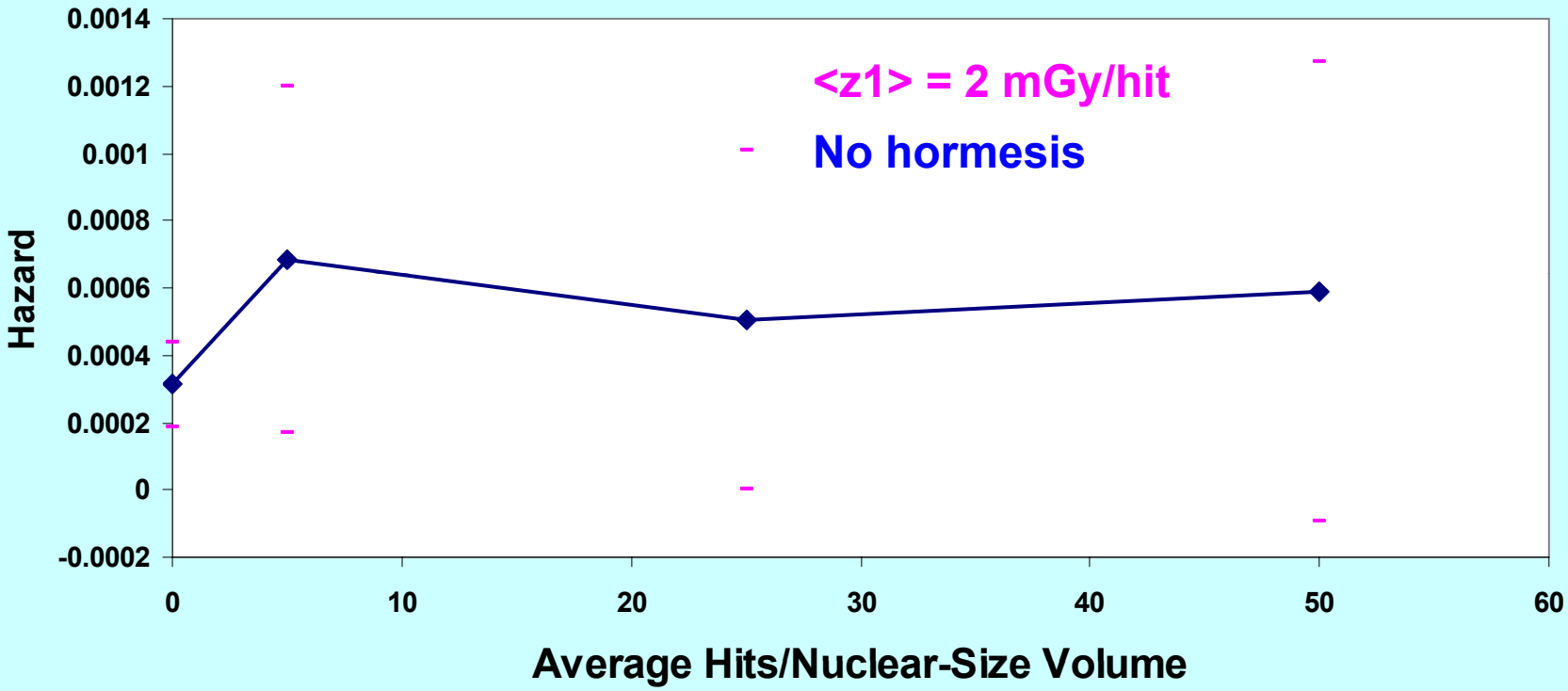
Data suggest that hormetic process can either eliminate or maintain a steady-state level in the number of cells with two or more micronuclei.

One or More Micronuclei per Cell: 250-kVp X Rays



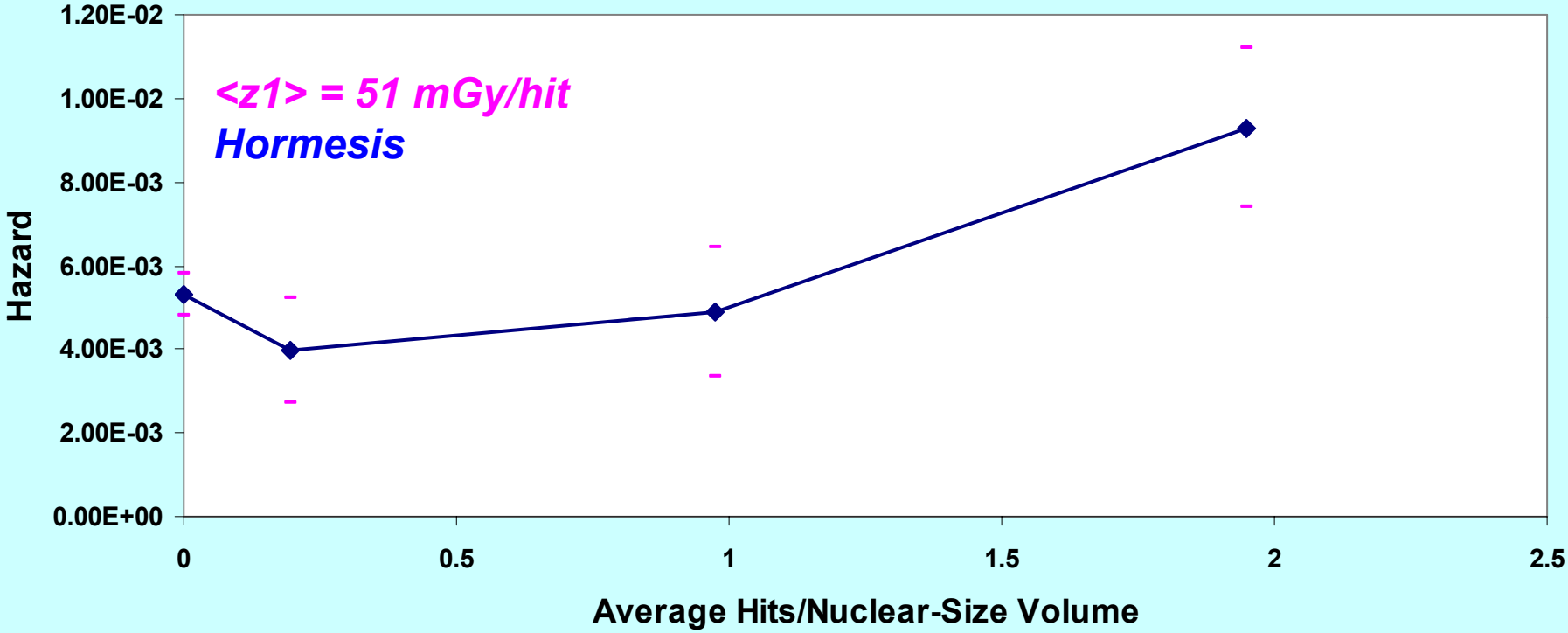
Significant increase in hazard relative to controls. No evidence for protective processes being activated.

Two or More Micronuclei per Cell: 250-kVp X Rays



None of the data points significantly greater than for controls ($p > 0.05$).
Data suggests that hormesis-related protective processes may be maintaining a steady state level in the number of cells with more than one micronucleus?.

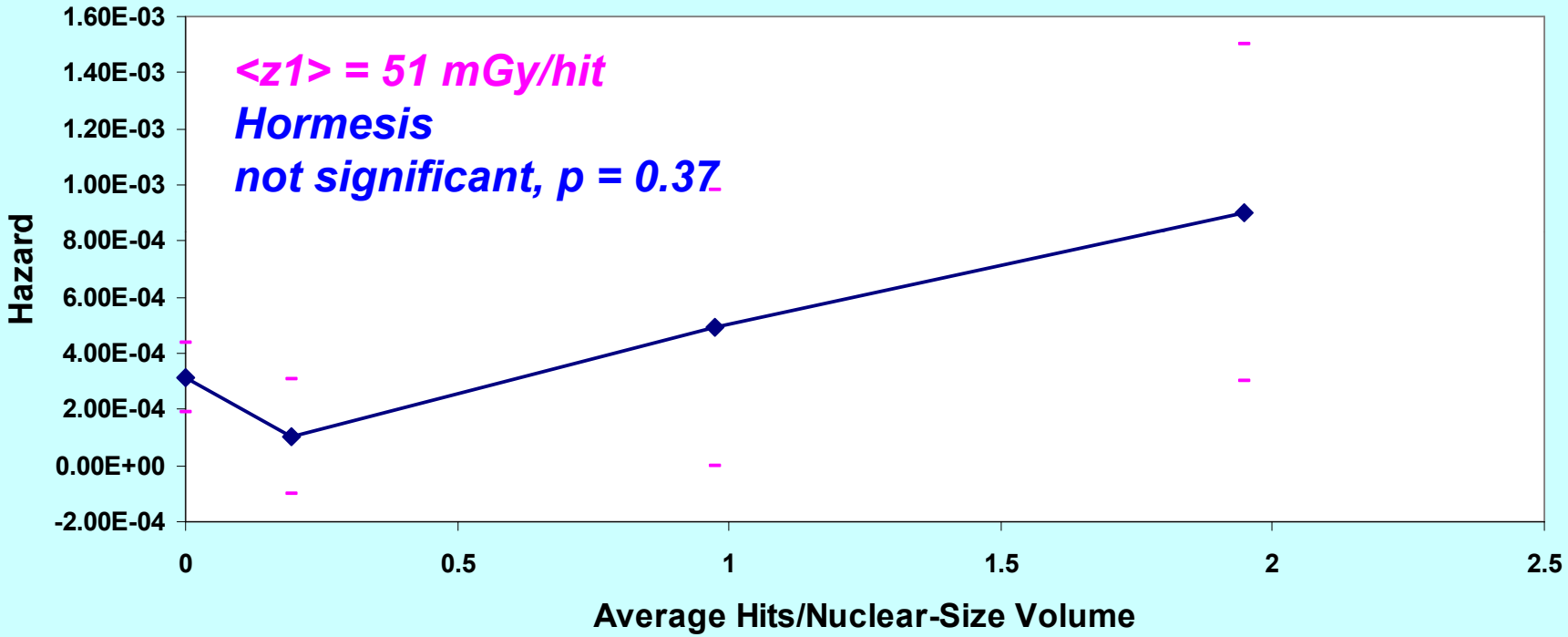
One or More Micronuclei per Cell: 13.7-MeV Neutrons



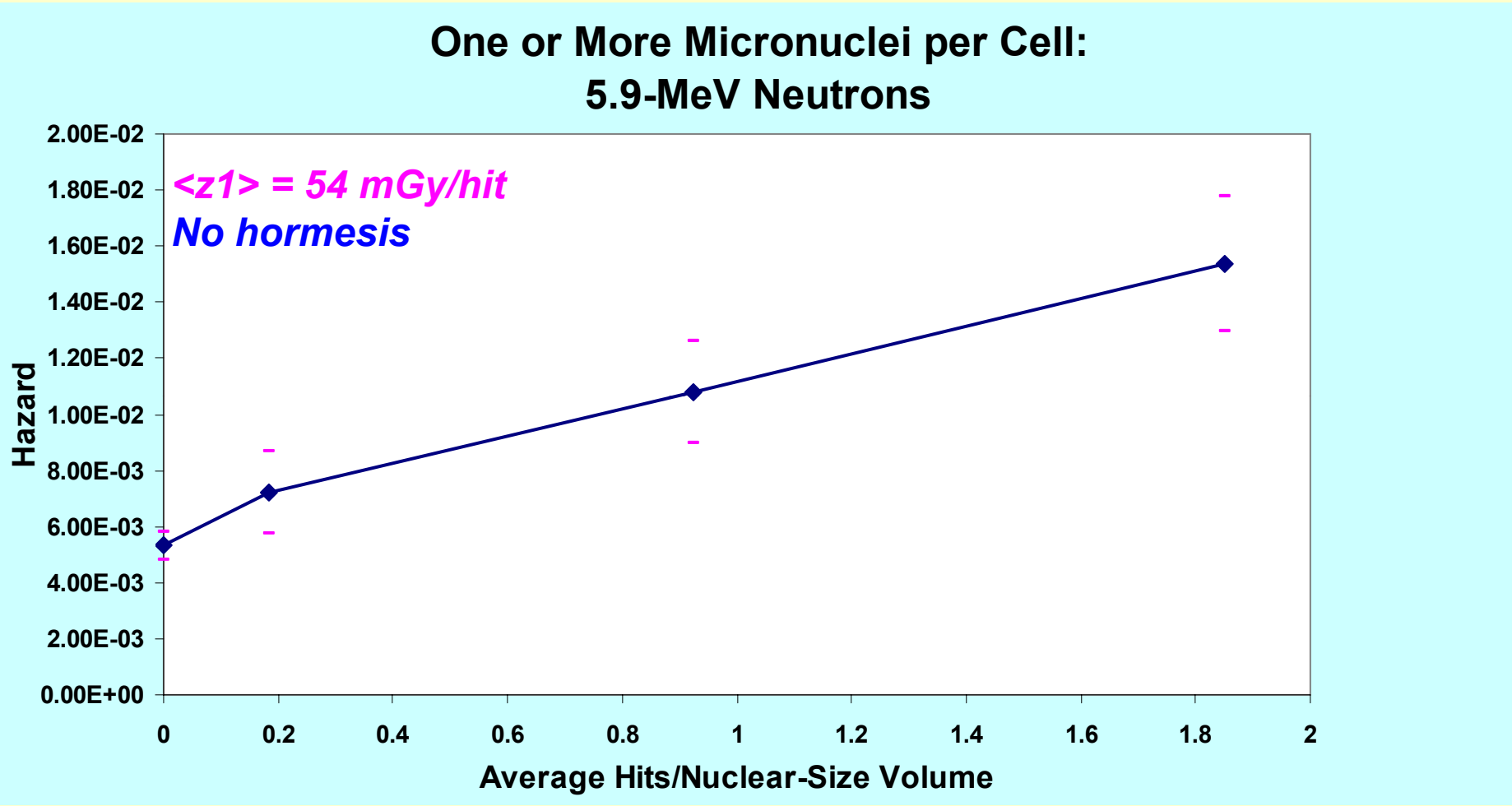
A significant hormetic effect was found for an average of 0.19 hits per NSV (p = 0.017) but not for an average of 1 hit per NSV (p = 0.3).

These results also implicate hormesis as arising from a protective bystander effect since only 19 out of each 100 cells on average are hit yet hormesis occurs. Protective bystander effect.

Two or More Micronuclei per Cell: 13.7-MeV Neutrons

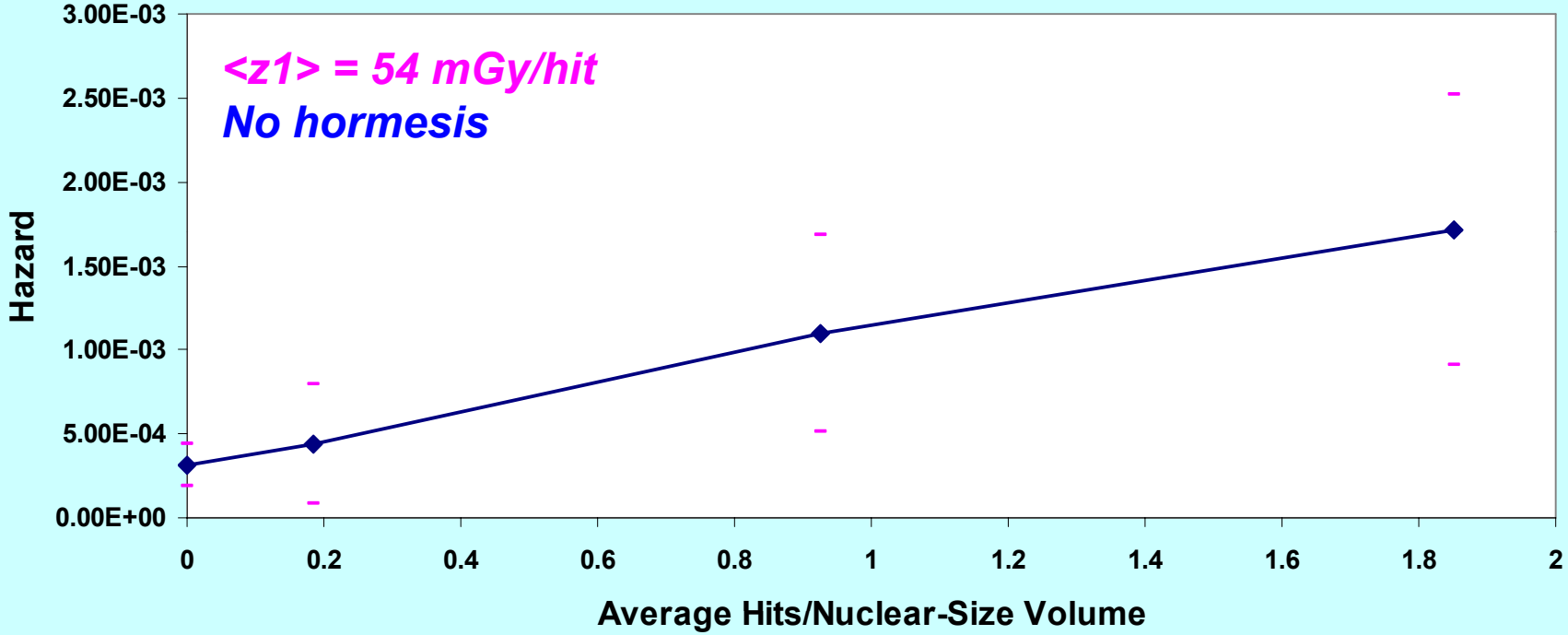


None of the data points significantly differ from controls. Thus, protective processes may be maintaining steady-state for the micronuclei hazard.

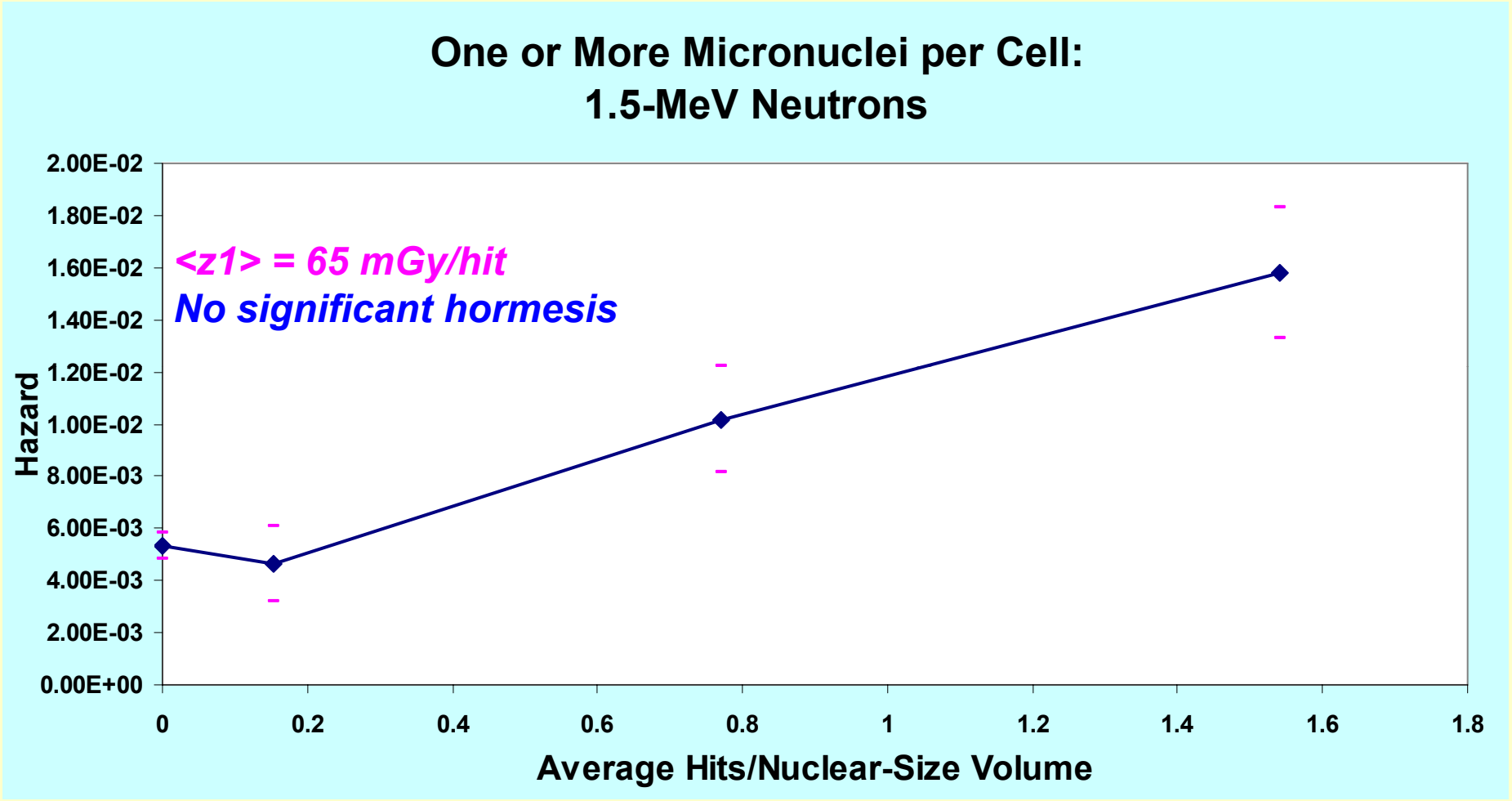


No evidence for protection activation.

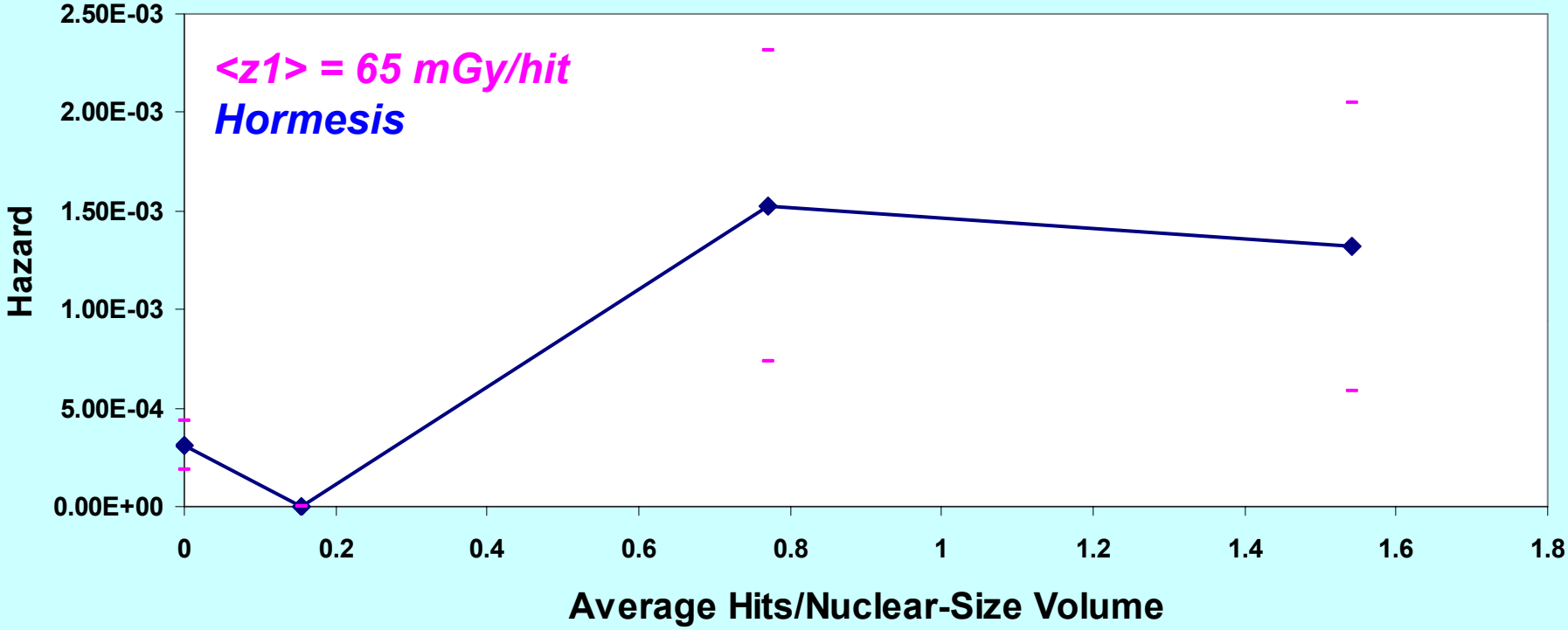
Two or More Micronucldi per Cell: 5.9-MeV Neutrons



No evidence for protection activation.

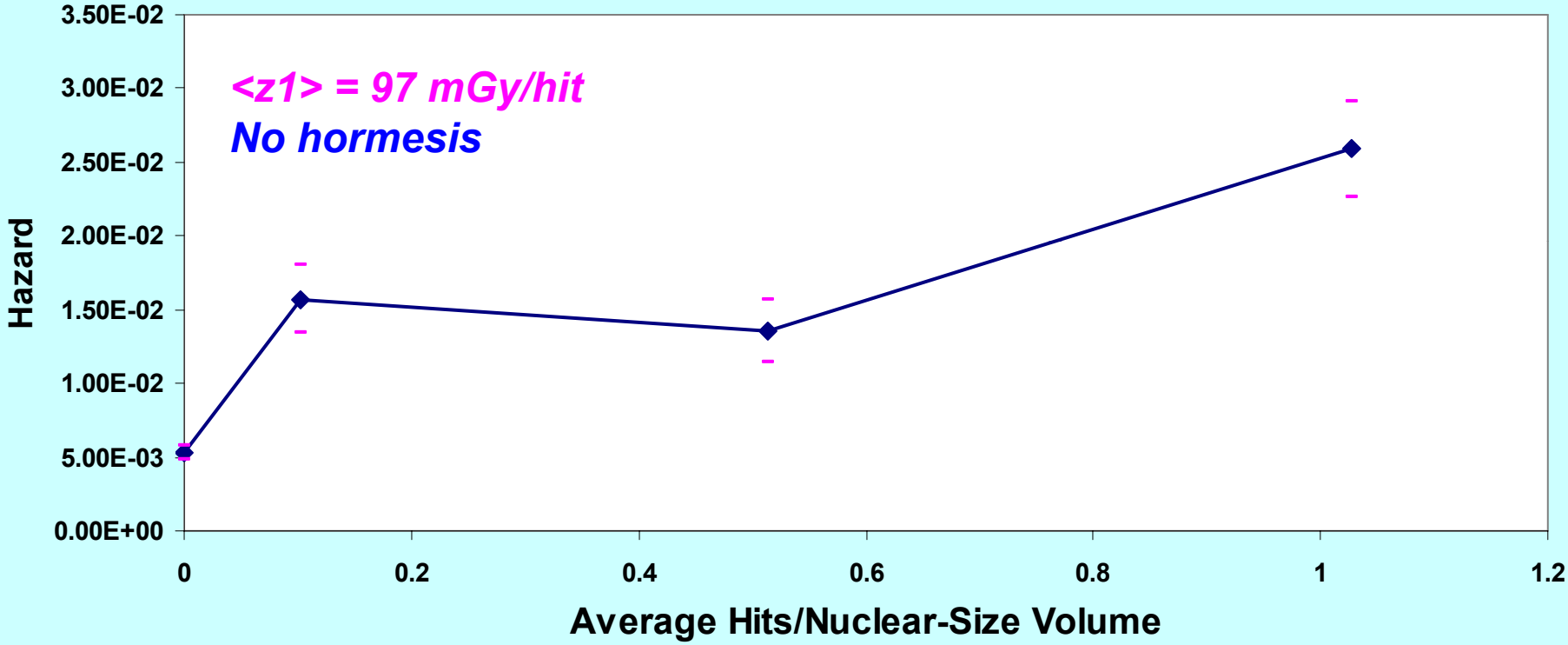

One or More Micronuclei per Cell:
1.5-MeV Neutrons
<z1> = 65 mGy/hit
No significant hormesis
Hazard
2.00E-02
1.80E-02
1.60E-02
1.40E-02
1.20E-02
1.00E-02
8.00E-03
6.00E-03
4.00E-03
2.00E-03
0.00E+00
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
Average Hits/Nuclear-Size Volume

Two or More Micronuclei per Cell: 1.5 MeV Neutrons



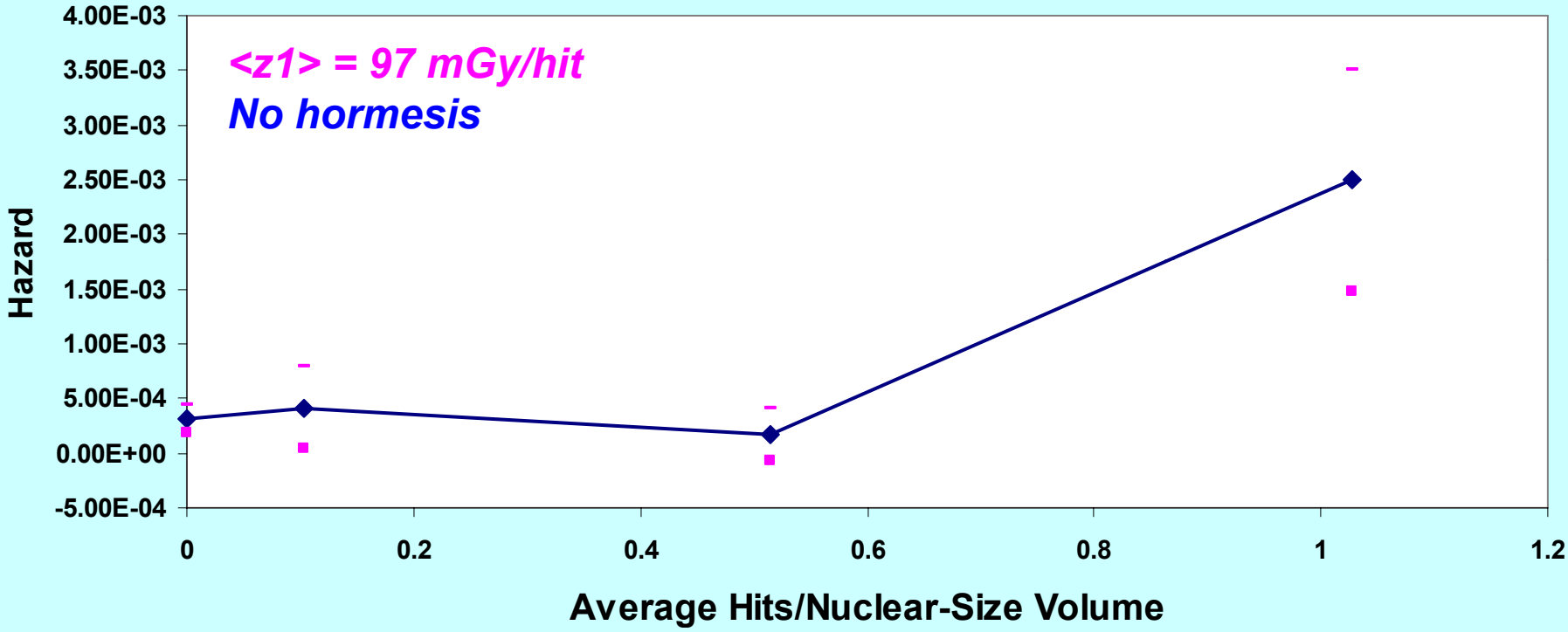
The hormetic effects at just below an average of 0.2 hits per NSV is highly significant ($p < 0.0001$) as all cells with the indicated lesions of interest (more than 1 micronuclei) were apparently removed from the population. This implicates apoptosis as the protective bystander effect. Protective bystander effect.

One or More Micronuclei per Cell: 0.44-MeV Neutrons



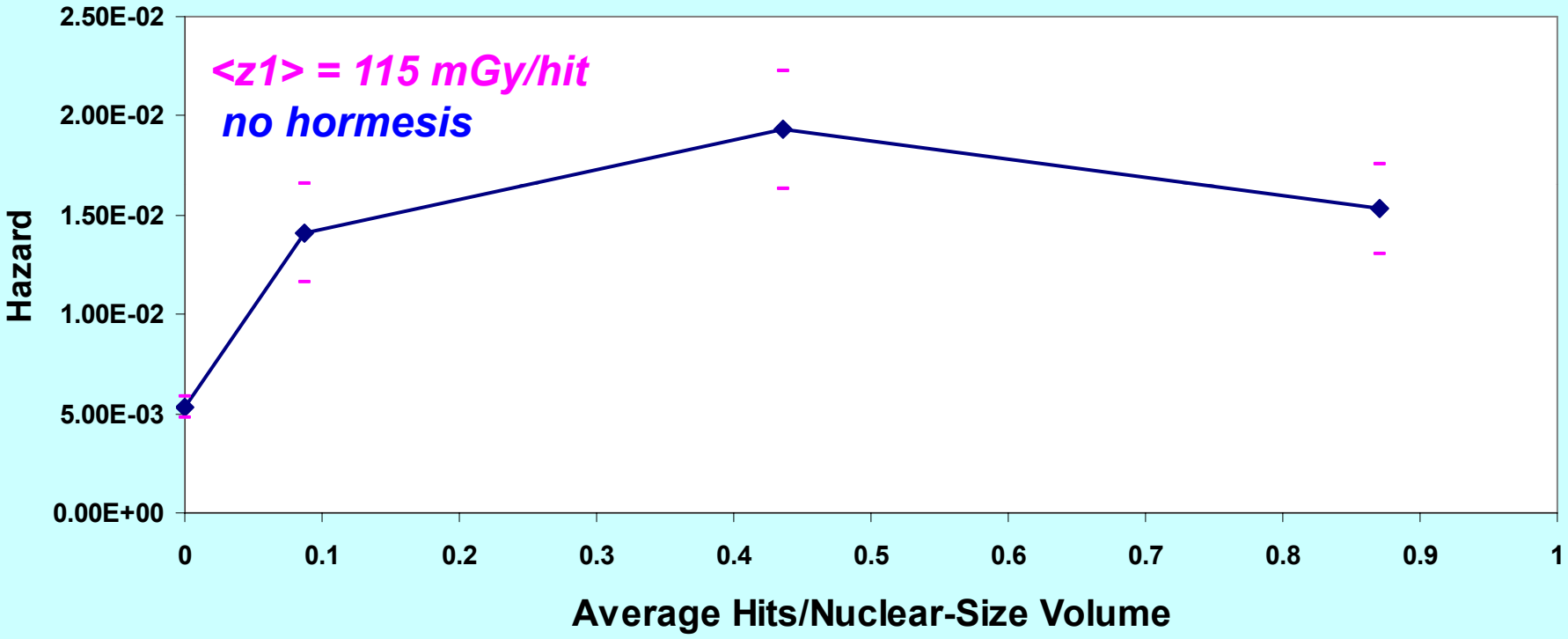
***Deleterious bystander effect** implicated since produced when as few as 1 in 10 cells hit.*

Two of More Micronuclei per Cell: 0.44-MeV Neutrons



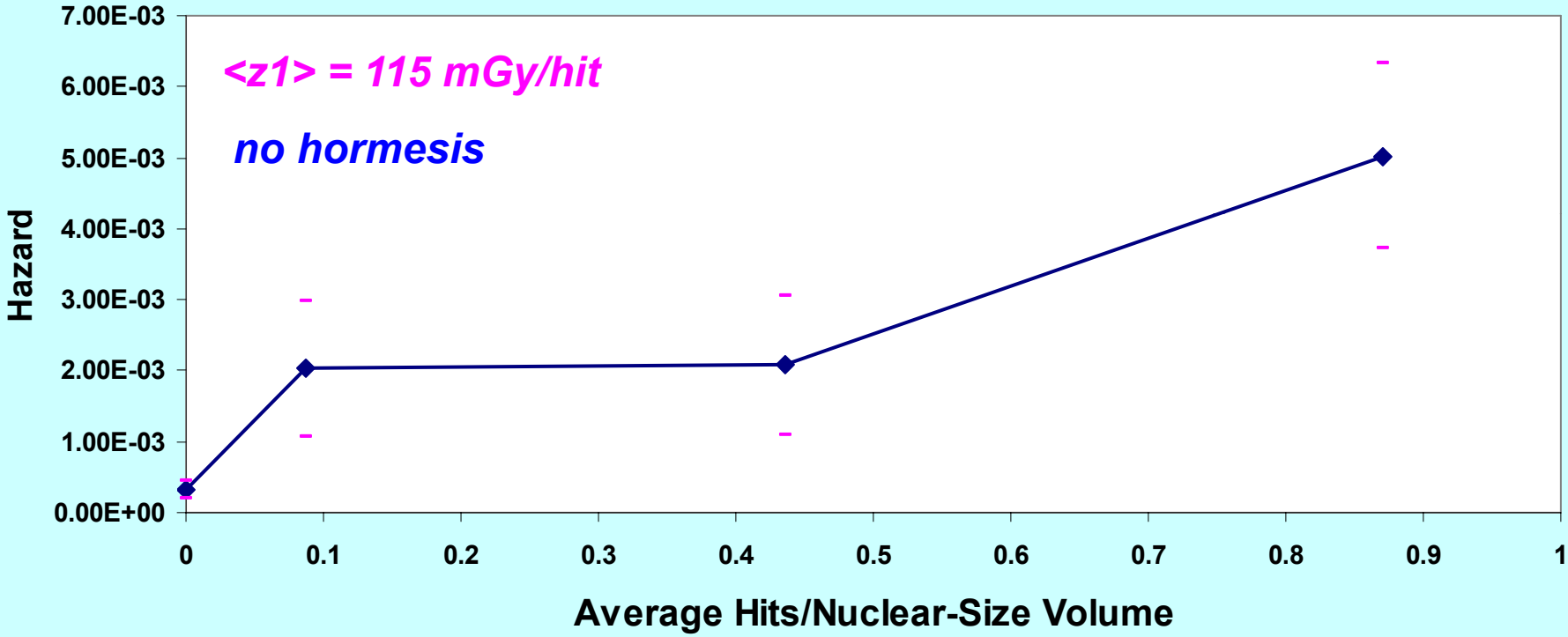
Data suggest that protective processes maintain steady state level for the micronuclei hazard for fewer than an average of 0.6 hit/NSV. This would be a protective bystander effect.

One of More Micronuclei per Cell: 0.22-MeV Neutrons



***Deleterious bystander effect** implciated since produced when as few as 1 in 10 cells hit.*

Two or More Micronuclei per Cell: 0.22-MeV Neutrons



***Deleterious bystander effect** implicated since produced when as few as 1 in 10 cells hit.*

SUMMARY

1. The data are suggestive of a protective hit-size effectiveness function for micronuclei occurrence.

2. Hormetic effects were induced by photons, *depending on the energy of photons.*

3. The data also suggest that neutrons induce either protective or deleterious bystander effects depending on neutron energy and the average microdose $\langle z_1 \rangle$ for the nuclear-size volume.

Future Directions

Determination of Molecular Biomarkers of Hormesis Induced by Low Doses of Radiation

Proteomic Approach

Protective Bystander Effects
Deleterious Bystander Effects

Working Hypothesis ……

Differential protein expression profiles in these two disparate bystander effects.

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Bobby Scott
Grant Number DE-FG02-03ER63657