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Relative biological effect of alpha particle radiation on low dose phenomena: lethal mutation, hyper-radiosensitivity and increased radioresistance

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## 20 **Abstract**

21           At high doses, the current recommended radiation weighting factors advise a significantly  
22 higher effectiveness of alpha particles relative to gamma radiation. However, at lower doses, the  
23 ratio of effectiveness between radiations of varying linear energy transfer values is complicated  
24 due to the relative importance of low dose phenomena such as genomic instability, bystander  
25 effects, low dose hyper-radiosensitivity and increased radioresistance (HRS/IRR). Radium is the  
26 most common source of alpha radiation exposure to humans, but the dosimetry is complicated by  
27 the decay chain which involves gamma exposure due to radon daughters. This study aimed to  
28 isolate the relative biological effect of alpha particles after low doses of radium to cells and their  
29 progeny. This was done by subtracting the survival values of a human keratinocyte cell line  
30 (HaCaT) and an embryonic Chinook salmon cell line (CHSE-214) exposed to gamma irradiation,  
31 from survival of the same cell lines exposed to mixed alpha and gamma radiation through  
32 chronic exposure to Ra-226 and its decay products. The human cell line showed increased  
33 radioresistance when exposed to low doses of alpha particles. In contrast the fish cell line, which  
34 demonstrated radioresistance to low dose gamma energy, demonstrated increased lethality when  
35 exposed to low doses of alpha particles. The results confirm the need to consider the dose-  
36 response relationship when developing radiation weighting factors for low dose exposures, as  
37 well as the need to be aware of possible cell line and species differences.

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## 41 **Introduction**

42           Linear energy transfer (LET), describes the amount of energy deposited to the interacting  
43 material, per unit of distance. Photons such as gamma rays are able to traverse great distances  
44 unchanged before being absorbed, however monoenergetic ions such as alpha particles cause  
45 frequent direct ionizations within a smaller range. Due in part to the clustered nature of damage  
46 caused, the relative biological effectiveness (RBE) of an alpha particle is often described to be  
47 significantly higher than that of a gamma ray. This is a result of the concentration of damage  
48 given the same amount of absorbed energy [1].

49           While this may be true for high doses, it has been shown that if a single alpha particle  
50 traverses a cell, it causes zero to small risk of oncogenic transformation [2]. Further, the work of  
51 Nagasawa and Little has shown significantly higher frequencies of mutation than would be  
52 expected through linear extrapolation from data for high doses, at doses where the mean number  
53 of alpha particle traversals per nucleus was significantly less than one [3]. At low doses, both  
54 alpha and gamma rays can cause non-targeted effects (NTE) like genomic instability where  
55 damage does not cause direct mortality and cells appear completely normal but de novo effects  
56 are seen in distant progeny and lethality occurs generations later (often referred to as lethal  
57 mutations or delayed reproductive death) [4]. Cell survival at sub-lethal doses of gamma  
58 irradiation has also been observed to differ from what is expected by the traditional linear-  
59 quadratic model, instead displaying a region of low-dose hyper-radiosensitivity (HRS) followed  
60 by increased radioresistance (IRR) [5]. Currently accepted recommendations for the radiation  
61 weighting factor ( $w_r$ ) of alpha particles, which apply the concept of RBE to derive equivalent

62 dose, are dose independent [6]. However, research has shown instances for RBE to be dose  
63 dependent when high dose biological effects are substantially different to low dose effects [7].

64       Of particular interest to this study is whether NTE amplify low dose effects such that they  
65 are higher than what would be expected from established linear no-threshold model (LNT)  
66 related RBE values following exposure to low doses of an environmental alpha emitter: radium-  
67 226. Despite being an alpha emitter by itself, it is known that the uranium decay chain of which  
68 radium is part of involves many gamma emissions, thereby making it difficult to measure pure  
69 alpha effects. To approach this problem, observations from gamma irradiation (through acute  
70 exposure to Cs-137) will be subtracted from mixed alpha and gamma irradiation (through  
71 chronic exposure to Ra-226 and its progeny).

72       This study will measure the acute survival and the lethal mutation phenotype assayed as  
73 reduced cloning efficiency in culture of a human keratinocyte cell line (HaCaT). In addition, due  
74 to the increasing relevance of protecting non-human biota from radium in hydrogeologic  
75 contaminations from mining, etc., this study will also investigate relative alpha exposure effects  
76 in the embryonic Chinook salmon cell line (CHSE-214).

77

## 78 **Materials and Methods**

### 79 **Cell culture**

80       The HaCaT cell line used in the study is an immortalized human keratinocyte cell line  
81 originally derived and characterized by Boukamp et al [8]. The cell line used in this study was  
82 obtained as a gift from Dr. Orla Howe (Dublin, Ireland). The cell line was routinely maintained

83 with RPMI-1640 medium supplemented with 10% fetal bovine serum (Invitrogen, Burlington,  
84 Canada), 5 ml of 200 mM L-Glutamine (Gibco, Burlington, Canada), 0.5 g/ml hydrocortisone  
85 (Sigma-Aldrich, Oakville, Canada), 25 mM HEPES buffer (Gibco), penicillin and streptomycin  
86 (Gibco). These cells were grown at 37°C in an incubator with 5% CO<sub>2</sub>.

87 The CHSE-214 is an embryonic cell line derived from Chinook salmon obtained as a gift  
88 from Dr. Neils Bols (Waterloo, Canada). CHSE-214 cells were cultured in Leibovitz's L-15  
89 medium supplemented with 12% fetal bovine serum (Invitrogen), 5 ml of 200 mM L-Glutamine  
90 (Gibco), 25 mM HEPES buffer (Gibco), penicillin and streptomycin (Gibco). These cells were  
91 grown at 19°C in an incubator without CO<sub>2</sub>.

92 Reduction in cloning efficiency was observed using the clonogenic assay technique  
93 developed by Puck and Marcus [9]. Cell stocks were maintained in T75 flasks with 30ml  
94 medium. Upon reaching 80-90% confluence, flasks were subcultured. Here cells were gently  
95 rinsed with calcium and magnesium-free DPBS in a biosafety level 2 laminar flow cabinet.  
96 HaCaT cells were detached using a 0.25% (v/v) trypsin-1 mM EDTA solution (Gibco) at 37°C  
97 for 8 minutes, while CHSE-214 cells were detached using a 0.125% (v/v) trypsin-1 mM EDTA  
98 solution (Gibco) at 19°C for 8 minutes. Trypsin was neutralized using fresh culture media, and  
99 the cell solution was centrifuged at 125g for 4 minutes. The pellet was resuspended, and cells  
100 were counted using an automated cell counter (Bio-Rad TC20). The cells were then seeded into  
101 fresh flasks with fresh culture media at the required cell density such that at least 100 viable  
102 colonies could be expected to form in control flasks.

103 Reporter T25 flasks were maintained in the incubator for 9 days. Following this  
104 incubation period, colonies in sham irradiated (control) flasks were visible to the naked eye.  
105 Flasks were stained using a 1:4 (v/v) dilution of Fuchsin-Carbol (Ricca Chemical Co., Arlington,

106 TX) in water, and macroscopically visible colonies (confirmed to have more than 50 cells when  
107 observed under a microscope) were scored as survivors.

108

## 109 **Chronic irradiation using Ra-226 in medium**

110 Stock solutions of medium containing the radioisotope Ra-226 were prepared using  
111 neutralized radium nitrate (Eckert and Ziegler, Valencia, USA). 100 ml L-15 or RPMI medium  
112 was mixed with 1000 Bq of Ra-226 solution. The concentration of Ra-226 in this stock medium  
113 was 10,000 mBq/ml. After filtering into storage tubes, serial dilutions were made to give the  
114 required final concentrations.

115 500 cells were initially seeded into T25 flasks containing 5 ml of medium with Ra-226 or  
116 control medium. 4 flasks were prepared for each respective concentration: 0, 0.1, 1, 10, 100, 200  
117 or 500 mBq/ml Ra-226. Flasks were maintained in the incubator for 9 days after which the  
118 radioactive medium was removed, and the cells were gently rinsed with calcium and magnesium-  
119 free DPBS. Ra-226 residues in the flasks were assumed to be insignificant. Flasks then received  
120 5 ml of fresh culture medium without Ra-226 and returned to the incubator. 3 flasks from each  
121 concentration were deemed reporter flasks, incubated for 9 days and stained as described above.  
122 Cloning efficiencies observed in these reporter flasks represented the initial plating efficiencies  
123 from direct chronic irradiation. The remaining fourth flask of each concentration was left to  
124 incubate until 80-90% confluency, after which it was subcultured as described above seeding 500  
125 cells into a fresh flask. From here on however no further irradiation was to be done and all flasks  
126 received fresh culture medium containing 0 mBq/ml Ra-226. The process was repeated as  
127 before, and cloning efficiencies observed in these reporter flasks represented survival fractions of

128 the progeny (P2). The process was repeated once more to observe further change in the cloning  
129 efficiency in subsequent generations (P3).

130

### 131 **Acute irradiation using a Cs-137 source**

132 As with the Ra-226 experiments, 4 T25 flasks were seeded with 500 cells for each  
133 respective dose: 0, 0.05, 0.1, 0.25, 0.5, 0.75 or 1 Gy. The flasks were incubated for 6 hours to  
134 allow for cells to adhere to the flask, after which they were exposed to their respective  $\gamma$ -ray dose  
135 using a cesium-137 source (Taylor source, McMaster University, Hamilton, Canada). Flasks  
136 were placed at 26 cm from the radiation source, irradiated at a dose rate of 0.273 Gy/min and the  
137 room temperature was around 26°C.

138 All flasks were placed back in the incubator immediately after irradiation. Similar to the  
139 Ra-226 experiments, 3 flasks were deemed reporter flasks and incubated for approximately 9  
140 days before being stained as described above (initial). The remaining fourth flask of each dose  
141 was incubated until cells became 80-90% confluent, after which they were subcultured as  
142 described above with fresh culture medium. This process was also repeated twice as above (P2  
143 and P3).

144

### 145 **Determining $\gamma$ dose from Ra-226**

146 All possible  $\gamma$  emission events during the decay of Ra-226 to daughters Pb-214 and Bi-  
147 214 were tabulated according to their energy (keV) and probability (%) [10]. Total  $\gamma$  energy  
148 emitted per decay was then found through the summation of each  $\gamma$  energy multiplied by its

149 emission probability. A system was then set up using MonteCarlito 1.10 to describe the starting  
150 source geometry: a plane of uniformly spread particles with a y-dimension twice as big as the x-  
151 dimension (similar to T-25 flask dimensions), with each particle representing emitted  $\gamma$  energy  
152 from the source. The radionuclide was assumed to be evenly distributed in the medium. The  
153 number of particles was calculated through the concentration of Ra-226 in each respective  
154 medium (dividing the activity by the decay constant). The average distance for one interaction by  
155 an emitted particle was calculated through its mean free path. Central particles conducted most  
156 interactions within the flask however only a quarter of interactions of particles closer to the  
157 corners of the flask, and half of interactions from particles immediately adjacent to flask walls  
158 contributed dose within the flask. Figure 1 shows one iteration of the Monte Carlo simulation  
159 describing the spatial distribution of  $\gamma$  emissions in a flask. Dose rate contributed by each particle  
160 was calculated using the following equation:

161 
$$\dot{D} = \frac{AE \mu_{en}}{4\pi r^2 \rho}$$

162 Where  $A$  represents the respective activity (Bq),  $E$  represents the respective  $\gamma$  energy emitted per  
163 decay (MeV) and  $\mu_{en}/\rho$  represents the mass energy-absorption coefficient (assumed to be 0.05 to  
164 represent cells). The final dose was determined in Gy through multiplying the average dose rate  
165 for all particles in the flask (determined with consideration to the previously defined geometry  
166 parameters through Monte Carlo simulation) by the time exposed to Ra-226 (9 days or  $7.8 \times 10^5$   
167 seconds).

168 **Fig 1. Spatial Distribution of  $\gamma$ -emissions.** One iteration of the Monte Carlo simulation  
169 determining the spatial distribution of Ra-226 particles emitting gamma energy per emission  
170 (500 mBq/ml shown). The gamma energy is shown in MeV.



171

## 172 **Curve modelling**

173 Survival fractions/plating efficiencies of cells are determined as cloning efficiency  
174 observed through staining: the fraction of colonies formed from the 500 cells plated. Residual  
175 survival fractions were calculated at each observed interval and for each dose through following  
176 the recorded cell numbers at the start and end of each interval, in accordance to previous delayed  
177 lethal effect assays by Mothersill et al. [11, 12]. Here, the product of the cells observed at the end  
178 of the current passage, with the total cell number at the end of the preceding passage, was  
179 divided by the initial number of cells seeded per passage corrected for plating efficiency. Finally,  
180 curves were fitted to the calculated residual survival values at each observed interval using the  
181 induced-repair equation taken from the model described by Lambin et al. [13]:

$$182 \quad S = \exp \left( -\alpha_r \left( 1 + \left( \frac{\alpha_s}{\alpha_r} - 1 \right) e^{-\frac{D}{D_c}} \right) D - \beta D^2 \right)$$

183 Here  $\alpha_r$  describes the traditional linear-quadratic dose-response model while  $\alpha_s$  describes a  
184 region of the curve showing resistance from the linear component. Curves were fit using the R  
185 Project for Statistical Computing [14] through the *nlsLM* function of MINPACK, which uses a  
186 modified Levenberg-Marquardt algorithm to perform non-linear regression. The residual sum of  
187 squares (RSS) was used to further observe the fit of the curve to empirical values, as well as  
188 verify the use of the induced-repair equation compared to the traditional linear-quadratic model.

189 To isolate the effect of  $\alpha$ -particles on the survival and genomic instability of cells, this  
190 study subtracted effects observed after  $\gamma$  irradiation (through acute exposure to Cs-137) from

191 mixed  $\alpha$  and  $\gamma$  irradiation (through chronic exposure to Ra-226). Once the empirical data of the  
192 study is represented through curves, this is simply done through subtracting the function of one  
193 curve from the other.

194

## 195 **Results**

### 196 **Human keratinocyte cell line (HaCaT)**

197 Fitted curves representing the residual survival fractions for HaCaT cells show markedly  
198 different responses in directly exposed cells and their progeny with acute exposure to Cs-137  
199 compared to those with chronic exposure to Ra-226 (Fig 2). Progenitor HaCat cells (*initial*)  
200 exposed to Cs-137 show a region of hyper-radiosensitivity (HRS) at very low doses with lower  
201 survival than would be expected by the traditional linear-quadratic model, followed by a region  
202 of increased radioresistance (IRR). The progeny of these cells continue to demonstrate such  
203 HRS/IRR behavior with further decrease in cloning efficiency thereby observing lethal mutations  
204 in those generations. In particular significant decreases in cloning efficiencies are observed in the  
205 first observation of progeny of cells (*P2, 8 population doublings*) irradiated at 0.05 Gy by 19% (  
206  $p = 0.007$ ), 0.1 Gy by 23% ( $p = 0.00007$ ), 0.25 Gy by 14% ( $p = 0.02$ ), 0.5 Gy by 15% (  
207  $p = 0.01$ ) and 0.75 Gy by 17% ( $p = 0.0004$ ). Further significant decreases are observed in the  
208 second observation of progeny (*P3, 16 population doublings*) at 0.05 Gy by 17% ( $p = 0.01$ ) and  
209 at 0.1 Gy by 14% ( $p = 0.001$ ). The residual sum of squares (RSS) values for the initial, P2 and  
210 P3 curves are 0.0003, 0.004 and 0.002 respectively, demonstrating noticeably better fit with the

211 induced-repair equation compared to the traditional linear-quadratic model (RSS values of 0.2,  
212 0.09 and 0.2 respectively).

213

214 **Fig 2. Residual Survival of HaCaT.** Residual survival fractions as represented through fitted  
215 curves following the induced-repair model. Green curves represent cells exposed to Cs-137 and  
216 their progeny while black curves represent cells exposed to Ra-226 and their progeny. The  
217 darkest/solid curves represent the initial survival of the progenitors directly receiving radiation,  
218 medium/dashed curves represent the first observation of progeny (not directly irradiated), and the  
219 lightest/dotted curves represent the second observation of progeny (not directly irradiated). There  
220 were roughly 7 days between observations (time taken to reach 80-90% confluency).

221

222 In comparison, progenitor cells exposed to Ra-226 show significantly greater survival  
223 with many observations of higher cloning efficiency compared to sham irradiated control flasks  
224 (denoted as survival values greater than 100%). As such no HRS region is observed, with little to  
225 no change in survival compared to control in cells exposed to concentrations greater than 0.1  
226 mBq/ml of Ra-226. At P2, survival of progeny in concentrations up to 10 mBq/ml of Ra-226  
227 observe significantly higher survival compared to control, while observations at P3 show similar  
228 survival values to what was observed in the progenitors. Despite lacking an HRS/IRR region, the  
229 induced-repair equation still shows greater fit with RSS values of 0.006, 0.03 and 0.005  
230 respectively for the initial, P2 and P3 curves (compared to 0.02, 0.08 and 0.03 respectively for  
231 the traditional linear-quadratic model), as it better matches the observed hyper increased  
232 radioresistance (HIRR) observed at very low doses.

233 Through subtracting the functions of fitted curves for cells exposed to Cs-137 from those  
 234 exposed to Ra-226 at each interval, the relative effect of alpha exposure to the residual survival  
 235 of HaCaT cells was isolated (see Table 1). Figure 3 describes the functions of the isolated effect  
 236 of alpha exposure to residual survival graphically at each observation.

237

238 **Table 1. Isolating the relative effect of alpha exposure to the residual survival of HaCaT**  
 239 **cells.** At each dose, “Expected Residual Survival due to  $\gamma$ ” was calculated using the gamma  
 240 component of the dose, and the function representing residual survival of cells exposed to Cs-  
 241 137. This was then compared to the residual survival observed when cells were exposed to Ra-  
 242 226. The difference ( $\Delta$ ) is the isolated effect of alpha exposure. Note negative difference values  
 243 indicate higher survival of cells in the presence of alpha particles, versus gamma exposure.

Dose (mBq/ml Ra-226)		Expected Residual Survival due to $\gamma$	Observed Ra-226 Residual Survival ( $\gamma+\alpha$ )	$\Delta$
0	<i>Initial</i>	1.00	1.00	0.00
	<i>P2</i>	1.00	1.00	0.00
	<i>P3</i>	1.00	1.00	0.00
0.1	<i>Initial</i>	1.00	1.09	-0.09
	<i>P2</i>	0.10	1.18	-0.18
	<i>P3</i>	0.99	1.09	-0.10
1	<i>Initial</i>	0.99	1.07	-0.08
	<i>P2</i>	0.97	1.16	-0.19
	<i>P3</i>	0.94	1.15	-0.21
10	<i>Initial</i>	0.95	1.07	-0.12
	<i>P2</i>	0.79	1.15	-0.36
	<i>P3</i>	0.65	1.06	-0.41

100	<i>Initial</i>	0.91	1.04	-0.13
	<i>P2</i>	0.76	1.03	-0.27
	<i>P3</i>	0.69	1.01	-0.32
200	<i>Initial</i>	0.86	1.07	-0.21
	<i>P2</i>	0.69	1.13	-0.44
	<i>P3</i>	0.65	1.01	-0.36
500	<i>Initial</i>	0.58	0.92	-0.34
	<i>P2</i>	0.62	0.79	-0.17
	<i>P3</i>	0.64	0.86	-0.22

244

245

246 **Fig 3. Residual Survival of HaCaT due to Alpha exposure.** Calculated relative effect of alpha  
247 particle exposure on residual survival of HaCaT cells, as a function of effective gamma dose to  
248 progenitor cells. The darkest/solid curve represents the initial survival of progenitors directly  
249 receiving radiation, the medium/dashed curve represents the first observation of progeny (not  
250 directly irradiated), and the lightest/dotted curve represents the second observation of progeny  
251 (not directly irradiated). There were roughly 7 days between observations (time taken to reach  
252 80-90% confluency).

253

### 254 **Embryonic Chinook salmon cell line (CHSE-214)**

255 In contrast to the studied human cell line, there was no significant cell death observed in  
256 the directly exposed cells of the CHSE-214 fish cell line and their progeny to acute Cs-137  
257 exposure (Fig 4). This shows an existing radioresistance when compared to human cell culture  
258 [15]. When CHSE-214 cells were exposed to Ra-226 however, progenitor cells show a marked

259 response with decreasing cell survival following an almost linear trend with respect to dose.  
260 Residual survival observed at P2 (8 doubling periods) show increased lethal mutation however  
261 residual survival observed in the subsequent progeny at P3 (16 doubling periods) demonstrate a  
262 return of radioresistance with survival values similar to initial values.

263

264 **Fig 4. Residual Survival of CHSE-214.** Residual survival fractions as represented through fitted  
265 curves following the induced-repair model. Green curves represent cells exposed to Cs-137 and  
266 their progeny while black curves represent cells exposed to Ra-226 and their progeny. The  
267 darkest/solid curve represents the initial survival of progenitors directly receiving radiation, the  
268 medium/dashed curve represents the first observation of progeny (not directly irradiated), and the  
269 lightest/dotted curve represents the second observation of progeny (not directly irradiated). Note  
270 significant overlap in residual survival of cells exposed to Cs-137 and their progeny due to  
271 minimal observed cell killing. There were roughly 40 days between observations (time taken to  
272 reach 80-90% confluency).

273

274 Using the same methodology as was done for the human cell line, the relative effect of  
275 alpha exposure to the residual survival of CHSE-214 cells was isolated (see Table 2). As  
276 exposure to gamma irradiation caused little to no effect in residual survival, the isolated relative  
277 effect of alpha exposure is significant, especially at the higher end of the low dose range. The  
278 dose dependent function for the isolated effect at each observation is shown graphically in Figure  
279 5.

280

281 **Table 2. Isolating the relative effect of alpha exposure to the residual survival of CHSE-214**  
 282 **cells.** At each dose, “Expected Residual Survival due to  $\gamma$ ” was calculated using the gamma  
 283 component of the dose, and the function representing residual survival of cells exposed to Cs-  
 284 137. This was then compared to the residual survival observed when cells were exposed to Ra-  
 285 226. The difference ( $\Delta$ ) is the isolated effect of alpha exposure.

286

Dose (mBq/ml Ra-226)		Expected Residual Survival due to $\gamma$	Observed Ra-226 Residual Survival ( $\gamma+\alpha$ )	$\Delta$
0	<i>Initial</i>	1.00	1.00	0.00
	<i>P2</i>	1.00	1.00	0.00
	<i>P3</i>	1.00	1.00	0.00
0.1	<i>Initial</i>	1.00	0.69	0.31
	<i>P2</i>	1.00	0.50	0.50
	<i>P3</i>	1.00	0.66	0.34
1	<i>Initial</i>	1.00	0.71	0.29
	<i>P2</i>	1.00	0.56	0.44
	<i>P3</i>	1.00	0.73	0.27
10	<i>Initial</i>	1.00	0.75	0.25
	<i>P2</i>	1.01	0.61	0.40
	<i>P3</i>	1.03	0.81	0.22
100	<i>Initial</i>	1.02	0.55	0.47
	<i>P2</i>	1.10	0.39	0.71
	<i>P3</i>	1.23	0.64	0.59
200	<i>Initial</i>	1.02	0.31	0.71
	<i>P2</i>	1.17	0.15	1.02
	<i>P3</i>	1.39	0.45	0.94

287

288

289 **Fig 5. Residual Survival of CHSE-214 due to Alpha exposure.** Calculated relative effect of  
290 alpha particle exposure on residual survival of CHSE-214 cells, as a function of effective gamma  
291 dose to progenitor cells. The darkest/solid curve represents the initial survival of progenitors  
292 directly receiving radiation, the medium/dashed curve represents the first observation of progeny  
293 (not directly irradiated), and the lightest/dotted curve represents the second observation of  
294 progeny (not directly irradiated).

295

## 296 **Discussion**

297 At sub-lethal doses of gamma irradiation through acute exposure to Cs-137, the HaCaT  
298 cell line displayed a region of low-dose hyper-radiosensitivity (HRS) followed by increased  
299 radioresistance (IRR). In addition, lethality was observed in subsequent generations (lethal  
300 mutation phenotype) with significant decreases in cloning efficiencies observed in unirradiated  
301 progeny cells. In contrast, only radioresistance was observed in the progenitor cells exposed to  
302 Ra-226 with significantly higher survival and no observable region of HRS. Further, the  
303 observed progeny of these cells showed increased survival and lowered lethal mutation. In  
304 comparison, the results of experiments using the non-mammalian embryonic fish cell line  
305 showed the reverse of what was observed in human cell culture. Survival data following  
306 exposure to gamma irradiation confirmed existing radioresistance in the CHSE-214 cell line  
307 compared to human cell culture, with no significant lethality. However, survival data for cells  
308 exposed to Ra-226 suggested that alpha particles promoted lethality at doses otherwise known to  
309 have no significant effect.



310           Considering the potential for sub-lethal doses from chronic exposure to radium and its  
311 daughters found in waste products, to remnants of historic commercial and medical usage of  
312 radium (ranging from self-luminous paints to cancer treatment), the unconventional behaviors  
313 observed in both cell lines of this study have potential importance in radiological protection.  
314 Further, the presence of radium in waste reaching the ecosystem from mining and nuclear  
315 applications is important given the currently growing interest for non-human radiological  
316 protection.

317           The radioprotective quality of sub-lethal doses of alpha radiation that was observed in  
318 the HaCaT cell line, where cells displayed significantly lower lethality in the presence of alpha  
319 particles greatly contrasts with RBE values found in the literature. Previous *in vitro* studies of  
320 alpha radiation effects at higher doses compared to this study have all consistently demonstrated  
321 a significantly higher biological effect of alpha particles relative to photons, with values ranging  
322 from <2 for the induction of double strand breaks, to 3.5-10 for cell lethality and transformation  
323 in different cell lines, to >25 for other endpoints assessed [16]. Research observing HRS/IRR  
324 behaviors suggest the activation of cell cycle checkpoints for increased cell repair, etc. as a  
325 possible mechanism for radioresistance [17]. Considering only radioresistance was observed in  
326 the presence of alpha radiation, the results suggest an ultra-low dose of alpha particles produces a  
327 sufficient level of genomic instability to activate the previously mentioned cell cycle  
328 checkpoints, inducing radioresistance. This effect was non-linear with dose with marked  
329 reduction as dose increased to the progenitor. The results observed in the CHSE-214 cell line on  
330 the other hand is in line with currently accepted descriptions on the effect of alpha particles at  
331 high doses. Here, the concentration of damage events is said to exceed a threshold at which  
332 effective repair becomes difficult [18]. Further lethality is seen in progeny as a de novo

333 appearance of non-clonal lethal mutations, indicative of genomic instability. However, this  
334 decreases with subsequent generations suggesting the ability for existing damage repair  
335 mechanisms eventually to counteract the heritable susceptibility to lethal damage.

336         The results of the study support the need to consider dose-dependence when describing  
337 the relative biological effect of different radiation qualities. Overestimation of the biological  
338 effect of sub-lethal exposure to radium in humans can result in unnecessary psychological stress  
339 and limit productivity in industry. In addition, the results of the fish cell line experiments  
340 confirm the need to be aware of species differences, confirming that protection for humans  
341 would not inherently protect ecosystems and non-human biota.

342         It should be noted however that the observed *in vitro* results cannot simply be translated  
343 to *in vivo* effects without further research. For example, while there is evidence for heritable  
344 NTE through *in vitro* and non-human studies, there has been no evidence for radiation-induced  
345 hereditary effects observed in epidemiological studies of human populations exposed to ionizing  
346 radiation [19]. In addition, further research needs to be done to isolate the effect of dose rate on  
347 sub-lethal exposure to high-LET radiation, as differences in time for cell repair can affect the  
348 level of radioresistance observed.

349

## 350 **Conclusion**

351         At sub-lethal doses, survival greatly depends on repair mechanisms. Since the HaCaT cell  
352 line demonstrates hyper-radiosensitivity to gamma energy at low doses, high-LET alpha particle  
353 radiation may be able to produce sufficient genomic instability to induce radioresistance. In such  
354 instances, the ratio of relative biological damage caused by alpha exposure is significantly lower

355 than an equivalent dose of gamma energy alone, and as such a lower radiation weighting factor  
356 might be considered. However, while the CHSE-214 cell line demonstrates increased  
357 radioresistance to gamma energy, the concentrated nature of energy deposited causes increased  
358 lethality when exposed to alpha particles. These cases would suggest a higher radiation  
359 weighting factor, similar to what is currently recommended. Further study is required to isolate  
360 the effect of dose-rate at sub-lethal doses. In addition, further consideration is required to  
361 translate the observed *in vitro* results to *in vivo* effects. As alpha-emitters are commonly found in  
362 industrial applications, the environment, as well as released in nuclear incidents, this knowledge  
363 would be particularly meaningful for risk management and radiation protection of human and  
364 non-human biota to low-dose high LET radiation.

365

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370

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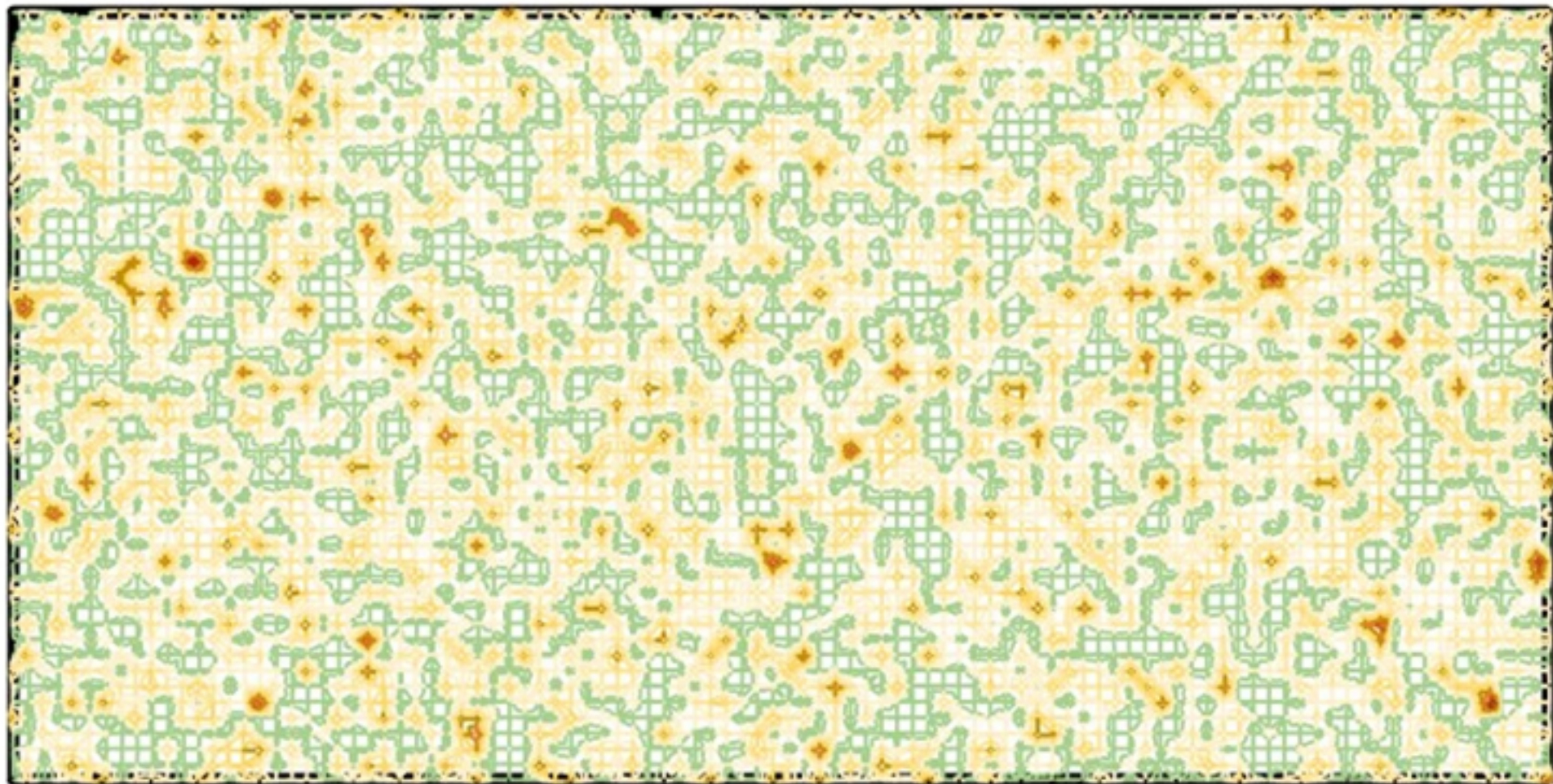
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$\gamma$  energy (MeV)

□ 0-1

□ 1-2

□ 2-3

□ 3-4

□ 4-5

□ 5-6

□ 6-7

□ 7-8

□ 8-9

