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Low dose ionising radiation produces too few ROS to directly affect antioxidant concentrations in cells

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Summary

It has been hypothesised that radiation-induced oxidative stress is the mechanism for a wide range of negative impacts on biota living in radioactively contaminated areas around Chernobyl. The present study tests this hypothesis mechanistically for the first time by modelling the impacts of radiolysis products within the cell resulting from radiations (low LET β and γ) and dose rates appropriate to current contamination types and densities in the Chernobyl exclusion zone and at Fukushima. At 417 μGy h^{-1} (illustrative of the most contaminated areas at Chernobyl), generation of radiolysis products did not significantly impact cellular concentrations of reactive oxygen species, or cellular redox potential. This study does not support the hypothesis that direct oxidising stress is a mechanism for damage to organisms exposed to chronic radiation at dose rates typical of contaminated environments.

Keywords Chernobyl, Fukushima, radiation, oxidative stress, biota, cell

1. INTRODUCTION

Oxidative stress "results from a mismatch between the production of damaging reactive oxygen species (ROS) and the organisms' capacity to mitigate their damaging effects" (1). At high dose rates, cellular oxidising stress plays an important role in cell damage from ionising radiation, and antioxidants may have a protective effect at such dose rates. For example, injection of vitamin E (α-tocopherol) increased 30-day survival rates of mice exposed to high dose rate (up to 6×10^7 μGy h^{-1}) of low linear energy transfer (LET) radiation (2).

Significant radiation-induced oxidative stress of flora and fauna at lower dose rates (here defined as up to ca. 400 μGy h^{-1} from internal and external sources) has also been hypothesised. Plant responses have been linked to oxidising stress and antioxidant capacity in field and experimental studies (3). Significantly lower antioxidant concentrations have been observed in birds (barn swallow, *Hirundo rustica*; great tit, *Parus major*) inhabiting areas contaminated by Chernobyl (4), an effect attributed to radiation-induced oxidative stress. This hypothesis is supported by observations of decreased levels of the antioxidants retinol, α-tocopherol and carotenoids in blood plasma, liver and egg yolk of barn swallows living near Chernobyl (4).

However, the relationship between antioxidant concentrations and oxidative stress is complex (1). For example, studies in plants have found increased concentrations of antioxidant enzymes with radiation exposure (5) at low dose rates. Other low dose-rate studies have found no changes in antioxidant concentrations either in plants (6) or birds (7), though the latter did observe a significant difference in metabolites produced by reactive oxygen (ROM). Recently, Bonisoli-Alquati et al. (8) found that "oxidative damage of sperm was negatively related to sperm motility" in birds exposed to radiation at Chernobyl, but that "the highest values [of high sperm motility] were associated with relatively high radiation levels".

The low radiation dose-rate oxidising stress hypothesis has not, to our knowledge, yet been tested at a mechanistic level. The present study tests this hypothesis (using previously published data on oxidising stress in birds at Chernobyl) by modelling, for the first time, the capacity of selected antioxidants to reduce radiolysis products at radiation dose rates appropriate to current contamination densities pertaining at Chernobyl and Fukushima.

2. MATERIAL AND METHODS

For given dose rate, D (Gy s^{-1}) we can calculate the rate of production of ion pairs per unit mass of an organism by considering the radiolysis products of water when exposed to ionising radiation. These include: H_2; H_2O_2; e^-_{aq}; $H\cdot$; $OH\cdot$; $HO_2\cdot$ (see ESM). For low LET radiation, G-values, giving radiolysis products per Joule of absorbed radiation energy are given in Table SM1 (from (9)).

The rate of production of each radiolysis product, r_p per unit mass of tissue (mol l^{-1} s^{-1}) is:

$$r_p = \theta D G \qquad (1)$$

where θ is the fractional water content of the tissue.

We here investigate the potential impact of radiolysis products on cellular antioxidant concentrations. Assuming that the rate of replenishment of antioxidant molecules occurs at a rate proportional to the difference between the current concentration, C_T (mol l^{-1}) and a "target" equilibrium concentration, C_{TE} (mol l^{-1}), the following equation describes the rate of change of C_T :

$$\frac{dC_T}{dt} = r_f(C_{TE} - C_T) - r_p \quad (2)$$

where r_f is the fractional replenishment rate of antioxidant molecules (s^{-1}). For boundary condition $C_T = C_{TE}$ at t = 0, this has solution:

$$C_T = C_{TE} - \frac{r_p}{r_f}(1 - e^{-r_f t}) \quad (3)$$

The impact of radiolysis on the redox status of the cell can be quantified by considering the impact on redox potential, E_h (Volts) of decreased glutathione (GSH) due to oxidation to oxidised glutathione (GSSG) concentrations (10, 11):

$GSSG + 2H^+ + 2e^- \rightarrow 2GSH$

$$E_h = E_m - \frac{RT}{nF} 2.3\, log\left(\frac{[GSH]^2}{[GSSG]}\right) \quad (4)$$

where E_m is the mid-point potential (-0.240 V at pH 7; (11)), R is the gas constant (8.314 J K^{-1} mol^{-1}) , T is temperature (Kelvin), F is the Faraday constant (9.6485 × 10^4 C mol^{-1}), n is the number of electrons involved in the redox of the couple, in this case, two.

3. RESULTS

Effect of radiolysis on cellular antioxidant concentrations

A study by Møller and coworkers (4) observed that barn swallow liver cell α-tocopherol and carotenoid concentrations decreased at sites near Chernobyl (contaminated with approximately 3.9 μGy h^{-1} external) compared with a control site. Equation (3) was used to determine whether radiolysis could account for these changes. Setting the equilibrium antioxidant concentration C_{TE} to that of the control site, and assuming that the cell has minimal anti-oxidant capacity (i.e. no other enzymatic or non-enzymatic antioxidants operate in the cell), the change in concentration, C_T can be calculated. Figure (1) shows the effect of ionising radiation of 417 μGy h^{-1} (much higher than the birds were exposed to, see ESM) on cellular α-tocopherol and carotenoid concentrations for three different fractional rates of replenishment, 0.001, 0.01 and 0.1 d^{-1}.

Effect of radiolysis on cellular redox potential

The effect of radiolysis on cellular redox potential was investigated by again assuming the cell has minimal anti-oxidant capacity, i.e. just GSH and no other enzymatic or non-enzymatic anti-oxidants. Equation (3) was used to determine reduction in GSH concentration as a function of time following chronic exposure to 417 μGy h^{-1} low LET radiation. Equation (4) was used to calculate the impact of this change in the GSH – GSSG balance on the redox potential of the cell. We have here assumed a hypothetical GSH concentration of 1mM and have calculated the GSH/GSSG concentration starting with 1% conversion of GSH to GSSG. Glutathione concentrations in cells typically range from 1 – 11 mM (11-13). Figure (2) shows the change in GSH concentration and cellular redox potential for 1200 days exposure to 417 μGy h^{-1} low LET radiation assuming an unrealistically low (0.001 d^{-1}) replenishment rate of GSH.

Comparison of rate of production of ROS with reactive oxygen metabolite concentrations

The rate of production of ROS from radiolysis (Table 1) is compared with measurements from barn swallows at a contaminated (up to 2.9 μGy h^{-1}) site at Chernobyl using data presented in (7). Table 2 compares the daily rate of production of ROS by 417 μGy h^{-1} radiation with the difference in ROM between a contaminated and a control site. It can be seen (Table 2) that daily rate of production of ROS by ionising radiation represents a minuscule fraction (*ca*. 10^{-5}) of the difference in ROM between contaminated (dose rate 2.9 μGy h^{-1} external) and control sites observed by Bonisoli-Alquati et al. (7). As this study (7) only measured hydroperoxides, the difference between radiation-induced ROS production and ROM would in reality be greater.

4. DISCUSSION

We calculated the direct effects of radiolysis on antioxidant concentrations at a total (external+internal) radiation dose rate of 417 μGy h^{-1} low LET radiation, representative of the highest doses to organisms in the Chernobyl zone (see ESM) and also relevant to current contamination densities and dose rates at Fukushima (14). No significant changes in antioxidant concentrations or cellular redox potential were calculated. Assuming that only single antioxidants were utilised to reduce radiolysis products, and that fractional replenishment rates were as (unrealistically) low as 0.001 d^{-1}, antioxidant concentrations observed in "control" birds are not reduced to those of exposed birds over 1200 days (Figure 1). Differences in ROM between contaminated and control sites (7) cannot be explained by direct effects of radiolysis since the observed differences are orders of magnitude larger than the rate of production of ROS by radiolysis. The functional replenishment rate of glutathione in a range of animal tissues under different dietary conditions ranges from 10-100s %/d (15) highlighting how conservative our assumptions are. Furthermore, Schafer and Buettner (11) suggest that changes in redox potential that cause cell changes including, sequentially, proliferation, differentiation, apoptosis and necrosis need to be of order 60 mV whereas changes calculated here are less than 5mV over a 1200 day period.

We note that, despite the minor direct impact of radiation on redox status of the cell and on antioxidant concentrations, it is well known that even low dose ionising radiation can cause negative effects via DNA damage. Such damage is direct, by strand breaks and deletions, or indirect, from the free-radical products of water radiolysis in the immediate vicinity of nucleotides. At dose rates of order 417 μGy h^{-1} (representing the most contaminated parts of the Chernobyl exclusion zone), radiation effects on organisms would be expected, and have indeed been observed (16, 17). The present study shows that observed effects are unlikely to be due to radiolysis products directly causing oxidative stress, significantly clarifying discussions about low-level radiation and oxidative stress. Thus, whilst some radiation effects on organisms are likely (though see, for example, (18)) at dose rates pertaining in the most contaminated sites at Chernobyl, the results of our study do not support the hypothesis (4, 7, 8) that direct oxidising stress is the damage mechanism. It may also help to explain the variety and inconsistency of radiation-induced antioxidant responses apparently observed at low doses. Though not directly tested against data from organisms other than birds, these results are also likely to apply to other organisms: direct generation of radiolysis products is not nearly high enough to affect oxidative stress even though there is variation in antioxidative capacity between different organisms. Some of the studies on birds take account of habitat differences between sites of different contamination level, but it seems more likely that differences in habitat, diet or ecosystem structure are associated with changed antioxidant concentrations rather than the direct effects of radiolysis products.

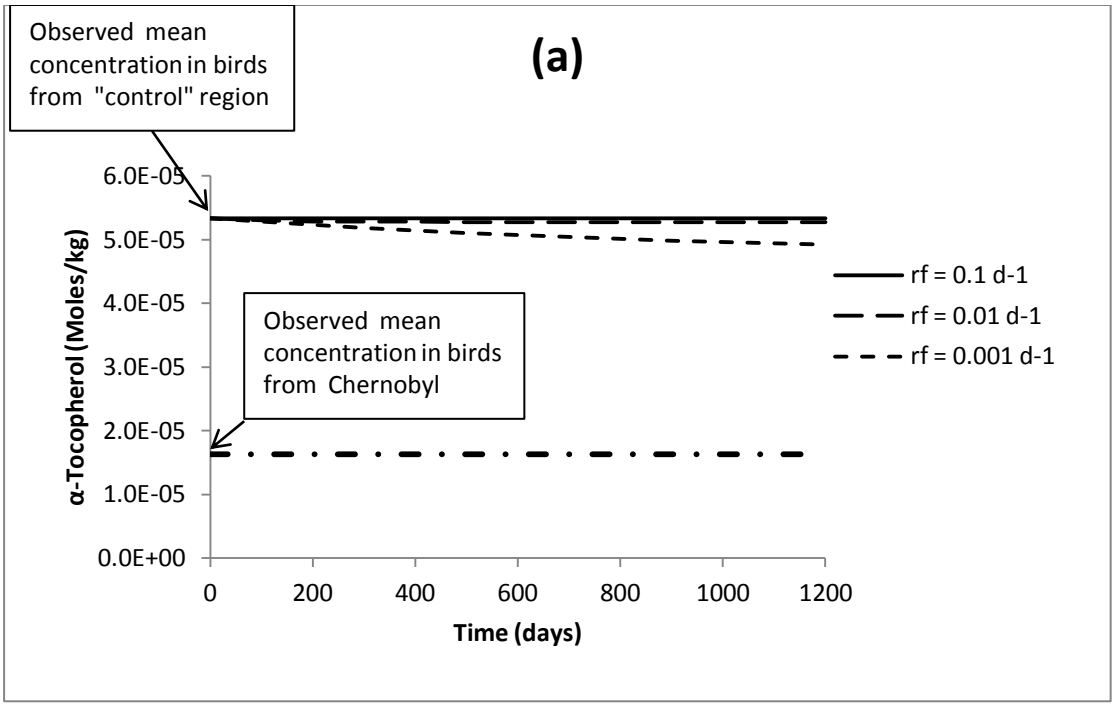
References

1. Monaghan P, Metcalfe NB, Torres R. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecology Letters. 2009;12(1):75-92.
2. Weiss JF, Landauer MR. Protection against ionizing radiation by antioxidant nutrients and phytochemicals. Toxicology. 2003;189(1-2):1-20.
3. Esnault M-A, Legue F, Chenal C. Ionizing radiation: Advances in plant response. Environmental and Experimental Botany. 2010;68(3):231-7.
4. Møller AP, Surai P, Mousseau TA. Antioxidants, radiation and mutation as revealed by sperm abnormality in barn swallows from Chernobyl. Proceedings of the Royal Society B: Biological Sciences. 2005 February 7, 2005;272(1560):247-53.
5. Zaka R, Vandecasteele CM, Misset MT. Effects of low chronic doses of ionizing radiation on antioxidant enzymes and G6PDH activities in Stipa capillata (Poaceae). Journal of Experimental Botany. 2002;53:1979-87.
6. Vandenhove H, Vanhoudt N, Cuypers A, van Hees M, Wannijn J, Horemans N. Life-cycle chronic gamma exposure of Arabidopsis thaliana induces growth effects but no discernable effects on oxidative stress pathways. Plant Physiology and Biochemistry. 2010;48(9):778-86.
7. Bonisoli-Alquati A, Mousseau TA, Møller AP, Caprioli M, Saino N. Increased oxidative stress in barn swallows from the Chernobyl region. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology. 2010;155(2):205-10.
8. Bonisoli-Alquati A, Møller AP, Rudolfsen G, Saino N, Caprioli M, Ostermiller S, et al. The effects of radiation on sperm swimming behavior depend on plasma oxidative status in the barn swallow (Hirundo rustica). Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology. 2011;159(2):105-12.
9. Choppin GR, Liljenzin J-O, Rydberg J. Radiochemistry and Nuclear Chemistry. Oxford: Butterworth-Heinemann; 1995.
10. Hancock JT, Desikan R, Neill SJ, Cross AR. New equations for redox and nano-signal transduction. Journal of Theoretical Biology. 2004;226(1):65-8.
11. Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radical Biology and Medicine. 2001;30(11):1191-212.
12. Meyer AJ, May MJ, Fricker M. Quantitative in vivo measurement of glutathione in Arabidopsis cells. The Plant Journal. 2001;27(1):67-78.
13. Smith CV, Jones DP, Guenthner TM, Lash LH, Lauterburg BH. Compartmentation of Glutathione: Implications for the Study of Toxicity and Disease. Toxicology and Applied Pharmacology. 1996;140(1):1-12.
14. Hosoda M, Tokonami S, Sorimachi A, Monzen S, Osanai M, Yamada M, et al. The time variation of dose rate artificially increased by the Fukushima nuclear crisis. Sci Rep. [10.1038/srep00087]. 2011;1.
15. Malmezat T, Breuille D, Capitan P, Mirand PP, Obled C. Glutathione Turnover Is Increased during the Acute Phase of Sepsis in Rats. The Journal of Nutrition. 2000 May 1, 2000;130(5):1239-46.
16. Chesser RK, Sugg DW, Lomakin MD, van den Bussche RA, DeWoody JA, Jagoe CH, et al. Concentrations and dose rate estimates of 134137cesium and 90strontium in small mammals at chornobyl, Ukraine. Environmental Toxicology and Chemistry. 2000;19(2):305-12.
17. Geras'kin SA, Fesenko SV, Alexakhin RM. Effects of non-human species irradiation after the Chernobyl NPP accident. Environment International. 2008;34(6):880-97.
18. Wickliffe JK, Bickham AM, Rodgers BE, Chesser RK, Phillips CJ, Gaschak SP, et al. Exposure to chronic, low-dose rate γ-radiation at Chornobyl does not induce point mutations in Big Blue® mice. Environmental and Molecular Mutagenesis. 2003;42(1):11-8.
19. LaVerne JA. OH Radicals and Oxidizing Products in the Gamma Radiolysis of Water. Radiation Research. 2000;153(2):196-200.

20. Kreipl M, Friedland W, Paretzke H. Time- and space-resolved Monte Carlo study of water radiolysis for photon, electron and ion irradiation. Radiation and Environmental Biophysics. 2009;48(1):11-20.
21. Beresford NA, Barnett CL, Brown JE, Cheng JJ, Copplestone D, Gaschak S, et al. Predicting the radiation exposure of terrestrial wildlife in the Chernobyl exclusion zone: an international comparison of approaches. J Radiol Prot. 2010 Jun;30(2):341-73.
22. Pungkun V. Chronic radiation doses to aquatic biota [PhD Thesis]: University of Portsmouth; 2012.
23. Prange HD, Anderson JF, Rahn H. Scaling of Skeletal Mass to Body-Mass in Birds and Mammals. Am Nat. 1979;113(1):103-22.
24. Dumont ER. Bone density and the lightweight skeletons of birds. Proc Biol Sci. 2010 Jul 22;277(1691):2193-8.
25. Kuncova P, Frynta D. Interspecific morphometric variation in the postcranial skeleton in the genus Apodemus. Belg J Zool. 2009 Jul;139(2):133-46.
26. Annunziata MF. Handbook of Radioactivity Analysis. 2nd ed. Amsterdam: Elsevier; 2003.
27. Beresford NA, Gaschak S, Barnett CL, Howard BJ, Chizhevsky I, Strømman G, et al. Estimating the exposure of small mammals at three sites within the Chernobyl exclusion zone – a test application of the ERICA Tool. Journal of Environmental Radioactivity. 2008;99(9):1496-502.

Figure 1 Changes in (a) α-Tocopherol and (b) carotenoids in birds' liver as a result of 10 mGy d^{-1} ionising radiation (Many times higher than the mean at the Chernobyl study sites, see ESM).

(Anti-oxidant concentrations were estimated from (4))



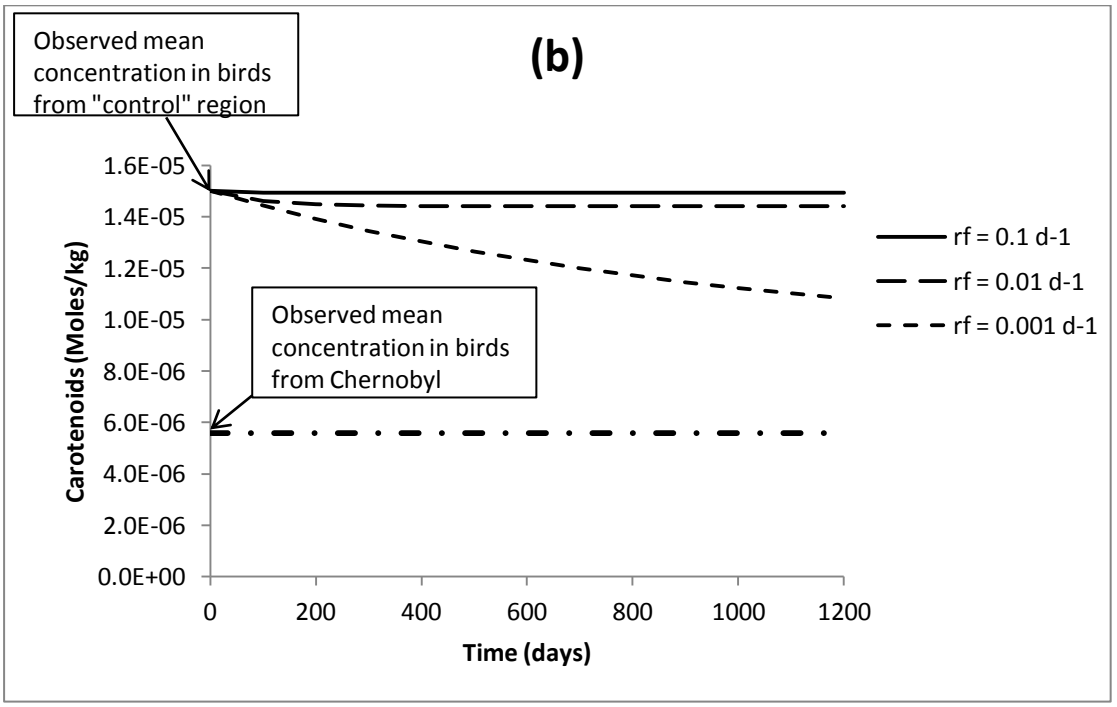


Figure 2 Predicted changes in GSH concentration and cellular redox potential following 1200 day exposure to 10 mGy d^{-1} (417 µGy h^{-1}) ionising radiation assuming initial 1 mM total (GSH + GSSG) and an unrealistically slow glutathione replenishment rate of 0.001 d^{-1}.

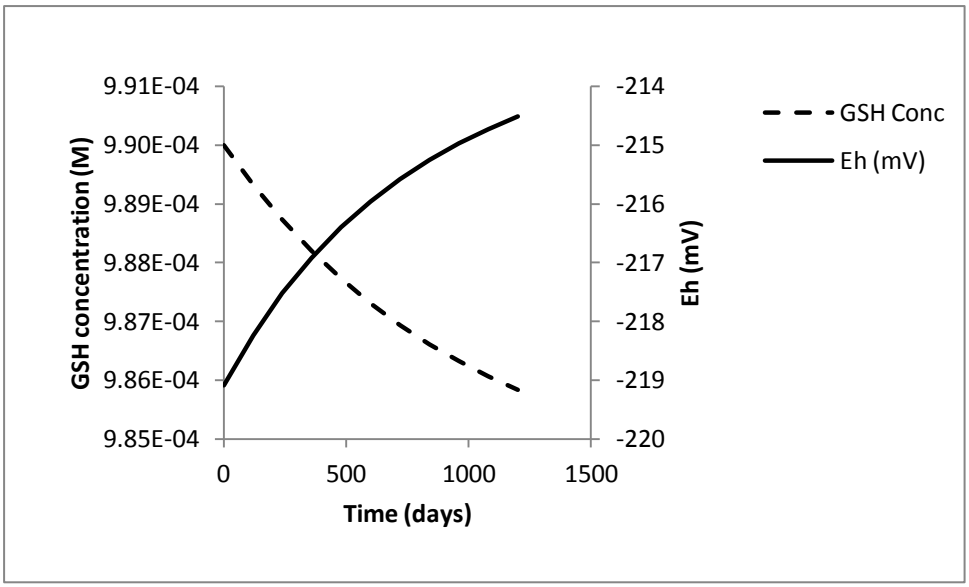


Table 1 Rate of production (mol l^{-1} s^{-1}) of radiolysis products of water at different exposures to γ- and high-energy β- radiation.

(A typical cellular water content of $\theta = 0.8$ is assumed.)

Dose rate mGy d^{-1}	Dose rate μGyh^{-1}	H_2	H_2O_2	e^-_{aq}	H·	OH·	HO_2·	ΣROS
1	41.7	4.35E-16	6.76E-16	2.59E-15	5.74E-16	2.59E-15	2.5E-17	**6.9E-15**
10	417	4.35E-15	6.76E-15	2.59E-14	5.74E-15	2.59E-14	2.5E-16	**6.9E-14**
100	4170	4.35E-14	6.76E-14	2.59E-13	5.74E-14	2.59E-13	2.5E-15	**6.9E-13**

Table 2 Daily production of ROS by radiolysis at 10 mGy d^{-1} (417 μGy h^{-1}) compared to differences in ROM (concentration of hydroperoxides) in the plasma of barn swallows between contaminated and control sites (data from (7)).

	ROM mM H_2O_2 equivalents				
Sex	Contaminated ca. 3 μGyh^{-1}	Control	Difference ΔROM	Rate of prodn. of ROS mM d^{-1}	$\frac{Rate\ prod.ROS}{\Delta ROM}$ d^{-1}
Males	2.45	2.03	0.42	5.96×10^{-6}	1.42×10^{-5}
Females	2.92	1.97	0.95	5.96×10^{-6}	6.27×10^{-6}

ELECTRONIC SUPPLEMENTARY MATERIAL

Radiolysis products of water

For low linear energy transfer (LET) radiation, the hydroxyl radical (•OH) and hydrogen peroxide (H_2O_2) are the primary oxidising species produced by water radiolysis, yields of other oxidising species, HO_2 and O_2 being negligible in comparison (19). At 1 ps, approximating to the end of the physical stage of radiolysis, yields of oxidising species are at a maximum, dominated by the OH radical. During the chemical stage (1 ps to 1 µs), yields (expressed as *G*-values, µmol J^{-1}) of H_2O_2 increase (19, 20) by the following reaction:

$$\bullet OH + \bullet OH \rightarrow H_2O_2$$

During the chemical phase, the molar yield of oxidising species decreases due to reactions of •OH with hydrated electrons (e^-_{aq}) and hydrogen radicals (•H) to form H_2O and H_2. Table SM1 shows radiolysis products at 10^{-7} s, i.e. during the chemical phase.

The yield of •OH at the end of the physical stage (ca 1 ps) of water radiolysis therefore represents the maximum number of oxidising species produced by radiolysis per Joule of absorbed radiation. Ten calculated *G* values of •OH summarised by Kreipl and co-workers (20) gave a mean of 0.630 µmol J^{-1} (S.E. 0.033; S.D. 0.100). For model calculations, the sum of radiolysis products at the end of the chemical stage (0.745 µmol J^{-1}, Table SM1) was used, this being an upper bound estimate, given the lower yield of •OH at the end of the physical stage.

Table SM1 *G*- values (µmol J^{-1}) for radiolysis products of water 10^{-7} s after an interaction from low- and high- LET radiation (from (9))

	H_2	H_2O_2	e^-_{aq}	H·	OH·	HO_2·
$G(\alpha)$	0.115	0.112	0.0044	0.028	0.056	0.007
$G(\beta/\gamma)$	0.047	0.073	0.28	0.062	0.28	0.0027

Relative importance of internal to external exposures

In assuming a representative high dose rate of 417 µGy h^{-1} to calculate generation of radiolysis products, we have not explicitly considered whether the radiation source is internal or external to the organism: the rate of generation of radiolysis products is independent of the source of radiation. However, the assumption that 10 mGy d^{-1} total (internal+external) dose needs to be assessed in the light of potentially higher internal than external dose rates in organisms, particularly since we are comparing model estimated ROM with antioxidant data linked only to an external dose measurement (3.9 µGy h^{-1}) (4). We have chosen a total dose rate of 417 µGy h^{-1}, more than 100 times higher, for comparison with the Moller et al. (4) data. We will demonstrate, below, that whilst internal exposures can exceed external, the difference is not great enough to invalidate our assumption of a 417 µGy h^{-1} total (internal+external) dose rate, even close to bone tissue which is contaminated with ^{90}Sr.

Radiocaesium (^{137}Cs) and radiostrontium (^{90}Sr) are the major contributors to dose in the Chernobyl area, with doses from transuranium elements being negligible in comparison (21). Using measurements of ^{137}Cs and ^{90}Sr in birds and small mammals at Chernobyl presented in Beresford *et al*. (21), we have calculated the internal dose to tissue close to bone for comparison with external dose

rates. Given the relatively even distribution of ^{137}Cs in tissue, and the high gamma contribution to total emitted decay energy, internal dose rates for ^{137}Cs were assumed to be evenly distributed throughout the body. Mean whole-body dose rates were calculated for the mass and activity concentration of each organism using dose conversion coefficients (DCC, μGy h^{-1} per Bq kg^{-1}) derived from DCC-mass relationships given in (22).

For ^{90}Sr, doses close to representative "large" bones (humerus for birds; femur for small mammals) were estimated. Total skeletal mass of each organism was calculated from allometric relationships given in Prange *et al*. (23) and bone masses and dimensions from data in Prange *et al*. (23), Dumont (24) and Kunkova and Frynta (25). Assuming that all of the internal ^{90}Sr in the organism is absorbed to bone tissue (giving the most uneven internal distribution of dose), we calculated the ^{90}Sr activity concentration in bone, then the dose to a region of tissue of radius 4 mm (based on mean range of the 0.934 MeV $^{90}Sr/^{90}Y$ beta in water, (26)) surrounding the bone.

Table SM2 compares the summed internal dose rate close to bone in various birds and small mammals living in the Chernobyl Exclusion Zone with external dose rates modelled using the ERICA model or measured by thermoluminescent dosimeter (21, 27). The ratio of internal:external dose rate ranges from 0.95 – 5.5 implying that internal contribution to dose does exceed external. However, assuming a ratio (internal:external) of 5.5, gives a total dose rate of 25.3 μGy h^{-1} for the barn swallows studied by (4) and 18.9 μGy h^{-1} for those studied by Bonisoli-Alquati et al. (7). Thus our assumption of 417 μGy h^{-1} remains conservative, even considering (potentially) small areas of tissue within 4 mm of more than one large bone. The highest estimated mean dose to tissue close to bone is 198 μGy h^{-1} for vole species inhabiting the highly contaminated Red Forest area (Table SM2).

Table SM2 Comparison of internal and external dose rates to birds and small mammals at Chernobyl based on data in Beresford et al. (21).

Species		Site ref and no. samples (N)	Assumed Mass (g)	Mean ^{90}Sr in body Bq/kg (N)	Mean ^{137}Cs in body Bq/kg	Internal dose from ^{90}Sr in tissue near bone μGy h^{-1}	Internal dose from ^{137}Cs, μGy h^{-1}	Total internal dose rate close to bone μGy h^{-1}	External dose rate, μGy h^{-1}	Ratio of int:ext dose rate
Barn swallow	*Hirundo rustica*	CT7 (1)	19	1.7×10^3	1.4×10^3	1.0	0.21	1.24	1.3	0.95
Robin	*Erithacus rubecula*	CT37 (8)	19	3.60×10^4	1.50×10^3	21.8	0.22	22	4	5.5
Great tit	*Parus major*	CT36a (26)	18	5.70×10^3	1.80×10^4	3.4	2.68	6.1	1.1	5.5
Starling	*Sturnus vulgaris*	CT10 (1)	75	9.70×10^3	2.90×10^3	9.5	0.46	10	1.8	5.5
Vole species	*Microtus spp.*	CT32a (11)	23	1.10×10^5	6.10×10^5	62.2	91.9	154	43.7	3.5
Bank vole	*Clethrionomys glareolus*	CT33a (39)	23	1.90×10^4	7.10×10^4	10.7	10.7	21.4	13.1	1.6
Yellow-necked mouse	*Apodemus flavicollis*	CT33b (10)	30	2.50×10^4	6.00×10^4	18.4	9.2	27.6	17.2	1.6
Bank vole	*Clethrionomys glareolus*	CT34a (3)	23	7.70×10^3	3.80×10^3	4.4	0.57	4.9	2.1	2.3
Yellow-necked mouse	*Apodemus flavicollis*	CT34b (18)	30	7.40×10^3	3.10×10^3	5.5	0.47	5.9	1.5	4.0