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ORIGINAL PAPER

Cytogenetic effect of low dose γ-radiation in *Hordeum vulgare* seedlings: non-linear dose–effect relationship

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Abstract The induction of chromosome aberrations in *Hordeum vulgare* germinated seeds was studied after ionizing irradiation with doses in the range of 10–1,000 mGy. The relationship between the frequency of aberrant cells and the absorbed dose was found to be nonlinear. A dose-independent plateau in the dose range from about 50 to 500 mGy was observed, where the level of cytogenetic damage was significantly different from the spontaneous level. The comparison of the goodness of the experimental data fitting with mathematical models of different complexity, using the most common quantitative criteria, demonstrated the advantage of a piecewise linear model over linear and polynomial models in approximating the frequency of cytogenetical disturbances. The results of the study support the hypothesis of indirect mechanisms of mutagenesis induced by low doses. Fundamental and applied implications of these findings are discussed.

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Introduction

Exposure to low doses and dose rates of ionizing radiation is an inevitable factor in the current environment, and all biota, including man, are subjected to chronic exposure. At many sites around the world high levels of natural background radiation occur, where environmental exposure can be significant. In addition, accidental releases of radionuclides at nuclear facilities can substantially increase exposure levels for the environment. Understanding the risks of low doses of radiation is also important with regard to various issues such as cancer screening, occupational exposure, frequent-flyer risks, and the future of nuclear power. For example, most radiological examinations produce doses in the range from 3 to 30 mSv [1]. Since the concept of radiation protection for humans and biota should be based on a clear comprehension of the consequences of low level exposure, a correct estimation of the effects of low doses is an important topic.

Since the early 1930s, it has been thought that the yield of mutations per unit of dose is the same at low and high doses (linearity of response) [2] and it was also then supposed that a charged particle, interacting directly with a chromosome, produces irreversible modifications in genes (the non-thresholdness) [3]. These postulates were taken as a background for the currently prevalent linear non-threshold (LNT) concept [4] which states the existence of an absolute risk at any radiation level.

Actually, the effect of ionizing radiation on a molecular level can be considered non-threshold, because the energy of any charged particle is far above the binding energy in biological macromolecules.

However, by the mid 1950s it became obvious that the notions used for the high dose effects could not be transferred mechanistically and applied to an analysis of the biological effects of low doses. Luchnik and Timofeeff-Ressovsky were, to our knowledge, the first who showed abnormal responses of different biological objects to a low dose exposure [5, 6]. Since then many experimental data have shown that the mutation processes may be modified by various factors, and also that cells possess a potential for repairing the primary radiation-induced damage in DNA molecules. This has radically changed the conceptual basis for the comprehension of the mutation process.

Many facts have now been accumulated which are impossible or very difficult to explain by means of the LNT hypothesis. With precise cell survival assays, a composite nonlinear relationship between survival and dose was shown for a variety of mammalian cells at doses below 1 Gy. The biphasic low-dose survival response is comprised of a region of hypersensitivity (in the range of 0–0.25 Gy) followed by an increased radioresistant response as the dose increases up to 1 Gy [7]. Additionally, it was shown that a small priming dose can cause an increased resistance to a subsequent higher dose, which becomes weaker with time. An induction of such resistance can be blocked by inhibitors of protein synthesis [8].

Another phenomenon is the bystander effect: an increasing level of cell death was observed in two human cell strains exposed to low doses of low-LET radiation [9, 10]. For the human cell lines studied, the magnitude of the effect was relatively constant in the range of 0.03–0.5 Gy. Moreover, the bystander effect was found to be predominant over the effect from direct radiation-target cells interaction at doses below 0.5 Gy [10], thus contradicting the expectations of linear dose dependence.

The lack of clear dose–response relationships for low-dose radiation is considered a paramount problem in radiation biology. Extensive information on the dose–effect relationship in the low dose region has been accumulated concerning the frequency of chromosome aberrations in human peripheral lymphocytes. Pohl-Ruling et al. [11] and Lloyd et al. [12, 13] carried out detailed studies of dose–response curves at low doses which showed that the number of radiation-induced dicentrics at doses below 40 mGy did not exceed the control level. Moreover, all six data points (in the dose range of 3–10 mGy) obtained in these three independent experiments were found to lie below (sometimes significantly [11]) the corresponding control values. This could point at the existence of a threshold; the other possible explanation lies in the large statistical uncertainties associated with low dose studies. At higher doses essential deviations from linearity in dose–response curves have also been found [14, 15].

The dose–effect dependencies in a range of 10–2,000 mGy were also investigated in Chinese hamster and human melanoma cells [16]. A nonlinear dose–response curve was observed in all cytogenetic assays used (metaphase and anaphase chromosome aberration analysis and the micronucleus test). It is important to note that the nonlinearity was found in both synchronized and non-synchronized strains of mammalian cells. Consequently, the shape of the dose–effect curve reflects the radioresistance of the whole population and is not determined by the response of a sensitive subpopulation of cells at a certain cell cycle phase. These findings are best supported by the results shown in [17, 18].

Non-linear responses and the presence of a plateau in dose–response curves were also demonstrated for Chinese hamster fibroblasts and *Vicia faba* seedlings (species belonging to different taxonomic kingdoms), when the dose dependencies for the yields of cytogenetic disturbances were studied in the dose range of 0–2.5 Gy [19]. Another important finding was recently published concerning the induction of chromosome aberrations by doses ranging from 0 to 10 Gy in root meristem cells of 6-day-old *Pisum sativum* plantlets [20]. The dose–effect curves showed non-linear responses, especially in the dose range from 0 to 1 Gy, and the biological effects at the low doses were considerably higher than expected. A steep increase in the number of aberrations was observed for cells exposed at 0.4 Gy, followed by a plateau for doses up to 1 Gy.

The objectives of this work were (1) to study the induction of chromosome aberrations at doses ranging from 0 to 1 Gy in *Hordeum vulgare* seedlings; and (2) to examine whether the cytogenetic disturbances observed depend linearly on the dose in this range.

Materials and methods

The test organism

Spring barley (*Hordeum vulgare* L., variety Zazerskiy 85), one of the most studied crops with regard to genetics [21], was used. Barley is a convenient object for studies of induced chromosome aberrations because of its few ($2n = 14$) relatively large (6–8 μm) chromosomes which are easy to identify.

Irradiation and root tip fixation

It is generally accepted that at the time of harvesting the initial cells of the root tip in barley are arrested in G_1 of the DNA cycle [22]. The seeds were soaked for 24 h in distilled water at 0°C in the darkness to provide evenness of swelling at the beginning of germination [23] and to synchronize their entry into germination. Presoaking of seeds at 0–1°C does not cause an advancement of cells from G_1- to S-phase [21]. It is known that DNA synthesis (S phase) begins about 16 h after the initiation of water imbibitions at +20 to 22°C [24, 25]. In this study, seeds were allowed to germinate for 12–16 h at +24°C and then irradiated, presumably at the late G_1-phase. For irradiation, a ^{137}Cs external source was used, which delivered γ-rays at a dose rate of 0.5 Gy h^{-1} (Lutch Irradiator, Latenergo, Latvia). The following doses were applied: control, 10, 50, 100, 150, 200, 300, 500, 750 and 1,000 mGy. After irradiation, the germination was allowed to continue until a root length of ≈10 mm was achieved. At such a root length, the first peak of mitotic activity in barley root tip cells was shown in [26], as well as in our pilot studies. Twenty to seventy seedlings per dose point were fixed in acetoalcohol (1:3).

Cytogenetic analysis

Squashed preparations of root meristem for every seedling were made and stained with aceto-orcein as described earlier [21, 27]. All slides were coded and examined blindly (i.e. the operator did not know the treatment used). For each slide, all ana-telophase cells (660–2,700 cells per dose point) were scored to determine the fraction of cells with aberrations, and the aberrant cell (AC) frequency was calculated. Chromatid (single) and chromosome (double) bridges and fragments, as well as lagging chromosomes were identified.

Statistical analysis

The frequencies of aberrant cells were calculated for every preparation, thus representing empirical distributions of the AC frequency at every exposure examined. Data were cleaned of outliers using the 4σ-test [28] and discordancy test for a single outlier in a normal sample with unknown expectation and variance [29]. To determine the significance of the difference between sample mean values, the Student's t test for independent variance as well as bootstrap percentile intervals [30] were applied. Bootstrap percentile intervals were calculated for $B = 2000$ bootstrap replications, as recommended in [30], to improve standard normal intervals.

A choice of the model with an optimum ratio of the complexity and goodness of data fitting was carried out using the criteria of structural identification [31]: $T = \frac{SS_{res}np}{N-np}$, where SS_{res} is the sum of squared residuals, N is the sample volume, and np is the number of parameters in a model.

To test the hypothesis whether mathematical model 2 fits the experimental data significantly better than model 1, the Hayek criterion [32] was applied:

$$H = \sqrt{\frac{\mu(R_2^2 - R_1^2)}{1 - R_2^2}}, \quad R_2^2 > R_1^2,$$

where R_1^2 and R_2^2 are multiple correlation coefficients for models 1 and 2, and μ is degrees of freedom in model 2. H-statistics follows the Student distribution.

Results

Frequency of aberrant cells

The cytogenetic analysis showed that the frequency of aberrant cells (AC) significantly exceeds the control level at a dose of 50 mGy and above, as follows from the t test. In the dose range from 50 to 500 mGy, the AC frequency was found to be almost constant (Table 1).

When increments of an observed effect are small and uncertainties are significant, which is the case at exposures to low dose ionising radiation, one should be careful when making statistical inferences. Routine approaches for two-sample statistical analysis such as the t test or standard equal-tailed confidence intervals based on parametric assumptions can lead to error-prone inferences if deviations from the Gauss distribution occur. Table 2 presents the results from testing the hypothesis of a normal distribution of the AC frequency obtained in the study, with the χ^2-test and the nonparametric Kolmogorov–Smirnov test [28]. The null-hypothesis is rejected at all doses except for 750 mGy, where there were not enough degrees of freedom. In addition, empirical distributions for the AC frequency are found to be clearly asymmetrical at low doses. Therefore, to reinforce findings on the cytogenetic damage level, the bootstrap percentiles were determined (Table 1). When there is a relatively small increment of an effect from control, even a slight shift in estimated values can be essential. The bootstrap approach is advantageous in this case because it

Table 1 Frequency of aberrant cells in irradiated barley seedlings, 95% confidence intervals calculated by routine and bootstrap percentile methods, and results of data fitting within three dose ranges with linear models ($Y = a + bD$)

Dose (mGy)	Cells scored	N_p	Frequency of aberrant cells (%)			Linear fitting
			Mean ± SE	Confidence interval[a]	Bootstrap percentile interval[a]	
0	1,667	46	0.34 ± 0.11	0.12–0.56	0.14–0.55	Range 1 (0–50 mGy)
10	1,264	32	0.27 ± 0.11	0.04–0.50	0.06–0.50	$a = 0.23 \pm 0.19$
						$b = (23.2 \pm 5.41)\ 10^{-3}$
						$F = 18.5$, $P = 0.003\%$
50	2,632	70	1.41 ± 0.25*	0.91–1.91	0.95–1.92	Range 2 (50–500 mGy) (plateau)
100	1,898	53	1.49 ± 0.29*	0.91–2.07	0.94–2.04	$a = 1.48 \pm 0.19$
150	1,604	43	1.58 ± 0.27*	1.04–2.13	1.08–2.12	$b = (0.03 \pm 0.84)\ 10^{-4}$
200	1,529	45	1.55 ± 0.29*	0.97–2.13	1.00–2.17	$F = 0.002$, $P = 96.7\%$
300	1,989	54	1.44 ± 0.22*	1.00–1.88	1.02–1.89	
500	655	29	1.47 ± 0.39*	0.68–2.26	0.78–2.23	
750	674	17	2.97 ± 0.66*	1.56–4.37	1.74–4.26	Range 3 (500–1,000 mGy)
1,000	1,315	45	4.02 ± 0.39*	3.23–4.81	3.28–4.79	$a = -1.01 \pm 0.97$
						$b = (5.07 \pm 1.17)\ 10^{-3}$
						$F = 18.6$, $P = 0.004\%$

N_p number of preparations, F Fisher statistics, P significance level

*Difference from the control is significant, Student t test; $P < 5\%$

[a] For 95% confidence level

Table 2 Testing the hypothesis of compliance of empirical distributions of AC frequency to the Gaussian distribution

Dose (mGy)	c_1	χ^2	$\chi^2_{0.05}$	c_2	D	$D_{0.05}$
0	5	87.9*	9.2	7	0.57*	0.24
10	5	69.8*	9.2	6	0.61*	0.23
50	4	70.8*	6.6	7	0.38*	0.20
100	4	60.3*	6.6	7	0.38*	0.22
150	4	39.2*	6.6	6	0.31*	0.25
200	4	51.4*	6.6	6	0.35*	0.24
300	6	91.7*	11.3	7	0.39*	0.22
500	4	41.2*	6.6	6	0.43*	0.25
750	3	3.9	[a]	5	0.28	0.32
1000	4	7.0*	6.6	6	0.21	0.24

c_1, c_2 the number of classes at χ^2- and Kolmogoroy–Smirnov-tests, D Kolmogorov–Smirnov statistics

*Null-hypothesis is rejected, $P < 5\%$

[a] There are not enough degrees of freedom, $f = c_1 - 3$, for χ^2-test

takes account of the empirical distribution skewness and extends the range of effectiveness of the standard intervals [30].

The improved bootstrap estimates (Table 1) support the conclusions made from parametric consideration that there is a significant increase in the cytogenetic damage at radiation exposure with 50 mGy and above, and a constant AC frequency in the dose range of 50–500 mGy. Thus, the whole dose range investigated here can be divided into three parts (Table 1) which differ in the dependences of the yield of cytogenetic disturbances on dose. In the range of 500–1,000 mGy (Range 3), there is a confident linear dependence of the response on dose, while this is not the case in the range of 50–500 mGy (Range 2). The absolute term, $a = 1.48 \pm 0.19\%$, of the linear regression model, $Y(D) = a + bD$, for Range 2, constructed on six dose points and 293 degrees of freedom, is significantly higher than the spontaneous level of the aberrant cell frequency; this confirms the presence of a plateau distinct from the control. These findings evoke the assumption of a non-linear character of the dose–effect curve, which follows from the experimental data only and not from any hypotheses, extrapolation models or other speculations.

Distribution of aberrations among cells

The data on empirical distributions (distribution type E) of the cytogenetic disturbances among root meristem cells are presented in Table 3. Lagging chromosomes were not taken into account in the distribution analysis because of their different origin (sources of chromosomal aberrations are affected chromosomes, while lagging chromosomes originate from injuries to the mitotic apparatus). The goodness-of-fit between the empirical distributions and a Poisson distribution can be tested based on the Poisson law's properties. In particular, for the theoretical Poisson distribution, a ratio between the average (λ) and variance (s^2) values is close to 1. Sachs [28] recommends to consider a distribution to agree with the Poisson when $9/10 < \lambda/s^2 < 10/9$, as an empirical rule. This requirement for the λ/s^2 ratio is violated at all doses above 50 mGy (Table 4). This finding is also fully consistent with conclusions from the asymptotic u test [33] application (Table 4). Thus, the distribution of cytogenetic disturbances

Table 3 Distribution probabilities of aberrations per cell at different doses and goodness of their fitting by the Poisson and confluent Poisson laws

Dose (mGy)	Distribution type	Cells containing different number of aberrations			χ^2	T
		0	1	2		
0	E	1,663	4	0		
10	E	1,261	3	0		
50	E	2,613	19	0		
100	E	1,888	6	4		
	P_c	1,888.0	6.8	2.4	0.054	6.24
	P	1,884.1	13.9	0.1	1.126	46.8
150	E	1,592	10	2		
	P_c	1,592.0	10.2	1.6	0.003	0.38
	P	1,590.1	13.9	0.1	0.272	11.3
200	E	1,515	13	1		
	P_c	1,515.0	13.0	0.9	0	0.02
	P	1,514.1	14.9	0.1	0.058	2.58
300	E	1,972	15	2		
	P_c	1,972.0	15.1	1.7	0.001	0.20
	P	1,970.1	18.8	0.1	0.195	10.9
500	E	641	12	2		
	P_c	641.0	12.2	1.7	0.002	0.29
	P	639.2	15.6	0.2	0.211	9.80
750	E	664	8	1		
	P_c	664.0	8.1	0.9	0	0.045
	P	663.1	9.9	0.1	0.088	2.57
1000	E	1,287	25	3		
	P_c	1,287.0	25.2	2.6	0.001	0.39
	P	1,282.4	30.3	0.4	0.232	20.9

E empirical, P_c confluent Poisson, *P* Poisson. *T* criteria of structural identification

among cells observed at doses of 0.1–1 Gy deviates from the Poisson distribution although this is commonly considered as a primary damage distribution. It is noteworthy that this deviation is caused by overdispersion, as at doses of 0.1–1 Gy at least one cell bearing two aberrations is found (Table 3).

Table 4 Parameters of the Poisson and confluent Poisson distributions

Dose (mGy)	Poisson				Confluent Poisson	
	λ (10^{-3})	s^2 (10^{-3})	λ/s^2	u	m	q
0	2.40	2.39	1.002	–0.06	–	–
10	2.37	2.37	1.002	–0.05	–	–
50	7.22	7.17	1.007	–0.25	–	–
100	7.38	11.54	0.64	18.05*	0.72	0.99
150	8.73	11.15	0.78	8.16*	0.32	0.97
200	9.81	11.02	0.89	3.56*	0.14	0.93
300	9.55	11.47	0.83	6.53*	0.23	0.96
500	24.43	29.94	0.82	4.25*	0.27	0.91
750	14.86	17.61	0.84	3.61*	0.21	0.93
1,000	23.57	27.58	0.86	4.45*	0.21	0.89

*Difference from the Poisson distribution is significant according to the asymptotic u test, $P < 5\%$

The distribution of cytogenetic disturbances is connected to recovery processes in the cells responding to irradiation, and it also implies some response mechanisms. Thus, systems facing a stressful exposure can respond in a life-or-death manner [34, 35] so that cytogenetic effects can be modified through decreasing the damaged cells' number instead of lowering the severity of their injury. The p53 protein plays a key role in this mechanism at the cellular level [35].

The life-or-death response could be described by the confluent Poisson distribution [34]. According to this law, the probability for a cell not to experience damage is equal to

$$p_0 = \mathrm{e}^{-m} + q(1 - \mathrm{e}^{-m}),$$

and a probability that a cell has n damage events is

$$p_n = p\mathrm{e}^{-m}m^n/n!, \quad (1)$$

where m is the mean number of primary damage events per cell; $p = (1 - q)$ is a probability of the primary damage realization. Parameters of the confluent Poisson distribution, q and m, were estimated as suggested in [34], and the distributions of the cytogenetic disturbances among cells expected from the life-or-death hypothesis are presented in Table 3, as calculated with formula (1). There are no calculated data for the control and 10 and 50 mGy doses because the parameters of the confluent Poisson distribution are impossible to assess when there are no cells with two or more damage events.

Since the value of χ^2 statistics is a measure of the deviation of a theoretical model (in this case, classical or confluent Poisson distribution) from the experimental data, the calculation results (Table 3) show that the confluent Poisson law describes the available empirical information better then the classical Poisson distribution. The criteria of structural identification, T [31], was applied to check whether the confluent Poisson is beneficial in data fitting due to its having more parameters (np = 2) than the classical Poisson (np = 1). The T criteria penalizes a model for the more additional free parameters it has; so that the lower the T value, the better is the ratio between the complexity and goodness of data fitting. From Table 3, the confluent Poisson model exhibits lower values of the T criteria despite its higher complexity than the classical Poisson model, at all exposures. Thus, the improved quality of approximation is reached not so much by making the model more complicated, but because of a better functional isomorphism between the model and the biological phenomenon. The results obtained indicate a contribution of indirect mechanisms

to the radiation-induced mutagenesis in a cell's response to low dose irradiation.

Spectrum of cytogenetic disturbances

The spectrum of cytogenetic disturbances and relative contributions from different types of aberrations are presented in Table 5. There are few chromatid type aberrations, and only at 1 Gy the frequency of single bridges exceeds the control value. Meanwhile, the occurrence of double bridges exceeds the control level at exposure with 50 mGy and above, and there is also a high frequency of lagging chromosomes, which exceeds the control at 50, 100, 150, 300, and 1,000 mGy. Relative contributions from different types of aberrations were calculated as a percentage of single and double bridges or fragments or laggings averaged through preparations, at certain dose (Table 5). These percentages do not show any transparent dependence on the dose value. Chromosome laggings and chromosome (double) aberrations are the prevailing types of cytogenetic disturbances with average contributions throughout the dose range of 45.5 and 44.1%, respectively. The significant percentage of lagging chromosomes indicates that radiation especially influences the mitotic spindle function. Indeed, mitotic spindles are known to be sensitive to radiation [20]. The only exception is the spectrum of disturbances at 500 mGy, where single aberrations contribute over 40% of the damage observed and only one chromosome lagging was found. Probably, this discrepancy from the effects observed at other doses is related to insufficient statistics, since only 655 ana-telophase cells were scored for this exposure.

When seeds of wheat and barley were exposed to acute γ-radiation [36, 37], chromatid aberrations were the predominant type of cytogenetic disturbances. The essential difference in the cytogenetic disturbances spectrum at seeds and seedlings' irradiation (Fig. 1) is probably linked to the biological singularities of the dormant seeds mostly bearing cells in G_1-phase. A part of the γ-induced G_1-phase primary damage is not only capable of forming mutations in other phases of the same cell cycle, but also of passing through a number of mitoses to form terminal mutations long after the exposure, as was shown in experiments on different plant species [38–40].

Analysis of dose–effect relationship

The data on the frequency of aberrant cells in Table 1 suggests that the dose dependence deviates considerably from linearity in the whole dose range and that in the range of 50–500 mGy the frequency is independent

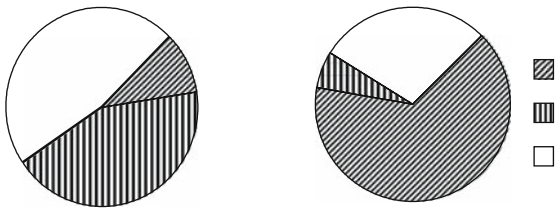

Fig. 1 Average percentages of cytogenetic disturbances of different types in irradiated spring barley seeds [36] and seedlings. *1* Chromatid (single) aberrations; *2* chromosome (double) aberrations; *3* lagging chromosomes

Table 5 Spectrum of cytogenetic disturbances in irradiated barley seedlings

Dose (mGy)	Cytogenetic disturbances total	Different types of cytogenetic disturbances					Percent contribution of different types of cytogenetic disturbances				
		g	*f′*	*m′*	*f″*	*m″*	*g*	*f′*	*m′*	*f″*	*m″*
0	10	5*	2	0	2	0	55.6	22.2	0	22.2	0
10	12	3	0	0	2	1	50.0	0	0	33.3	16.7
50	39	20*[a]	1	3	7	8*[a]	51.3	2.5	7.7	18.0	20.5
100	34	20*[a]	0	0	4	10*[a]	58.8	0	0	11.8	29.4
150	26	12*[a]	0	2	2	10*[a]	46.1	0	7.7	7.7	38.5
200	20	11*	0	0	9*	6*[a]	42.3	0	0	34.6	23.1
300	35	16*[a]	0	1	6*	12*[a]	45.7	0	2.9	17.1	34.3
500	18	1	4	3	2	7*[a]	5.9	23.5	17.6	11.8	41.2
750	20	10*	0	0	4*	6*[a]	50.0	0	0	20.0	30.0
1,000	63	30*[a]	2	10*[a]	8*	11*[a]	49.2	3.3	16.4	13.1	18.0

f′, *m′* chromatid fragments and bridges; *f″*, *m″* chromosome fragments and bridges; *g* chromosome laggings

*Frequency of aberrations is different from zero, $P < 5\%$

[a] Frequency of aberrations is different from control, $P < 5\%$

of dose. The validity of the observed non-linearity is tested by comparing the goodness-of-fit of the AC frequencies versus radiation dose, using mathematical models of different types and complexities (Table 6). A set of polynomial models $\left(y_m = \sum_{i=0}^{m} a_i x^i\right)$, including the linear function as a special case ($m = 1$), and a piecewise-linear (PL) model were used. The PL model pre-supposes a non-linearity of a dose dependency, including a dose-independent plateau in a dose range $[D_1, D_2]$, and has five free parameters:

$$F(D) = \begin{cases} a_1 + b_1 D, & D < D_1; \\ a_2, & D_1 < D < {}_2; \\ a_3 + b_3 D, & D > D_2; \end{cases} \qquad (2)$$

where $F(D)$ is the AC frequency (%), and b_1 and b_3 are defined from boundary conditions, with $b_1 = (a_2 - a_1)/D_1$, $b_3 = (a_2 - a_3)/D_2$.

For the polynomial models, free parameters are found from generalized linear regression [41]. In particular, the intercept and slope for the linear model $(Y(D) = a + bD)$ are $a = (0.74 \pm 0.21)\%$, $b = (3.00 \pm 0.46)\ 10^{-3}\%\ \mathrm{mGy}^{-1}$, correspondingly. Approximation of the data with the linear model is illustrated in Fig. 2.

Free parameters for the PL model were defined from the minimization of the following functional

$$U(a_1, a_2, a_3, D_1, D_2) = \sum_{i=1}^{N} (F_i(D) - F(D))^2, \qquad (3)$$

which is the sum of the squared differences between the experimental data $F_i(D)$, $i \in \overline{1, N}$ and a family of specified parametric piecewise linear functions $\{F(D)\}$, by the method of a coordinate-wise descent [42]. From the calculations, the dose-independent plateau for the given data set lies within the limits $D_1 = 83.4$ mGy and $D_2 = 513.7$ mGy, the levels of the spontaneous cytogenetic disturbances and disturbances within the plateau are $a_1 = 0.37\%$ and $a_2 = 1.45\%$, respectively. Fitting the data on the AC frequency in barley root meristem with the PL model is also shown in Fig. 2.

Table 6 Comparison of approximation qualities of experimental data by various models

Model	np	SS_{res}	F	R^2 (%)	T	H
Linear	2	1.80	41.7	83.9	0.45	–
Polynomial of degree 2	3	1.69	39.3	84.9	0.72	0.50
Polynomial of degree 3	4	1.20	49.7	89.2	0.80	1.25
Polynomial of degree 4	5	0.21	258.8	98.1	0.21	4.35*
Polynomial of degree 5	6	2.70	12.5	75.8	4.06	
Piecewise linear	5	0.30	180.2	97.3	0.30	3.55*

np the number of parameters in a model, SS_{res} sum of squared residuals, F Fisher statistics, R^2 multiple correlation coefficient, T criteria of structural identification, H Hayek criteria

*Model is better than the linear model, $P < 5\%$

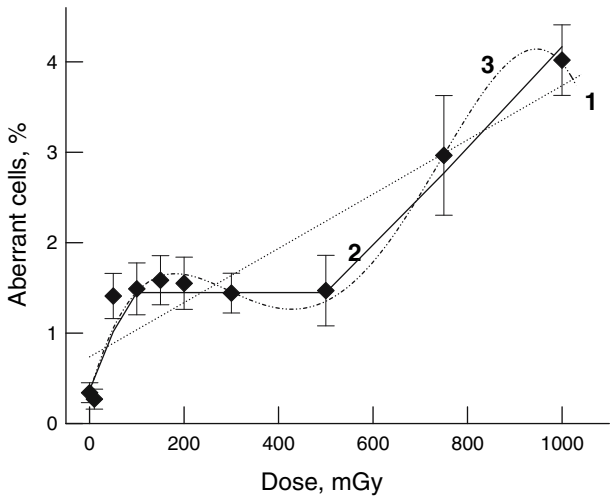

Fig. 2 Frequency of aberrant cells in barley seedlings (mean ± SE) exposed to low radiation doses and approximation of the data with linear (*1*), piecewise linear (*2*) and fourth degree polynomial (*3*) models

In general, the predictive power of a model breaks down when the number of free parameters increases [43]. Therefore, a linear model is preferred, since it is one of the simplest models. The current practice of plotting data on a linear dose scale for a wide range of doses masks any effects occurring at low doses that deviate from a linear or linear–quadratic dose–effect relationship and thus obscures any underlying relationship. Indeed, the dose–effect curve observed in this study at sufficiently low doses would not be detected if plotted together with high-dose data in a linear scale. Hence, one of the main advantages of the linear non-threshold (LNT) theory originates from these, purely mathematical properties of a linear function. This provides apparent support for the current application of the LNT for radiation protection purposes, but its biological fundamentals are quite contradictory [44–47].

The approximation quality of the models applied for the cytogenetic damage data set was compared using several statistics which are presented in Table 6. All six models are able to fit the data satisfactorily (F not less than 12.5, $P < 5\%$). However, the polynomial models of 2, 3, 4 degrees and the PL model show lower values of residual sum of squares, SS_{res}, than the linear model, which leads to higher Fisher statistics, F, and multiple correlation coefficient, R^2 (Table 6). Since all these models have more parameters than the linear one, which can cause their better flexibility in fitting the data, the criterion of structural minimization, T, is

calculated penalizing the model complexity (Table 6). From the *T*-values, the polynomial model of fourth degree, $Y = 0.34 + 0.018x - 8.02 \times 10^{-5}x^2 + 1.27 \times 10^{-7} x^3 - 6.13 \times 10^{-11}x^4$, as well as the PL model with np = 5 parameters are, nevertheless, advantageous as compared to the linear model with np = 2. The improvement in data fitting with the fourth-degree polynomial and PL models (see SS_{res} values in Table 6) is supported by the Hayek criteria ($P < 5\%$). A common feature for the best two functions is a tendency to fit a 'plateau' in the cytogenetic disturbances occurrence (Fig. 2). The fourth-degree polynom is, however, consistent with a biological response only in the dose range studied, i.e. 0–1 Gy, while at increasing dose this function soon takes on negative values and, thus, can not be considered as an appropriate model. This illustrates that formalistic approach to data verification can be erroneous. On contrary, the good fit with the PL model is provided through conformity achieved between a biological phenomenon and its mathematical model. Consequently, the piecewise linear model, which assumes nonlinearity of the dose–effect dependence and the presence of a plateau fits the data on cytogenetic disturbances in the low dose range much better than any other among the tested models (and, in particular, the linear).

Discussion

The use of the LNT extrapolation in radiological protection has been justified until now by a lack of reliable data on the effects of low doses, which in part is due to the difficulties of epidemiological studies and dosimetry at low doses and dose rates [1, 48]. The LNT model is not supported by epidemiological data at doses lower than about 50 mSv or at chronic dose rates up to at least 100 mSv per year [1, 49, 50]. In fact, the radiological protection practices require the extrapolation of risk estimation by more than four orders of magnitude in terms of dose, and over an even greater range in terms of dose rate, below the levels at which risks have been reliably measured [49]. This approach, reducing the complex to the simple, has been favoured by the decision-makers, who are subject to the requirements of establishing an easily applicable regulatory framework. However, the existence of inducible defence systems suggests that any simple relationship between exposure to a mutagenic agent and the incidence of mutations is unlikely [8, 46, 47, 51]. The validity of the LNT concept needs to be verified since its experimental and epidemiological backgrounds are highly controversial [44–47, 50]. The findings of this study give further evidence that models founded on the LNT concept are inconsistent with the available experimental data. Consequently, it is necessary either scientifically justify the use of the LNT, or, having proved it is not scientifically robust, to develop a new approach that would be fair at different levels of radiation exposure.

It has also become apparent that the existing paradigm governing radiobiology cannot suggest a satisfactory explanation for the phenomena of radiation-induced genomic instability [52, 53] and the bystander effect [10, 53]. While there is experimental evidence to support the existence of both effects, epidemiological studies are particularly difficult to interpret since the relevant aspects of the genetic background have not yet been characterized. Future studies will provide a greater clarification of the complex interrelationships that are involved.

For human lymphocytes, an increase in the aberration frequency above the control values is evident at low LET radiation doses of about 30–50 mGy [11–13]. In the present study, a steep increase of the irradiation effect is observed at a similar dose (50 mGy; see Table 1). For higher doses between 50–500 mGy, a plateau was observed in the dose–effect relationship. It is commonly acknowledged that the primary damage to chromosomes from sparsely-ionizing radiation is randomly distributed between cells, according to the Poisson law. The complicated processes of DNA damage and repair, however, can be modified depending on both the dose and dose rate. Firstly, efficiency of the repair systems in a cell is influenced by the type, quantity, and location of the damage in the spatial patterns of the chromatin, and intracellular medium conditions. Secondly, in a low dose range, the effect of cell-to-cell communication (bystander effect) might also be relevant [10]. Therefore, the final distribution of the cytogenetic disturbances between cells can differ from the distribution of the initial damage, and, correspondingly, from the Poisson law. The more appreciable the role of recovery systems and cell-to-cell communication is, the more the distribution of the cytogenetic disturbances is expected to differ from the Poisson law. In this study considerable deviations of experimental data from the classical Poisson distribution were revealed (Table 3), suggesting that the deviation from linearity and slope modification of the dose–response relationship might be due to an efficient repair system [51], triggered at a certain dose below 50 mGy. By the inclusion of inducible radioprotective mechanisms in the radiobiological models it is possible to explain the plateau in dose–response relationships for cytogenetic disturbances [54] and neoplastic transformations [55]. It is, therefore, not surprising that the

dose–response relationships for cytogenetic disturbances can significantly deviate from linearity and that the dose–effect dependence for the range of 0–1,000 mGy cannot be considered linear. This relationship type is similar to the dose–effect dependences described elsewhere in animals and plants [7, 14–16, 19, 20], which are characterized by a pronounced effect (hypersensitive response) in the range of low doses. Therefore, such a shape should not be considered accidental, but rather reflects qualitative singularities of a cell's response within the low dose range.

Up to now, it has been assumed [56] that the direct induction of alterations in the genetic structure is paramount for the deleterious biological effects of radiation in a wide dose range, including low doses. However, a number of experimental studies emphasize that regulation of gene expression plays a very important role for the adequate cell response to low dose exposure [51, 52, 57, 58]. As gene expression can be influenced by signals from neighbouring cells [57], perturbations of the gene expression pattern do not necessarily have to be caused by damage to DNA. Mutual influence of cells and their involvement in generating response to a low dose exposure essentially weakens an interpretation from the stochastic mutagenesis-based theory to the effects observed. The pronounced nonlinearity at low doses testifies that the genetic effects within this range originate from peculiar features of cellular response realisation and transformation after weak exposures rather than from direct damaging effect of ionizing radiation. A number of previous studies carried out on different species and test systems support this statement [8, 10, 16, 58–60].

An adaptive response to a low dose exposure is a fundamental biological phenomenon shown by many species belonging to different taxonomic kingdoms (bacteria, plants, insects, fish and mammalians) both in vitro and in vivo [51, 61]. If an external agent is able to cause DNA damage, low exposures to this agent should principally be considered as able to stimulate the physiological DNA defence systems. Since spontaneous DNA damage far outweighs [47, 62, 63] damage from a low dose or low dose rate of low-LET radiation (for example, the ratio of metabolic DNA damage to radiation DNA damage from a low-LET background of 10 mGy per year is estimated as 10^6 [47]), the low-dose induction of the DNA defence systems is expected to mainly affect the DNA damage from non-radiation sources. Thus, the dose–effect curve in this range definitely cannot be considered linear.

Radiation-induced structural mutations appear in accordance with fundamental principles of eukaryotic genome organization and functioning. Then, hypersensitive response to low doses observed for a number of physical and chemical factors [7, 10, 16, 60] contrasts with a high genomic conservation intrinsic to eukaryotic organisms [62]. Such a sensitivity to weak disturbances of environmental parameters is of adaptive importance and directed [51] towards an increase in the probability of cell population survival by means of: (1) activation of the processes reducing the amount of genetic damages (adaptive response); (2) programmed death of ''unwanted'' cells, that are no longer needed for an organism or bearing potentially dangerous alterations (apoptosis); (3) increase of the genetic diversity in cells (SOS-repair, coherent transposition of mobile genetic elements, genomic instability) with a subsequent selection of the most suited variants.

Cellular response to low-dose irradiation and weak exposure to other external factors is based on fundamental and evolutionary conserved mechanisms for ensuring stability of living systems that had developed long before multicellular organisms emerged. The non-specificity of the regularities studied and the wide range of species for which these are observed provide evidence that this is a general biological phenomenon. In fact, ionising radiation has become a widely used tool for studying cellular and organismal responses to low-level external impacts, and serves to improve our understanding of the adaptive process as well. Just as the phenomenon of DNA molecule repair discovered by radiobiologists has gone far beyond the scope of radiobiology and taken on general biological significance, the regularities found for biological effects of low-dose ionizing radiation are not artefacts or some exotic ''anomalous'' reaction, but one of the natural manifestations of the fundamental (i.e. being a basis for life) mechanisms for ensuring resistance and possibility of adaptation to the varying habitat conditions.

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