Cytogenetic Effect of Low Dose γ-Radiation in Plant Test-Systems: Non-Linear Dose-Effect Relationship

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Over several decades, modelling the effects of ionizing radiation on biological system has relied on the target principle [Timofeeff-Ressovsky et al., 1935], which assumes that cell damage or modification to genes appear as a direct consequence of the exposure of biological macromolecules to charged particles. It is assumed that there is no threshold for the induction of biological damage and that the effects observed are proportional to the energy absorbed. Following this principle, the average number of hits per target should increase linearly with dose, and the yield of mutations per unit of dose is assumed to be the same at both low and high doses. This principle has served as the scientific background for the linear no-threshold (LNT) concept that forms the basis for the radiological protection for the public and the environment [ICRP, 1990]. It follows from the LNT that there is an additional risk for human health from exposure to any radiation level, even below natural background.

Since the mid 50s, however, the scientific basis for the LNT concept has been challenged as experimental data have shown that, at low doses, there was a non-linear relationship in the dose response. Luchnik and Timofeeff-Ressovsky were the first who showed a non-linear response to a low dose exposure [Luchnik, 1957; Timofeeff-Ressovsky & Luchnik, 1960]. Since then, many data have been accumulated which contradict the LNT model at low doses and dose rates. However, the hit-effect paradigm has become so strong and indissoluble that it has persisted even under the growing pressure of scientific evidence for phenomena at low dose exposure that can not be successfully accounted for by the LNT concept.

In recent years, additional information on non-targeted effects of radiation has been accumulated following the first reports of an adaptive response in human lymphocytes [Olivieri et al., 1984] as well as bystander mutagenic effect of alpha-particles [Nagasawa & Little, 1992; Mothersill et al., 1995]. Other phenomena including genomic instability, low-dose hypersensitivity, and increased radiation resistance effects are also under study [Marples et al., 1997; Kadhim et al., 2004; Bonner, 2004].

The nonlinearity of the dose-effect relationship with low level exposures has been demonstrated in a number of studies where chromosome aberrations were considered as the endpoint of interest. For example, the number of radiation-induced dicentrics in human peripheral blood lymphocytes found in [Pohl-Ruling et al., 1983; Lloyd et al., 1988, 1992] did not exceed the control level at doses below 40 mGy, with some experimental points lying significantly below control values. Essential deviations of chromosome aberrations appearance from linearity in mammals were also shown at higher doses of 100-300 mGy [Luchnik & Sevankaev, 1976; Takahashi et al., 1982].

In other species, deviations of cytogenetic effect induced by low doses from linearity have also been reported. For example, the dose response for cytogenetic effects in Chinese hamster fibroblasts and *Vicia faba* germs at doses from 0 to 2.5 Gy was shown to be non linear with a plateau at low doses by [Zaichkina et al., 1992]. Frequency of chromosome aberrations in root meristem cells of *Pisum sativum*

plantlets in the dose range of 0-10 Gy also showed non-linear responses with a plateau for doses up to 1 Gy [Zaka et al., 2002].

However the available information on dose-effect relationships at low doses for non-human species is scarce despite its importance. In their natural environment, some non-human species may be at a higher risk of impact than humans because of differences in ecological niches occupied [Geras'kin et. al., 2003]. Currently, radiation protection of the environment and maintenance of ecosystem sustainability is of a special concern and the development of a harmonized approach to human and biota protection has been recognized as a challenge for modern radiobiology and radioecology [Copplestone et al., 2004; Pentreath, 2002]. In this context, much more information on non-human species response to low level exposures is needed.

This paper summarizes findings of several studies on the cytogenetic effects induced by low level exposure to external γ-radiation in plant meristem cells.

Materials and Methods

Four of the experiments presented here were carried out on spring barley (*Hordeum vulgare* L., variety Zazerskiy 85) and one with *Tradescantia* (clone 02).

Barley is one of the most genetically well-studied crops and has been identified as an excellent organism for studies of induced chromosome aberrations [Constantin & Nilan, 1982]. Barley has 14 (2n) relatively large (6-8 μm) chromosomes which are easy to identify. In addition, a general procedure for root-tip aberration bio-assay is well-known, relatively quick and inexpensive to undertake. The following protocols were assigned for treatment and exposure of barley seeds and germs:

Protocol I. Dry seeds of *H. vulgare* L. were acutely irradiated with ^{60}Co γ-rays (in a "Gamma-Cell") at doses of 0.1, 0.5, 1, 5, 10, 25, 50, 100, 200, and 300 Gy. 24 h after exposure, the exposed seeds were placed in Petri dishes on distilled water-moistened filter paper for germination. Sections of main roots (approximately 5-10 mm long) were cut from 20-40 seedlings per dose point and fixed in acetic alcohol (1:3).

Protocol II. Seeds of *H. vulgare* L. were soaked for 24 h in distilled water at +4 ^{0}C in the darkness to synchronize cell division and provide evenness of swelling by beginning of germination [Konzak and Narayanan, 1977]. The seeds were removed from cold storage and maintained on moistened filter paper at 24 ^{0}C. After 12-16 h of germination, barley seedlings were irradiated with ^{137}Cs γ-rays at doses of 10, 50, 100, 150, 200, 300, 500, 750 and 1000 mGy at a dose rate of 0.5 Gy/h (Lutch Irradiator, Latenergo, Latvia). The germination continued until a root length of ≈ 10 mm was achieved and then 20-70 seedlings per dose point were fixed as above.

Protocol III. Seeds of *H. vulgare* L. were kept for 24 h in distilled water at +4 °C to synchronize cell division and exposed to ^{60}Co γ-rays at doses of 3, 5, 10, 50, 100, 150, 250, and 300 mGy at a dose rate of 60 mGy/h immediately after removing from cold storage, then allowed to germinate at +24°C in Petri dishes on filter paper wetted with distilled water. When the main root length reached about 10 mm, root tips of 20-80 seedlings per dose point were cut and fixed.

Protocol IV. Prior-exposure treatment of seeds was the same as in Protocol II. Doses of 5, 10, 50, 100, 150, 300, 500, 750, and 1000 mGy were delivered to seedlings 24 h after germination at +24°C at three dose rates of 120, 300 and 900 mGy/h with ^{60}Co γ-ray source ("Lutch Irradiator", Latenergo, Latvia). The germination continued until a root length of ≈ 10 mm was achieved. 15-50 seedlings per dose point were fixed as above.

Preconditioning of seeds in cold storage provides a simultaneous initiation of barley seeds' germination. So at the moment of irradiation, the cell population would be nearly homogeneous in terms of the cell cycle. Maximal frequency of scorable chromosome aberrations can then be registered in the first mitosis. However, it is not always possible to say precisely at what time cells come into mitosis as, in

exposed cells, the cell cycle can be influenced not only by the treatment received (preconditioning, temperature, etc) but also by the type and severity of any impact (source of irradiation, dose rate, etc). From published data [Sandhu et al., 1994], as well as from our pilot studies, the first peak of mitotic activity in barley root tip cells was found at the moment when the main root reaches a length of approximately 10 mm, which was then taken as the point for samples to be fixed for cytogenetic analysis in the barley studies.

In all four studies with *H. vulgare* L., temporary squash slides for cytogenetic analysis were prepared from the root apical meristem of every seedling, coded and stained with aceto-orcein. In each slide, all ana-telophase cells (from 700 to 9800 cells at dose point in various studies) were scored to determine the fraction of cells with aberrations.

In the study with *Tradescantia* (2n=12), plants were grown under controlled conditions (t = 22 ± 1 °C; 18 hours per day of illumination at 11580 Lux; 44% air humidity). Irradiation was started at a moment when the first blossoming appeared in an inflorescence. At least 5 plants per dose point were exposed to γ-rays of ^{226}Ra during 72 hours with various dose rates so that the resultant doses amounted to 0.2, 1.1, 2.5, 9.1, 22.2, 54.9, 91.0, 267.4, and 422.4 mGy, as measured at the inflorescence position. Different dose rates were achieved by varying the distance of the plants from the source. Stamen hairs were analyzed from 4 up to 30 days of blossoming. Somatic mutations were registered according to [Ichikawa et al., 1978].

Results and discussion

1. Aberrant cells in barley root tips after seeds exposure (Protocol I)

Crops are an important component of food chains and have been extensively studied by the Russian Institute of Agricultural Radiology and Agroecology (RIARAE). Numerous radiobiological studies have been carried out in crops, including the study of the effect of ionizing radiation on reproduction potential of seeds (pre and post sowing) [Alexakhin & Korneyev, 1992]. With this historical background and the well-established techniques in RIARAE for seed irradiation, acute γ-exposure of seeds was used to study cytogenetic effects in crops induced by low and moderate doses to investigate the application of the LNT concept in non-human species [Geras'kin et al., 1993; 1995; 1997a].

Generally, the range of plants' radioresistance is higher than that of mammals, with dormant seeds being more radioresistant than during crop vegetation [Sarapultzev & Geras'kin, 1993]. The dose range in Protocol I was chosen to compare the plants radiosensitivity for different stages of ontogenesis. For example, it is known that, for Monocotyledons, radioresistance of dormant seeds is 4.8-5.6 times higher than that for vegetating plants [Sarapultzev & Geras'kin, 1993]. Thus, the doses from 0.1 to 300 Gy chosen for exposure of barley dry seeds could be considered as small (up to 5-10 Gy) and moderate dose values, regarding to high radioresistance of seeds.

Fig. 1 shows the frequency of aberrant cells (AC) observed in the root meristem of barley seedlings following exposure of the dormant seeds [Geras'kin et al., 1997a]. There is no significant difference from the control level following exposure to doses of 0.1 and 0.5 Gy. However at higher doses, the AC frequency is elevated compared with an unirradiated control, but the relationship shows no dependence on exposure applied in a range from 1 to 25 Gy, despite the 10 times increase in absorbed dose. Only at doses above 25 Gy is there an apparent linear increase in cytogenetic damage with increasing radiation dose.

Despite all the criticism directed towards the LNT concept, the fundamental principles regarding the interaction of radiation with DNA and other biological macromolecules is known to induce primary damage. Thus the effect of ionizing radiation at a molecular level is actually non-threshold as the energy of any charged particle is far above the binding energy in biological macromolecules. Consequently, a dose is specified as being low if a critical target receives not more than one radiation 'hit' [Kellerer, 1976]. Being aware of the critical target volume (i.e. size of cell or cell nucleus), it is possible to determine the

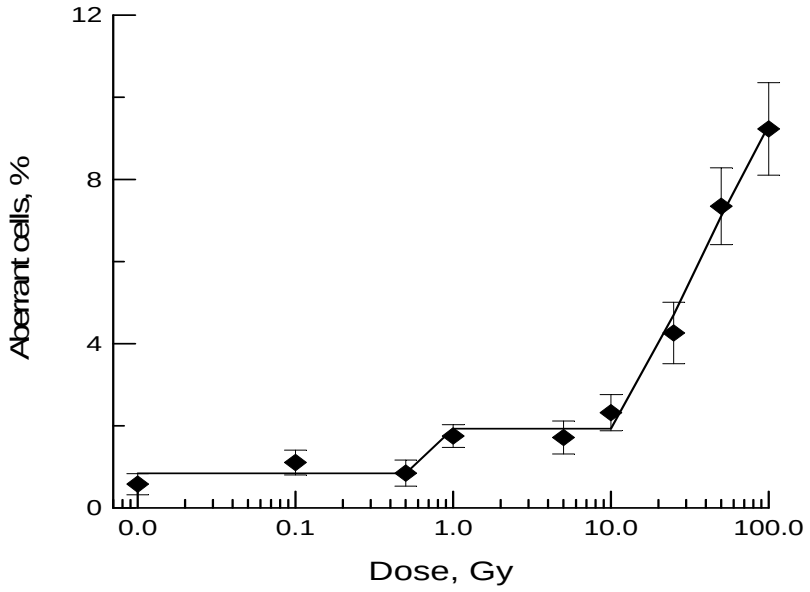

Figure 1. Frequency of aberrant cells (mean ± s.e.) in barley root meristem in dependence on dose absorbed by dormant seeds.

upper margin of low dose range based on the stochastic theory of radiation track distribution in a cell population [Spitkovskij et al., 1994b; Oudalova et al., 2002]. At doses from natural background up to this upper limit of the low dose region, the mean energy deposited in a cell's sensitive volume is a constant; with discordances from 'one-track' events being negligibly small. As the dose increases within this range, only the fraction of targets experiencing the radiation-absorption event increases linearly but not the energy absorbed in a single affected target volume [Bond et al., 1988]. Therefore, there seems to be no reason for an induction of response patterns different from background effect appearance, and an AC frequency should not differ significantly from the spontaneously observed level. This consideration underlies the first part of the dose-response curve in Fig. 1, i.e. between the control and 1 Gy, where there is no increase of the AC frequency over the control level.

At doses from 1 to 25 Gy, a 'plateau' in dose curve is observed. A dose-independence of cytogenetic effect in a low dose region of dose curves, 'plateau', has been reported in many studies, in particular, for human peripheral blood lymphocytes and melanoma cells, Chinese hamster fibroblasts, *Vicia faba* and *Pisum sativum* seedlings [Sevankaev, 1991; Shmakova et al., 2002; Zaichkina et al., 1992, Zaka et al., 2002]. To explain this deviation from linearity, most authors propose a hypothesis on radiation-induced repair system, which is triggered by a certain dose or certain level of biological damage [Luchnik & Sevankaev, 1976; Geras'kin, 1995a; Zaka et al., 2002]. In [Geras'kin, 1995b], induction of the mutagenic SOS-response, which is normally repressed, was suggested as providing restoration of cell survival but at the cost of an elevated frequency of genetic defects. At low doses, the contribution from radiation-induced effects compared with authentic spontaneous ones is rather small [Sarapultzev & Geras'kin, 1993; Pollycove & Feinendegen, 2001a] and so an increase in cytogenetic damage through misrepair appears important. The observation of the "plateau" in the dose dependency is a sign of the activation of repair mechanisms in response to the exposure received. These could be registered through alterations in other response patterns such as chromatin structure transformation or modifications in gene expression [Spitkovskij et al., 1994a]. Within the plateau range, the occurrence of scorable chromosome abnormalities reflects complex cellular responses that are triggered by the external insult (i.e. radiation exposure) but they do not depend on its value. As a result, an anomalous dose range is observed experimentally within which 2-10 fold changes in dose is not accompanied by any significant increase in chromosome aberration frequencies. As the dose increases further, the potential of the SOS-response systems to respond effectively decreases until it is overwhelmed and the dose-response curve then shows a

continuous increase in cytogenetic effect with increasing dose.

2. Aberrant cells in root tips after germs exposure (Protocol II)

To reduce misinterpretation and uncertainty resulting from the influence of physiological processes which may occur during germination after seeds exposure to ionising radiation and prior to scoring of chromosome aberrations in seedlings, other experimental protocols were designed (Protocols II-IV) to expose germinated and not dormant seeds. Actively dividing cells of meristem tissues are the most radiosensitive parts of a plant. Previous work has demonstrated that the test-system of "AC frequency in the intercalary meristem of spring barley" is as sensitive to γ-radiation as the generally accepted test of "aberrations in lymphocytes of human peripheral blood" [Geras'kin et al., 1996].

The results of cytogenetic analysis in barley root meristem after irradiation of 12-16 h seedlings (Protocol II) at doses from 10 mGy to 1 Gy are presented in Fig. 2 [Geras'kin et al., 1999]. The relationship between AC frequency and dose is obviously non-linear. A significant difference in AC frequency from the control was found at doses > 50 mGy ($p<5\%$, Kolmogoroff-Smirnov test [Sachs, 1976]). There are three parts to the dose curve which essentially describe differences in the dependences of the cytogenetic disturbance on dose. Thus, at doses from 500 mGy and higher, the AC percentage significantly differs from the control level ($p<1\%$) and increases with increasing dose. However, within the dose range 50-750 mGy there are no significant differences in the AC occurrence, although the effect observed is higher than the spontaneous level of the AC frequency ($p<5\%$). Hence, a 'plateau' in this study is revealed, and a non-linear relationship in dose-cytogenetic effect dependence is observed in barley root tip cells.

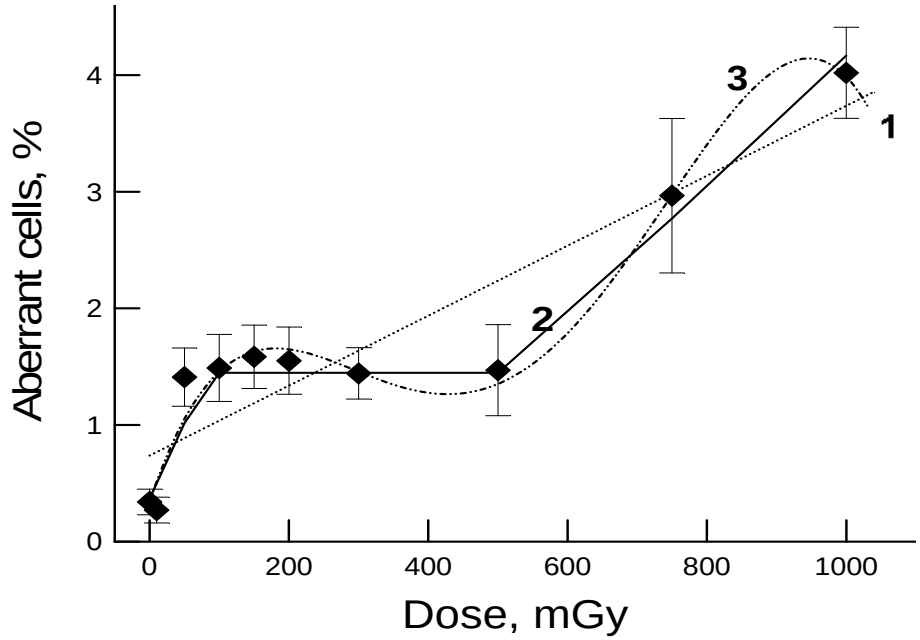

Figure 2. Frequency of aberrant cells (mean ± s.e.) in barley root meristem in dependence on dose absorbed by 12-16 h seedlings and approximation of the data with linear (1), piecewise linear (2) and 4th degree polynomial (3) models.

The use of the linear extrapolation from high doses into the low-dose region for an estimation of health risk and, correspondingly, in radiological protection, has been justified by a lack of reliable data, which are increasingly difficult to obtain at lower exposures because of large sample size needed [Upton, 2003; Brenner et al., 2003; Bonner, 2004; Tubiana, 1998]. At present, many authors have expressed the opinion that the validity of the LNT concept needs to be tested [Bonner, 2004; Tubiana, 1998, Mothersill & Seymour, 2004]. Under the increasing weight of experimental evidence which supports the non-

linearity at low doses, there has been a shift in a perception of the LNT hypothesis from the indisputably correct dogma to one which is the most convenient, prudent and transparent operational tool in radioprotection and radiotherapy practise [Tubiana, 1998; Mothersill & Seymour, 2004]. In the interim, it is useful to test the application of the LNT concept and to continue to challenge its superiority from not only biological but also a mathematical viewpoint.

With this in mind, a comparison of goodness-of-fit of AC yields versus radiation dose using mathematical models of different types and complexities was performed on an example of the data obtained under Protocol II [Geras'kin et al., 1999; Oudalova et al., 2005]. A set of polynomial models ($y_m = \sum_{i=0}^{m} a_i \cdot x^i$) including 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, which consists of several linear parts with different slopes, including a dose-independent plateau in a dose range [D_1, D_2], and has five free parameters.

For the polynomial models, values of free parameters were found from generalized linear regression [Draper & Smith, 1981]. In particular, an intercept and slope of the linear model ($y = a + b \cdot x$) are a = (0.74 ± 0.21) %, b = (3.00 ± 0.46) 10^{-3} %/mGy, correspondingly. Free parameters of the PL model were found using an iterative regression tool based on the method of coordinatewise descent [Vasiliev, 1988]. Important findings from the PL model verification are the lower and upper limits of the 'plateau' that are defined as D_1 = 83.4 mGy and D_2 = 513.7 mGy for the experimental data obtained in this study. Fitting the data on the AC frequency in barley root meristem with the linear and PL models is illustrated in Fig. 2.

The various models applied for the data approximation were followed by a quality review of these approximations with several different criteria, and the results are shown in Table 1. 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 the lower values of residual sum of squires, SS_{res}, than the linear model, resulting in higher Fisher statistics, F, and multiple correlation coefficient, R^2 (Table 1). It is known that the predictive reliability of a model breaks down as the number of free parameters increases [Algorithms..., 1988]. To test the relative complexity of the models, the criteria of structural identification, T [Geras'kin & Sarapult'zev, 1993] was used. This method penalizes a model for the more additional free parameters it has; so that the lower T-value the more optimum the ratio between the complexity and goodness of data fitting the model has. From Table 1, the lowest value of the T-criteria is acquired by the polynomial model of 4th degree as well as PL model despite their 5 free parameters (np=5) and its correspondingly high complexity. This result means that the improved quality of approximation is reached not so much by making the model more complex, but due to its more adequate mathematical description (or, in other words, functional isomorphism) of the biological phenomenon.

To check the significance of the approximation improvement, a hypothesis was tested whether the linear model fits the experimental data significantly worse than other models, using the Hayek criterion, H, [Gofman, 1990]. From the calculated values of H (Table 1), the goodness's of experimental data fit by both the PL model and 4th degree polynom is significantly higher than by the linear model (H>$H_{0.95}$=1.96).

A common feature for two the best functions is a tendency to fit a 'plateau' in the cytogenetic disturbances occurrence (Fig. 2). The 4th-degree polynom is, however, consistent with the biological response observed only in the dose range studied, 0 – 1 Gy, while at higher doses this function drops to -∞ very fast, which illustrates an errancy of formalistic approach to data verification. On contrary, the good fit with the PL model is provided through conformity achieved between a biological phenomenon and its mathematical model. Consequently, this study shows that the PL model which assumes non-linearity of the dose-effect dependency, and implies the presence of a plateau, fits the cytogenetic disturbances

occurrence in barley root meristem cells within the low dose range significantly better than any other among the tested models (and, in particular, better than the linear approach).

Table 1. Comparison of approximation quality of aberrant cells frequencies with different models. Data are obtained in dose range 0-1000 mGy (Protocol II) and 0-300 mGy (Protocol III)

Model	***np***	***0-1000 mGy***					***0-300 mGy***				
		SSR	***F***	***R^2, %***	***T***	***H***	***SSR***	***F***	***R^2, %***	***T***	***H***
1. Linear	2	1.80	41.7	83.9	0.45	-	0.41	24.4	77.7	0.12	-
2. Piecewise linear	5	0.30	180.2	97.3	0.30	3.55*	0.03	228.9	98.3	0.04	4.92**
3. Polynom (m= 2)	3	1.69	39.3	84.9	0.72	0.50	0.24	40.7	87.1	0.12	1.53
4. Polynomial (m= 3)	4	1.20	49.7	89.2	0.80	1.25	0.06	161.5	97.0	0.05	4.04**
5. Polynomial (m= 4)	5	**0.21**	**258.8**	**98.1**	**0.21**	4.35**	0.02	377.5	99.0	0.02	6.38**
6. Polynomial (m= 5)	6	2.70	12.5	75.8	4.06		0.01	459.5	99.4	0.02	7.09**
7. Piecewise linear II	3						0.11	91.3	93.8	0.06	2.84*

** – α<1%, * – α<5% - model fits the data significantly better than the linear model;

3. Aberrant cells in root tips after germs exposure (Protocol III)

The study of AC frequencies induced by low doses up to 300 mGy (Protocol III) was initiated to get more information on dose-cytogenetic effect relationship at doses below 50 mGy. The lack of such information became obvious at analysis and modeling of the previous experiment data at doses below 100 mGy, as there were only two experimental point below 83.4 mGy, the plateau lowest limit (Fig. 2).

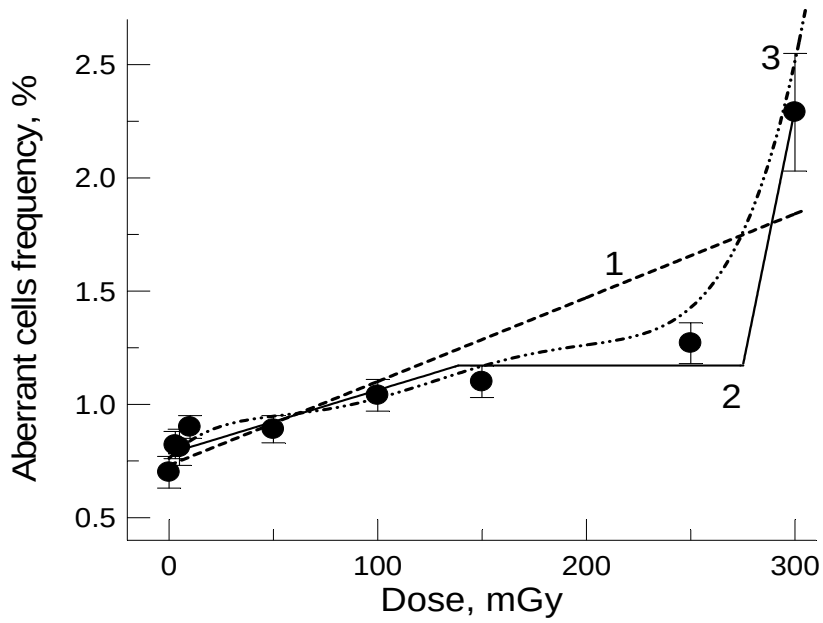

Figure 3. Frequency of aberrant cells (mean ± s.e.) in barley root meristem in dependence on dose absorbed by germs (Protocol III) and approximation of the data with linear (1), piecewise linear (2), and polynomial of 5th degree (3) models.

The common difficulty in assessing effects observed at low level exposures and making statistical inferences is related to the lack of a suitable quantity of available data to overcome uncertainties resulting from "experimental error" or "inter-species variation" when the induced effect itself has a small magnitude. In an attempt to provide a lower uncertainty of AC frequency estimates, exposure in this experiment was delivered to initiated barley germs immediately after their removal from cold storage,

assuming that in this case a cell population would be found in the most synchronized state. Moreover, attempts were made to provide experimental volumes that were as large as reasonably possible. Thus, 2000-9800 ana-telophase cells were scored at different doses as opposite to 1300-2600 cells in the previous study.

Data from AC scoring are presented in Fig. 3. Significant increase of cytogenetic damage over the control level is found at a γ-rays exposure of > 100 mGy as follows from Student *t*-test, or > 50 mGy as follows from non-parametric Kolmogorov-Smirnov test ($p<5\%$), which is in a good accordance with the previous study. There are no significant changes in AC occurrence within the dose range from 3 to 100 mGy. AC frequencies at doses of 100, 150 and 250 mGy are significantly different to the control level, but do not differ from each other. The yield of cytogenetic damage at 300 mGy increases significantly over the values obtained at all other doses, even 250 mGy ($p<5\%$).

To test a validity of the LNT extrapolation, a goodness-of-fit of the data on the AC frequency approximation by different models was performed. A set of polynomial models, and several piecewise-linear models of different shape were used. The results are presented in Table 1. Piecewise linear model II assumes an absence of dose dependence below a threshold dose (D_0) and a linear dose-dependence at higher doses. All models were able to fit the data satisfactorily (*F* not less than 24, $p<0.1\%$). However the best models (Table 1) were the polynomial 5- (Fig. 3, curve 3) and 4-degree functions as well as the PL model that includes a 'plateau' (Fig. 3, curve 2). A common feature for these three best functions is an absence (for the PL model) or very slight dependence (for the polynomial models) of AC yield on dose at low doses, as can be seen from plotting the best-of-fit functions (Fig. 3). The linear function shows the worst characteristics of the data fitting (Table 1) obtained in this study.

4. Dose-cytogenetic effect relationship at different dose rates (Protocol IV)

It is well understood that the dose rate is of importance in assessing radiation-induced effects and the risks at low doses. Numerous studies have reported direct dose-rate effects, with an absence of dose-dependence and even an inverse dose-rate effect being found as well [Lyon et al., 1972; Sorensen et al., 2000; Min et al., 2003]. In the LNT concept, the risk of detrimental effects per unit absorbed dose is smaller for lower doses than at higher exposures, which is acknowledged by an application of the dose-rate effectiveness factor [Tubiana, 1998; Sorensen et al., 2000]. In territories contaminated after severe radiation accidents, both humans and the biota have been exposed to radiation at dose rates that varies through time and space, and the doses absorbed during the cellular and ontogenetic cycles of development are consequently a function of the dose rate. Therefore, an issue of varying dose rate is of high relevance in regard to radiological regulation and practice policy.

To study an effect of dose rate value on cytogenetic damage level, barley germs were exposed at three dose rates of 120, 300 and 900 mGy/h (Protocol IV) and ACs were scored in seedling root meristem [Oudalova et al., 2002]. Frequencies of ACs obtained in this study are presented in Fig. 4. Dose dependences at different dose rates keep the shape with a characteristic 'plateau' region of 10-300 mGy, and can be approximated with the PL model much better then with the linear one. There is a change in magnitude of the AC yield with a change of dose rate used. Remarkably, in the exposure range investigated, the induced cytogenetical disturbances appeared in a reverse dependence on dose rate, and the lower the dose rate the higher the AC frequency (Fig. 4). Significant increase over the control ($p<5\%$) was found from dose of 10, 100, and 500 mGy and above for dose rates of 120, 300 and 900 mGy/h, correspondingly.

Observations of a reversed dependency of mutation frequency [Lyon et al., 1972] and DNA damage [Min et al., 2003] on dose rate at low-level exposures are known. For example, more point mutations and chromosome aberrations per unit dose at low level radiation than at higher levels were found in [Shevchenko et al., 1972]. The most generalised explanation refers to an assumption of a

diminished activation of repair at very low dose rates [Min et al., 2003]. In other words, the inducible repair system is probably activated by a certain threshold value of damage [Shevchenko et al., 1972; Calkins, 1973]. This means that, at low exposures, the accumulation of damage needs to reach a certain value or threshold before the repair mechanism is switched on. The lower the intensity of impact (i.e. dose rate) the more damage is required, which results in an inverse dependence of AC registered on dose rate in this exposure range.

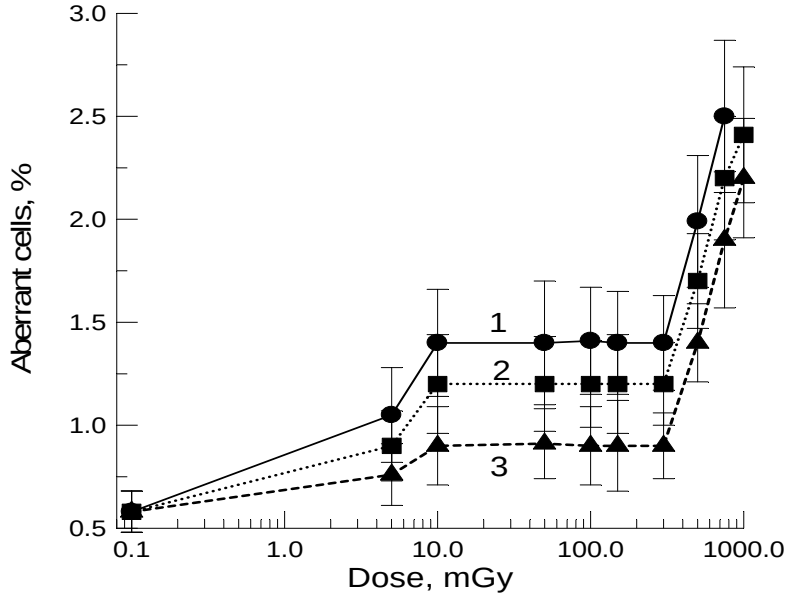

Figure 4. Frequency of aberrant cells (mean ± s.e.) in barley root meristem in dependence on dose absorbed by 24 h seedlings at dose rate of 120 mGy/h (1), 300 mGy/h (2), and 900 mGy/h (3).

An important finding of this study is that a non-linear shape of dose-effect dependence with plateau in low dose region is confirmed, and the independence of dose curve profile on dose rate in the studied exposure range is shown. An inverse dependence of chromosome aberration yield and dose rate is demonstrated for the first time in plant meristem cells, and requires further investigation. To obtain more information on the role of dose rate in cytogenetical damage induction patterns, a study identical to the work done under Protocol III has been launched but with several different dose rates.

5. Somatic mutations in *Tradescantia* stamen hair cells

To corroborate finding obtained with another plant system, *Tradescantia* (clone 02) was chosen as test-species in next experiment [Evseeva & Geras'kin, 2001]. *Tradescantia* has known as the best object for genetic and radiobiological studies [Sax, 1938; Ma et al., 1996] and been useful for detection of the genetic effects of both ionizing radiations and chemical mutagens at low exposure levels [Ichikawa, 1992]. The Tradescantia-Stamen-Hair-Mutation (Trad-SHM) bioassay uses the mitotic cells of stamen hairs for mutation induction in which the stamen hair cells mutate from dominant blue to pink constitutes a system unique in sensitivity; it can register doses of X-rays as low as several mGy [Sparrow et al., 1972].

Exposures in the study of radiation-induced somatic mutations in *Tradescantia* stamen hair cells [Evseeva & Geras'kin, 2001] were planned to provide detailed information at very low doses by choosing a dose interval between experimental points that was as small as possible. Experimental dependency of somatic mutation frequency on dose value presented in Fig. 5 demonstrates an obvious non-monotonous shape showing common features with dose dependencies obtained in barley studies (Fig. 1-4). Three dose ranges could be separated. At the exposure to doses of up to 9.1 mGy, the registered frequencies of

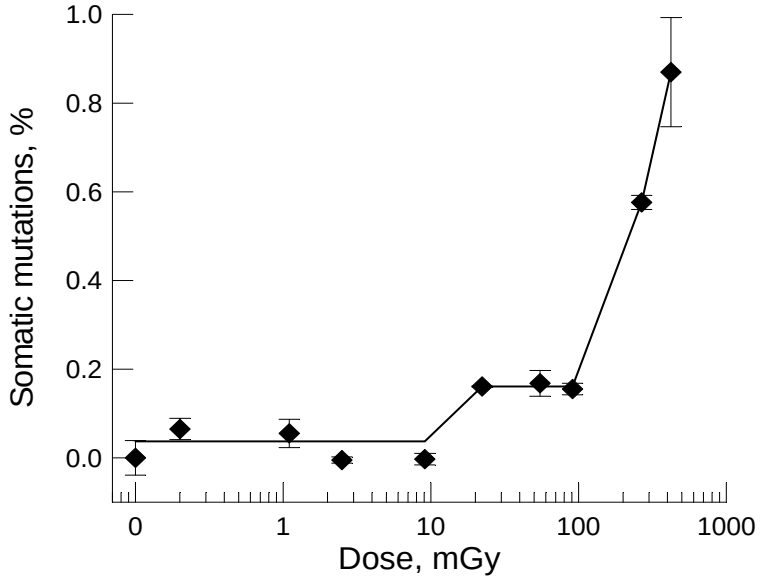

Figure 5. Frequency of somatic mutations (mean ± s.e.) in stamen hair cells from γ-ray-exposed *Tradescantia* plants

mutations do not differ from the level of spontaneous disturbances and are not significantly different from the control. In the second dose range, namely at doses of 22.2, 54.9 and 91.0 mGy, occurrence of somatic mutations is significantly higher than the control level and the values observed at the lower doses (up to 10 mGy) but does not significantly change within this dose range, which indicates a 'plateau'. When the dose increases over 100 mGy, a steady increase of the mutation frequency is observed; somatic mutation occurrence at doses of 267.4 and 422.4 mGy is significantly over the previous values at 0-9.1 mGy and 9.1-91.0 mGy.

Conclusion

In the present study, attempts were made to investigate the biological effect of ionising radiation at levels similar to those that may be observed in the environment. A correct assessment of risk of low doses for human health is impossible on a basis of current epidemiological data because of large uncertainties and experimental difficulties. There is a need to develop a more comprehensive understanding of the processes which govern cellular responses at low level exposures to ionising radiation. These processes are likely to have a lot in common, at least for eukaryotic organisms, because of uniformity in fundamental principles of genome organization and functioning. It is becoming increasingly clear that at low doses indirect and non-targeted effects play a crucial role in cell response that are, in some ways, similar to systematic stress [Mothersill & Seymour, 2004; Kadhim et al., 2004] and relevant to environmental toxins other than radiation. In this context, high sensitivity, low level of spontaneous mutagenesis, accessibility to large volumes of compatible experimental data, and acknowledgement in environmental genotoxins testing make those plant-based systems excellent models for studying low-dose effects.

The non-linearity and presence of plateaus in dose dependencies for cytogenetic disturbances have been reported in a number of works carried out with different species and test-systems. On the basis of these information and data obtained in several studies on cytogenetic effects in plant meristem, a concept of biological effect of low-level radiation on cell was suggested [Geras'kin, 1995b; Geras'kin et al., 1997b]. It postulates uniformity for different species relating to an essentially non-linear dose dependence shape, with only species-specific variations in critical doses at which slope modifications appear and that these critical doses depend on species radiosensitivity. The results of several studies presented in this review confirm the main postulates of this concept [Geras'kin, 1995b]. For example, the 'plateau' limits in

dose curve observed by the Trad-SHM bioassay, 20-200 mGy (Fig. 5), are shifted down to smaller doses in comparison to 80-500 mGy for barley (Fig. 2), which is in a perfect accordance with a higher radiosensitivity of *Tradescantia* stamen hairs than that of barley cells. Furthermore, the concept [Geras'kin, 1995b] supposes an existence of one more dose-independent part that should not differ from the level of spontaneous cytogenetic variability. With the Trad-SHM bioassay, this theoretical prediction has, for the first time, received an empirical confirmation on vegetative object. Indeed, the high sensitivity and low level of spontaneous mutagenesis in *Tradescantia* made it possible to reveal such a dose range as low as 0.2 – 9.1 mGy (Fig. 5).

Ignoring the fact that the non-linear character of dose–response curves leads to a substantial underestimation of cancer risks, if high doses and linear dose–response curves are used for estimation of the hazard of low-level radiation, the following points can be made:

- Although many researchers believe that the estimation of cancer risks by the linear extrapolation of high-dose data to the range of low radiation doses may only give overestimated values [Tubiana, 1998], the data presented in this paper suggest that the LNT concept should not be used for estimating the risks of genetic defects induced by low-level radiation, since this concept has no sound biological underpinning and comes into contradiction with available experimental and epidemiological data [Gofman, 1990; Pollycove & Feinendegen, 2001b; Wei et al., 1990].
- The linear model is presumed to be advantageous as simple and prudent at modeling ambiguous biological effects at low doses, so its benefits follow from mathematical properties of the linear function, while the biological background of the LNT has been admitted as controversial. Findings of this work demonstrate, however, that it is not hard to call in question advantages of the LNT in fitting available experimental data from mathematics point of view, as well.
- New data obtained in these studies concern a perception of fundamental mechanisms governing cell response to low level exposures. These findings are of general biological interest, since response to low level exposures is one of the manifestations of basic laws determining and ensuring the living systems resistance and their adaptation potential under varying habitat conditions. An accumulation of information on cell responses to low level exposures and validating LNT hypothesis eligibility are relevant not only to an improvement of radiation safety standards but to the development of a general ideology for the public and environment protection. It should be acknowledged that more studies are needed for further validation of concepts for risk assessment at environmentally relevant levels.

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