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LETTER TO THE EDITOR, NEWS AND VIEWS

First report of X-ray induced somatic mutation by Muller's department chair fails to support Muller's linearity hypothesis

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Abstract

This paper reevaluates the first report of X-ray-induced somatic gene mutations. It was undertaken by John Patterson, Department Chair of Hermann Muller, using the same biological model, methods and equipment of Muller. Patterson reported X-ray induced mutation frequencies for X-chromosome-linked (sex-linked) recessive gene mutations in somatic cells of *Drosophila melanogaster* that resulted in color changes in the ommatidia of the eyes. Results were based on color changes detected in both male and female offspring irradiated while in egg, larval or pupal stages and for unirradiated controls. Patterson claimed that the observed dose response displayed linearity, with a clear implication that the linear response extended to background exposure levels of unirradiated controls. This reanalysis disputes Patterson's interpretation, showing that the dose response in the low-dose zone strongly supported a threshold model. The doses in the experiment, which were not clearly presented, were so high that it would preclude the assumption that the experiment provided any information of relevance to radiation exposures of humans at low doses, or even at high doses delivered at low-dose rates. Induced phenotypical changes that occurred at the higher doses, especially in female offspring, overwhelmingly resulted from X-ray-induced chromosome breaks instead of point mutations as initially expected by Patterson. The Patterson findings and linearity interpretations were an important contributory factor in the acceptance of the linear non-threshold (LNT) model during the formative time of concept consolidation. It is rather shocking now to see that the actual data provided no support for the LNT model.

Keywords Gene mutation · X-rays · Linear dose response · LNT · Cancer risk assessment · Threshold dose response

Introduction

The striking findings of Hermann J. Muller (1927) that high doses/dose rates of X-rays induced gene mutations in the reproductive cells of *Drosophila melanogaster* (fruit flies) soon raised the question of whether similar effects would occur in somatic cells. Considerable research on this issue was undertaken by Professor John Patterson, Muller's department chair at the University of Texas at Austin. He also used fruit flies from Muller's laboratory, as well as the same X-ray equipment as Muller, and he began his experiments soon after Muller made his startling claims about inducing high frequencies of gene mutations in reproductive cells of male flies. Patterson (1928, 1929a, b) published a series of papers demonstrating that recessive mutations could be induced in the somatic cells of the eyes of fruit flies by exposure to high doses of X-rays early in development. The present paper reevaluates the data that Patterson published and the gene mutational and linearity dose response claims that he made.

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Background information and methods for somatic mutation study

The compound eyes of fruit flies are made up of hundreds of individual ommatidia, each one of which contains a lens. The outer surface of each ommatidium is called a facet, and the color of each facet is genetically determined. A somatic

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mutation occurring during mitosis early in the development of an eye can change the color of a single facet or, if it occurs very early in the cell line of a particular facet, a contiguous group of facets. In evaluating his experiments, Patterson assumed that the average numbers of ommatidia in male and female fruit flies were 1700 and 1720, respectively (based on Krafka 1920a, b), or approximately 855 per eye.

Numerous mutations have been discovered–and propagated in stocks–that determine the color of all the facets and thus of the entire surface of the eye. Many such mutations are in genes that are located on the X chromosome and are referred to as sex-linked. (The X chromosome is called the sex chromosome because female flies have two X chromosomes while males have only one). A wild-type fruit fly has red eyes because of a dominant mutation in one particular gene on the X chromosome. The wild-type allele at the gene of interest for Patterson's experiment is designated here as *R* (with *R* indicating a dominant mutation causing a red phenotype). A recessive mutation (symbolized here as *wh)* at that same gene produces a white eye unless it is in a fly carrying *R*. Females of genotype *R/R* and *R/wh* and males that are *R* all have wild-type (red) eyes. Females of genotype *wh*/*wh* and males of genotype *wh* have white eyes.

Patterson mated *R*/*R* females with *wh* males so that, in the absence of any spontaneous mutation in the reproductive cells, all female progeny would be *R*/*wh* and all male progeny would be *R*. Patterson then exposed the eggs, larvae, or pupal stages of those progeny to X-rays. If one of the *R* alleles in the cell lineage of one of the ommatidia mutated in such a way that it lost its function, the resulting ommatidium would be white. If that mutation occurred early enough in development, it might result in a whole group of congruent ommatidia becoming white.

In his papers on somatic mutations (Patterson 1928, 1929a, b), Patterson never reported anything about the dosimetry of the doses of X-rays that he administered. It was only stated that the X-ray machine was operated at 50 kV and 5 mA with the irradiated eggs, larvae, or pupae being at a distance of 12 cm and protected by an aluminum filter one mm thick. Numerically, the doses were then presented merely as ten different durations of exposure ranging from D-1 to D-10 (designated D1 to D10 in the present paper). D1 was a 5-min exposure, and each remaining exposure in the series increased by 5 min.

Reference to a later paper (Patterson 1931) on induction of sex-linked lethal mutations in reproductive cells of male fruit flies almost certainly reveals what doses were used in his experiments discussed in the present paper (Patterson 1928, 1929a, b). That paper presented doses in "r" units that were calculated from sample readings on a Victoreen dosimeter. Paul Selby notes that this dosimetry method is similar to what was used for measuring R (Roentgens) as ionizations in air for the massive mouse experiments at Oak Ridge National Laboratory starting in 1947. Patterson (1931) reported that his flies were exposed "with the machine operated at 50 kv., peak 10 ma, target distance 12 cm., and a 1-mm. aluminum filter." These treatment details were reported to yield a total dose of 1654 r units in 16 min for a dose rate of 103.4 r/min. Five different treatments were administered with the same machine setting with different durations or fractionation regimen. The calculated dose rates for the five treatments were 103.4 (three times), 108.0, and 109.0, for a mean of 105.4 r/min. Keep in mind that this dose rate was for the machine being set at 10 mA instead of the 5 mA reported to have been used in the experiments on somatic mutations.

With reference to the above information on dosimetry, it is important to note the following quotation from the *Journal of Experimental Zoology* (Patterson 1929b) regarding his X-ray machine:

> "In the more recent series of experiments the milliamperage was increased from 5 to 10, but the machine was operated at the same voltage and target distance as before. This change in the method of running the machine allows a reduction to one-half the time for a given dose, and is a great advantage in any series of experiments requiring the treatment of many cultures. The effect of variations in dosage in the production of mutations in eye color is a matter of considerable importance."

Since there is uncertainty raised by not knowing what Patterson meant by the wording "the more recent series of experiments", it will be assumed that the dose rate in his experiments on somatic mutations (Patterson 1928, 1929a, b) was 53 r/min based on the use of 5 mA and not the ~ 106 r/min reported for 10 mA. He discusses many experiments in his (Patterson 1929b) paper and continues to identify the doses as being from D1 through D10 in all of them, those being of 5 min (or multiples thereof) duration. Presumably, if he had made the shift in milliamperage from 5 to 10 mA within the set of experiments described in that paper, he would have shifted to treatments lasting only 2 1/2 min. Therefore, assuming a dose rate of 53 r/min for durations of 5 min (or multiples thereof), the doses that were used ranged from 265 to 2650 r. The dose rate of 53 r/min is ~ 170 million times background.

Patterson (1928, 1929a, b) summarized his findings as follows

In the early experiments in which flies with red eyes were examined for white facets, all cases in which white facets were found in males were concluded (assumed) to have resulted from gene (point) mutations. At least ten times as many

females had white facets as males. It was assumed that the frequency of induced somatic gene mutations is identical in both sexes and that the great excess in the females occurred because of chromosomal breakage followed by physical or functional loss of the portion of the chromosome containing the *R* gene. Either that part of the chromosome was broken off and lost during subsequent mitosis, or there was a translocation in which, because of position effects, the *R* gene ceased to function. Similar findings were reported in experiments with crosses in which wild-type females were crossed with males of the yellow-white, white-forked and white-lozenge strains (presumably slightly different alleles at the same gene as white) as well as for three other allelomorphs of white in various combinations, those being tinged, eosin, and apricot. Because the phenotypes of some of those variants do not stand out on a red background as clearly as the crosses in which the facets turned white, classification was sometimes not as clear cut. Also, for some of the other strains the irradiated flies do not survive as well. The findings from the other strains were generally similar. Of the groups irradiated, the younger ages (eggs and early larval stages) were far more susceptible to showing the presence of white facets. Irradiation of pupal stages showed no evidence of induction of somatic mutations.

R/*R* females irradiated in early stages of development, unlike their *R* brothers, showed no white spots in their eyes, which demonstrated that a mutation or loss of only one of the two normal genes had no effect, with the presence of the remaining *R* gene being sufficient to produce red pigmentation, just as it normally does in *R* males. Mutant areas varied in size, from a single ommatidium (usually) to the whole eye (in one instance). The size of the white areas appeared to be determined by the age of which the flies were treated. It was estimated that for a dose of D4.6 (i.e., [4.6 × 5 min of exposure]) there would be one gene mutation among 9891 genes and one chromosome break among every 713 X chromosomes carrying the dominant gene. An attempt was made to induce reverse mutations, but only one certain case was found in eyes of 4661 irradiated flies.

The assumed gene mutation response for somatic mutations was said to be proportional to the X-ray dose. On page 364, Patterson (1929b) stated that the rate of mutation "is influenced by the strength of the dose, probably being directly proportional to the dose." This conclusion was reinforced in the final paragraph on page 352 of the same paper as follows. "In fact, to double the dose results in practically doubling the rate at which modified areas [i.e., white ommatidia zones] are produced."

Assessment of the Patterson findings

Dose patterns in the nine Patterson experiments

The present assessment of the Patterson 1929b study involved evaluating the changes in the coloration of facets in males for each experiment separately and then combining the data from all the experiments by dosage in order to derive a robust dose–response assessment. The most methodologically valid assessment is the evaluation of separate experiments because there was more experimental consistency with respect to use of similar age when exposed. Nonetheless, the basic patterns of dose response relationships that occur within individual experiments also occur when the data are combined. Figure 1 displays the doses tested for each experiment. It shows that the nine experiments used a wide range of doses but varied greatly as to the number of doses tested. Four experiments (i.e., Experiments #5, #7, #8 and #9) employed only the two doses D4 and D5. Two experiments (i.e., Experiments #1 and #2) tested only the two doses D5 and D10. Experiment #6 emphasized the high dose range, testing only doses D4, D5, D7 and D10. In contrast, only two experiments (i.e., Experiments #3 and #4) assessed the broad range of doses, from low to high. Thus, the most useful experiments for the present assessment are Experiments #3 and #4 because they present findings across the entire dose–response spectrum. Experiment #3 is the most relevant since it is the only study including exposures in the lowest dose tested and also having much larger sample sizes than Experiment #4 for the lower doses.

Effect of X-ray treatment during development on adult survival pattern

Figure 2 provides the findings regarding survival to adulthood following irradiation at different stages of development for each experiment for the combined age groups within each exposure group. That is, eggs were typically exposed to the X-rays from approximately 2 h of age until about 82 h later, often within about 12-h intervals. There was variability between experiments with respect to the age when exposure first occurred and when it ended, as well as the length of the age intervals. There is additional variability because eggs vary as to the extent of development when they are laid. In general, Patterson appeared to make these study design changes in attempts to optimize the likelihood of obtaining a treatment effect. For example, he irradiated younger aged developmental stages when testing a strain in which the older stages were likely to be far less susceptible. It seems likely that Patterson used

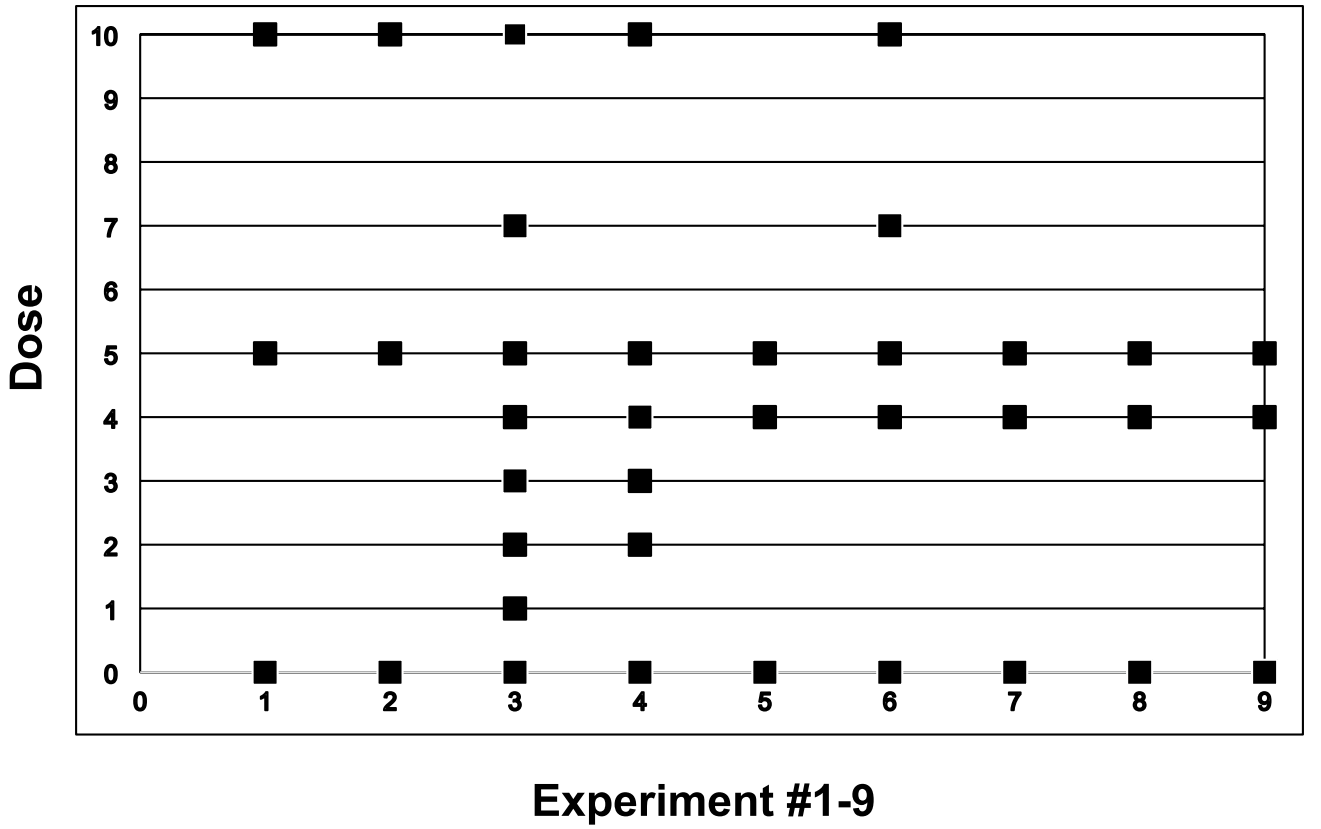

Fig. 1 Doses used in X-ray fruit fly mutation studies (Source: Patterson 1929b)

doses #4 and #5 more than others based on the results of his preliminary experiments that he published earlier (Patterson 1928). In those experiments, Patterson noted that the lower doses failed to have any treatment effect, whereas he could demonstrate effects at higher doses, especially at D5. The use of very high doses was also problematic because of high toxicity, that is, the killing of a high proportion of the developmental stages tested. Figure 2 shows the percentage of the irradiated developmental stages that survived to become adults for the different doses tested from combined experiments in which wild-type females were mated with white-forked males. The figure shows that the survival was slightly better than in the controls at the lower two doses and, as the doses got higher, survival decreased becoming only approximately 25% at D10. A threshold-like effect is seen for survival with a clear increase in toxicity occurring by D4. As will be shown below, this is also roughly correlated with the occurrence of color changes in the facets.

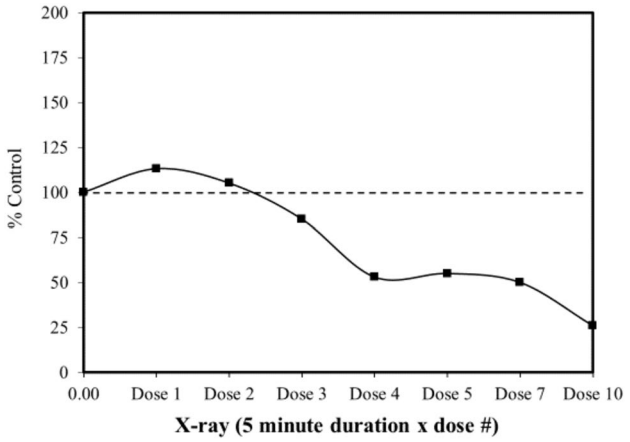

Fig. 2 Effects of X-ray treatment of egg/larvae/pupal stages and subsequent survival as adult flies (females and males) in the wild-type female x white-forked male experiments (Experiment #3, Tables 3–5, Patterson 1929b)

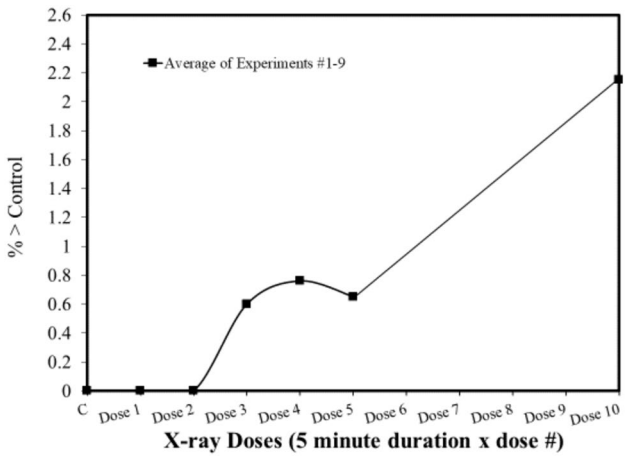

Fig. 3 Effects of X-ray treatment on phenotypic changes (white ommatidia) in adult male fruit flies treated during early developmental stages (Source: Patterson 1929b) [Note that data for Dose 7 is not shown in the graph because of the low sample size (with the results being 0/114), and data for Experiment # 8 is not shown because Patterson combined his data for doses D4 and D5.]

Effect of X-ray treatment on phenotypic changes (occurrence of white ommatidia) in adults whose somatic cells were irradiated early in their development

Figure 3 provides a dose–response relationship for the combined findings across all experiments. The data strongly suggest that there is a threshold dose response for the occurrence of what Patterson claimed to be radiation-induced gene mutations based on male flies showing color changes in the ommatidia. The data shown in Fig. 3 are from the detailed paper in which he presented data including nine different experiments (Patterson 1929b). The data points shown by black squares include all of the data tabulated for seven of those experiments. The data for D7 were excluded by us because of the small sample size, with the results being 0/114. The data in the figure were also excluded for experiment eight in which 3 of 305 males had single white ommatidia, indicative of gene mutations, as the combined results for doses D4 and D5. For unexplained reasons, Patterson reported the data for experiment eight, in which only those two doses were studied, with the data combined. The data from experiment eight are therefore not included in Fig. 3.

The data from D3 and above show obvious induction of some type of genetic damage that tends to increase with dose, but with no clear indication of how linear the response is. While these experiments demonstrated clear differences between different developmental stages in responding to the types of damage evaluated, there was obviously no indication of any such damage at the lowest doses (D1 and D2) in any stages.

This observation is important because it provides supportive justification for the combination of data across developmental stages at the higher doses. Thus, the data from the preliminary study as published in *Science* (Patterson 1928) and the much more extensive series of experiments published a year later (Patterson 1929a,b) are consistent, showing no evidence of X-ray induced somatic mutations that produced white ommatidia in male flies in the low dose part of the dose response. Our reanalysis is focused on data possibly relevant to gene mutations, and Patterson clearly showed that vastly more females than males showed effects in ommatidia, presumably as a result of chromosomal damage. The situation was entirely different for females because he was using X-linked mutations and female flies have two X chromosomes. Patterson assumed that the response that he observed in male flies resulted from somatic gene mutations and that radiation-induced gene mutations would occur at the same frequency in somatic cells of both sexes. Thus, a separate analysis of the much more complex data in the female is beyond the scope of this paper.

Discussion

The findings from Patterson's extensive experiments on radiation-induced somatic mutations affecting coloration of ommatidia are consistent with the more limited experimentation presented by Patterson (1928) in his initial paper on that topic that showed no treatment effects at doses equal to or less than D5. Patterson (1928) stated that "It was evident that the lighter dosages were not sufficient to bring about much change. Consequently, in all the succeeding experiments, the longer [duration] treatments were given. I have

since used almost exclusively the D-5 and D-10 doses." It is of interest that Patterson (1928) reported 0 areas of white ommatidia in 378 males in the D5 exposure group. However, he did report that females showed white ommatidia (D5: 16/382 and D10: 11/2080).

The experiments of Patterson discussed in this paper are of particular interest because they deal with somatic cells and thus might have relevance to cancer or other types of somatic damage. The great majority of experiments dealing with such genetic changes have been done in reproductive cells that would relate to damage in future generations. Subsequent research, mostly based on treatments of different germ-cell stages, has estimated the proportion of genetic changes induced by X-rays for recessive linked genes of *Drosophila* due to gene mutations or other causes (Muller and Altenburg 1930; Oliver 1930, 1932; Herskowitz 1946, 1951; Demerec 1937; Demerec and Fano 1941; Lea and Catcheside 1945; Haldane and Lea 1947; Catcheside 1948). Building upon this previous research, Herskowitz (1951) estimated that approximately 3/4 of these genetic alterations were due to chromosome breakage alone with another 5–6% being due to the position effect. Thus, a strong majority of these changes were not considered to have been due to gene mutation. The remaining ~ 20% of the genetic changes still remained to be characterized but could be related to actual point mutations or other changes such as gene deletions, transposon-based mutations and other factors. In 1946, Herskowitz (1946) concluded:

> "...that the great majority of x-ray-induced recessive lethals are probably produced at points of breakage and relegate to position effect and non-breakage mutation a relatively small part in the production of recessive lethals."

Later research with modern nucleotide measurement technologies indicated that most of the residual genetic changes were due to genetic deletions (See Calabrese 2017, page 780—right column and page 781—right column for an extensive discussion and citation series). These analyses, in retrospect, supported the perspective of Stadler (1954) that Muller (1927) had confused an observation (i.e., transgenerational phenotypic change) with a mechanism. In addition, Lefreve (1948, 1950) reported that the pattern of such genetic changes was both qualitatively and quantitatively similar between reproductive and somatic cells. These findings strongly suggest that the changes in coloration of ommatidia that Patterson observed in males and attributed to gene mutations was far from accurate, with actual changes within individual genes likely accounting for a small part of the total. Because Patterson found markedly higher levels of somatic effects in females, which he attributed mostly to chromosomal damage, his data now suggest that extremely little of the genetic damage that occurs in chromosomes present in two copies results from damage occurring within individual genes. If, indeed, almost all genetic damage occurring in somatic cells results from chromosomal damage, radiation would not be expected to give a linear dose response. The data reanalyzed in this paper are consistent with a threshold rather than a linear dose response. Furthermore, the threshold occurs at an extremely high dose delivered at a massively high dose rate. It is somewhat curious that major journals in the late 1920s would publish papers that made suggestions about dose responses without including any dosimetry. The defining of doses in terms of minutes of exposure might have kept some readers from realizing that the experiments had no relevance to health effects in humans.

The present paper is of considerable historical importance because it provides no evidence of a linear dose response related to X-ray induced gene mutations causing somatic effects. Despite such contrary findings by a close colleague, Hermann Muller in 1930 would proclaim the existence of a Proportionality Rule asserting that the dose response was linear down to a single ionization, with this claim soon leading to the creation of the LNT single-hit model (Calabrese 2017, 2019, 2022a, b, 2024). One can only wonder whether Muller would have issued his Proportionality Rule if Patterson (1928, 1929a, b) had presented a more detailed assessment of the dose response in the low dose zone of his experiments analyzed in this paper, thereby showing support for the threshold model with data from his own model and research team member. Nonetheless, Muller did this despite the fact that the exposures used by Patterson were quite high with D1 and D2 being in the 250–500 r area with a dose rate that exceeded background by ~ 170 million-fold. It also becomes relevant to know that George Snell, a mouse geneticist working in Muller's laboratory as a postdoc in 1931, failed to show that X-rays would induce gene mutations in reproductive cells (Calabrese and Selby 2024). Neither Patterson's nor Snell's negative findings were noted by Muller despite his very close relationship with the authors and their acknowledgement of his support in their studies. It is suggested that the failure of Patterson to put the results of his fascinating and substantial study of radiation-induced genetic changes in the somatic cells of the fruit fly eye into proper perspective, as well as a failure of the peer-review process and the lack of attention by the scientific community contributed to the development of the belief that the dose response following X-ray exposure was linear at low doses. Such factors had a contributing role in the inappropriate acceptance of the LNT model and its later application to hereditary and cancer risk assessment.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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