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SEASONAL VARIATION OF X-RAY-INDUCED CHROMATID ABERRATION FREQUENCIES IN CULTURED POLLEN TUBES OF *Tradescantia*

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ABSTRACT

Cultured pollen tubes of *Tradescantia* were X-irradiated (310 R) during mitosis of the generative nucleus. For experiments conducted intermittently through the seasons of the year, X-irradiation was applied at interphase (G_2), 5-7 min after sowing the pollen grains on the medium. For experiments conducted only in summer and designed to treat prophase and metaphase chromosomes, X-irradiation was applied 8 hr and 18 hr, respectively, after sowing. Treated and control cultures were fixed after 18 hr of growth for scoring chromatid aberrations in metaphase figures. Results from year round studies showed seasonal variation of aberration frequencies similar to those observed in hydroxyurea and SO_2 studies previously reported. The average aberration frequency, expressed in terms of aberrations per 100 cells, was 140 in summer, 95 in fall, 48 in winter and 19 in spring. Cultures treated at later stages of mitosis in summer showed 38 aberrations per 100 cells in prophase and 11 in metaphase, while the controls for all experiments has around 5. The discrepencies between seasons and between stages of mitosis could be interpreted as an implication of changing frequencies of sister chromatid crossingover, a spontaneous process of breakage and fusion of chromatids. Results of a comparative analysis of the seasonal changes of mutagenic efficiencies of X-rays of present study with those of hydroxyurea and 10_2 of previous studies further substantiate the concepto proposed.

RESUMEN

Cultivos de tubos polínicos de *Tradescantia* fueron tratados con rayos X (310 R) durante la mitosis del núcleo generador. En los experimentos conducidos intermitentemente a través de las estaciones del año, los rayos X fueron aplicados en interfase (G_2), 5 a 7 minutos después de sembrar los granos de polen en el medio. En los experimentos conducidos solamente en verano y diseñados para tratar a los cromosomas en profase y metafase, la irradiación X fue aplicada 8 y 18 horas después de la siembra, respectivamente. Los cultivos tratados y testigos fueron fijados después de 18 horas de crecimiento para registrar las aberraciones cromatídicas en metafase. Los resultados obtenidos en el año mostraron variaciones estacionales en las frecuencias de aberraciones similares a las observadas en los estudios con hidroxiurea y SO_2 descritos previamente. La frecuencia promedio de aberraciones expresada en términos de aberraciones por 100 células, fue de 140 en el verano, 95 en otoño, 48 en invierno y 19 en primavera. Los cultivos tratados en los estados tardíos de la mitosis en el verano, mostraron 38 aberraciones por 100 células en profase y 11 en metafase, mientras

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que en los testigos fue alrededor de 5 en todos los experimentos. Las discrepancias entre estaciones y entre estados de la mitosis pudieron interpretarse como una implicación del cambio de frecuencias de entrecruzamiento ("crossing over") de cromátidas hermanas, un proceso espontáneo de rompimiento y fusión de cromátidas. El análisis comparativo de los cambios estacionales en la eficiencia mutagénica de los rayos X con la de la hidroxiurea y el SO_2 de trabajos anteriores apoyan el concepto propuesto.

INTRODUCTION

Studies of chemical mutagen induced chromatid aberrations using pollen tubes of *Tradescantia* (Ma *et al.*, 1973; Khan and Ma, 1974), indicated that aberration frequencies vary from season to season, with higher frequency in summer and fall and lower frequency in winter and spring. Similare findings were reported by Tomkins and Grant (1976) in herbicide-induced chromosome aberrations in *Vicia* roots. Kato (1971) in his ten year study of spontaneous aberrations in *Clivia miniata* found a similar pattern of seasonal variation. Light condition tests conducted during hydroxyurea Khan and Ma, 1974), and SO_2 (Ma, *et al.*, 1973) studies in our laboratory revealed that high sensitivity of pollen tube cultures of *Tradescantia* can be nullified by keeping the summer plants under low intensity artificial light conditions for 7 or more days. Summer sunlight and its accompanying heat energy play an essential role in aberration formation in mitotic cells. A recent study on meitotic crossingover in maize under different light conditions Ma, 1976) indicated that exposure to low intensity light in shaded greenhouse sunlight or artificial light in growth chamber reduced the crossingover frequency as much as 30% of the original value. Thus summer sunlight is responsible for the activity of meiotic crossingover. Sister chromatid crossingover is known to occur spontaneously in somatic cell cultures of humans (Latt, 1974) animal (Ikushima and Wolff, 1974) and mitotic and meiotic cells of several plant species including *Tradescantia* (Schwartz, 1953; Taylor, 1959; Vig, 1973; Kato, 1974). Under the "Mutual Exchange" hypothesis, sister chromatid crossingover, like meiotic crossingover, requires breakage and fusion of chromatids in order to exchange and restitute to normal chromatids. The kinetics and enzimatic reactions of sister chromatid crossingover would also be light dependent as in meiotic crossingover. Based upon the aforementioned evidences, we postulate that seasonal variation of chromatid aberration frequencies could be attributed to seasonal change of frequencies of sister chromatid crossingover. High frequency of sister chromatid crossingover in summer and fall provide a greater opportunity for mis-joining or failure of fusion if the process were interfered by a treating agent. Under this hypothesis, we offer an explanation for the high frequency of aberration induced by very low dose of chemical mutagens during the summer and fall seasons, but the same low dose of chemical mutagen fail to do so in winter and spring.

In the present study, X-ray was utilized in place of chemical mutagens to further test the validity of the proposed hypothesis. According to "breakage first" hypothesis (Sax, 1940), X-ray presumably is able to break chromosomes by its excision force. Radicals and ions produced in the path of ionization of X-rays

also can interfer with proper fusion in a maner similar to chemical mutagens. By using X-rays, a constant base level of aberration, above the background, should be induced independent of seasonal change, and a seasonal variation of aberration frequencies should also be expected over and above the base level aberration frequency. Therefore, a comparison among the X-rays, hydroxyurea and SO_2 studies would help to differentiate between the two possible means of aberration induction, namely direct excision and interference of proper fusion.

In separate experiments, cultures were treated at prophase and metaphase stages to compare with the results obtained by treating cultures at interphase (G_2). If sister chromatid crossingover took place only in interphase when newly doubled chromatids were closely associated, as in pachytene of meiosis, a higher frequency of aberration would be expected only in cultures treated at interphase. A comparison of summer to winter change of mutagenic efficiencies among X-rays, hydroxyurea and SO_2 was made. The magnitude of decrease of mutagenic efficiencies of each of these agents from summer to winter would also help to substantiate the hypothesis proposed.

MATERIALS AND METHODS

Mature pollen grains of *Tradescantia paludosa* Anders. Sax clone-3 were utilized throughout all the experiments conducted intermittently during different seasons over a period of two years. New plants were vegetatively propagated in flower pots every two months. A crop of 60 plants was planted in the field during the summer and fall seasons in order to re-establish plant vigor. The plant population of the winter and spring was propagated from the field stock. In order to meet the requirement of a 16-hr photoperiod for *Tradescantia*, 8 hr of incandescent, light was supplemented each day beginning October 1 and ending April 1 each year. To minimize environmental variables, pollen grains were collected from greenhouse-grown plants. On days of experiments, pollen grains were collected from fully opened flowers around 11 a.m. and desiccated in the dark with silica gel for 4 hr. Dry pollen grains were sown on the surface of microslides coated with lactose (12%) agar (1.5%) medium. Colchicine (0.04%) was added to the medium for accumulation of metaphase figures of the mitotic generative nuclei.

Techniques of pollen tube culture and permanent slide preparation was based upon the procedures described in a previous publication (Ma, 1967) except that the cultures were fixed in Gate's fluid instead of aceto-alcohol. X-rays utilized in this study were generated by a Model E (Standard X-ray Co.) therapeutic unit operated at 80 Kvp, 5 ma with a 1.2 mm thick aluminum filter. Pollen cultures were irradiated at 13 cm distance from the filament of the X-ray tube with an exposure field size around 10 cm in diameter. Dose rate determined by a Victoreen Condenser R-meter (Temperature 24 $\pm$ 1°C, Pressure 75 $\pm$ 5 mm Hg), at this distance, was about 155 R/min, and a total dose of 310 R was administered to the samples in a plexiglass treatment chamber. The treatment chamber is a flat square box with the capacity to hold 8 microslides exposing their pollen culture surfaces side-by-side in two rows of 4 slides. All the culture-bearing slide surfaces were inside

the exposure field. The difference of X-ray intensity within the sample exposing area was negligible. The chamber contains sheets of absorbent paper moistened with distilled water and is sealed with Saran Wray (thin plastic film) covering the chamber top but allowing penetration of X-rays during the two-minute treatment time. All treatments of the year-round studies were administered to the pollen culture 5-7 min after sowing. The generative nucleus of the binucleated pollen grains were in early G_2 stage of interphase of mitosis at this time. This was known to be the most sensitive stage, resulting in chromatid aberrations, observable in metaphase figures, about 18 hr later. Thus the experimental culture materials of major part of this study were fixed 18 hr after treatment. Three experiments carried out in this study having their cultures treated at late prophase, thus the metaphase figures were fixed 8 hr after treatment. Cultures of another 3 experiments were trated at metaphase, therefore, they were fixed within 1 min after treatment. These 6 experiments were designed to study the difference of aberration frequencies induced at 3 different stages of mitosis and to explore the possible relationship between chromatid aberration and sister chromatid crossingover.

Generally, 50 metaphase figures were observed at random from each microslide for chromatid aberration frequency, and 6-8 microslides were scored from each of the treated and control groups. Aberration frequencies were expressed as the number of aberrations per 100 cells. Each chromatid deletion or isochromatid type of aberration was counted as one aberration, and each chromatid exchange was counted as two aberrations. Standard deviations of the summer experiments on 3 different stages of mitosis were derived from 3 repeated experimentos for each mitotic stage.

RESULTS

Although almost all types of chromatid aberrations were encountered in this investigation, approximately 90% of the aberrations were simple chromatid or isochromatid types, and the remaining 10% fell into the category of chromatid exchanges. Samples of aberrations are shown in Fig. 1.

Results of year round studies carried out in summer (July, August), fall (September, October), winter (March) and spring April) are shown in Fig. 2.

The general decrease of aberration frequency from summer through spring indicates the typical seasonal variation pattern as observed in previous studies on the effect of SO_2 (Ma *et al.*, 1973) and hydroxyurea (Khan and Ma, 1974) on chromosomes in the same material.

Results of repeated experiments carried out in summer season alone for comparison of stage sensitivity among interphase, prophase and metaphase are shown in Fig. 3.

The step-wise decrease of aberration frequencies in prophase and metaphase treated groups may have been partially due to the increasing quantities of protective nucleoprotein, but more likely due to the absence of sister chromatid crossingover during these stages. The aberrations in prophase and metaphase treated groups were most likely formed through the excision mechanism.

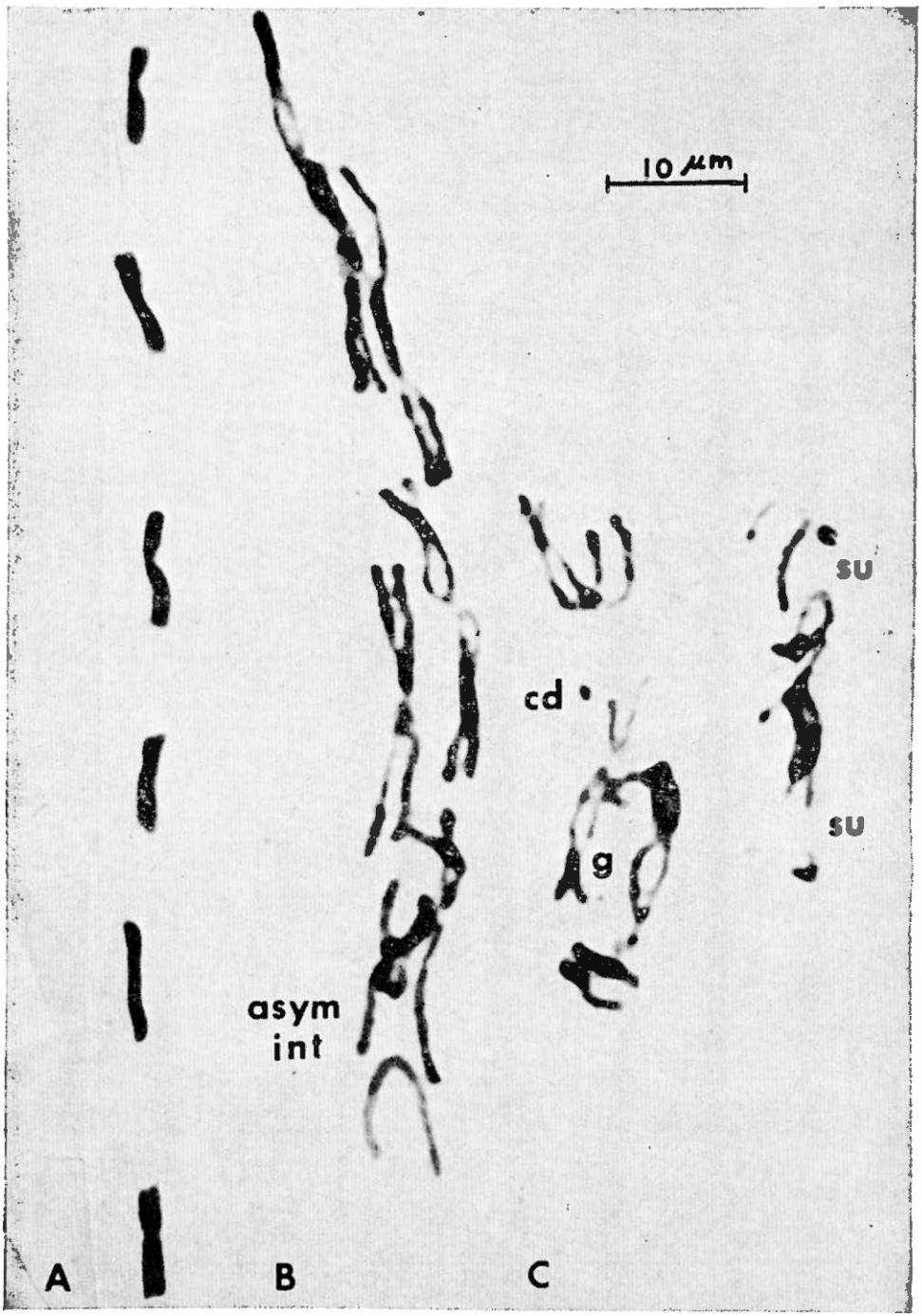

Fig. 1. Chromatid aberrations induced by X-rays (310 R): A, Control; B, Induced aberrations (asym. int., asymmetrical interchange); C, Induced aberrations (cd, simple chromatid break; g, gap; su, sister chromatid union).

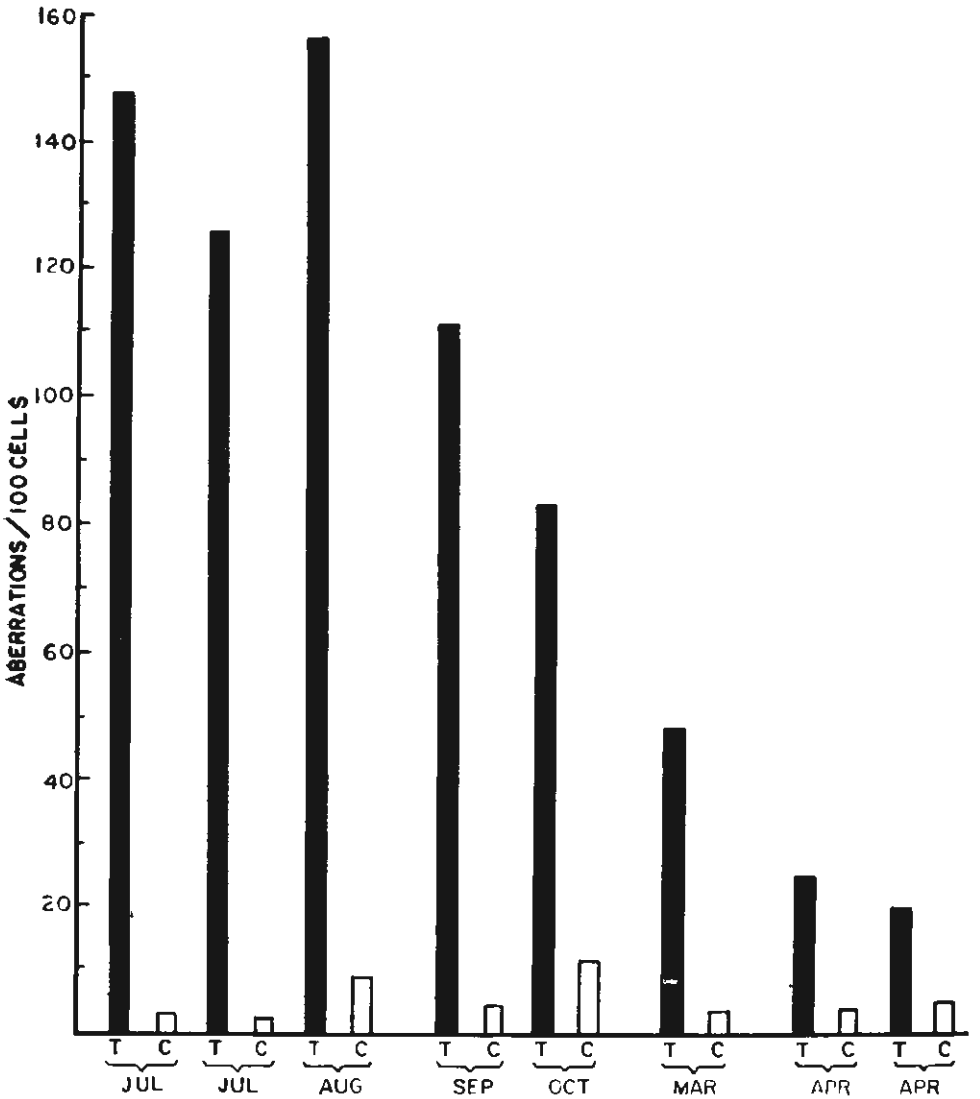

Fig. 2. Histograms showing seasonal variation of X-ray-induced aberration frequencies, T, treated; C, control.

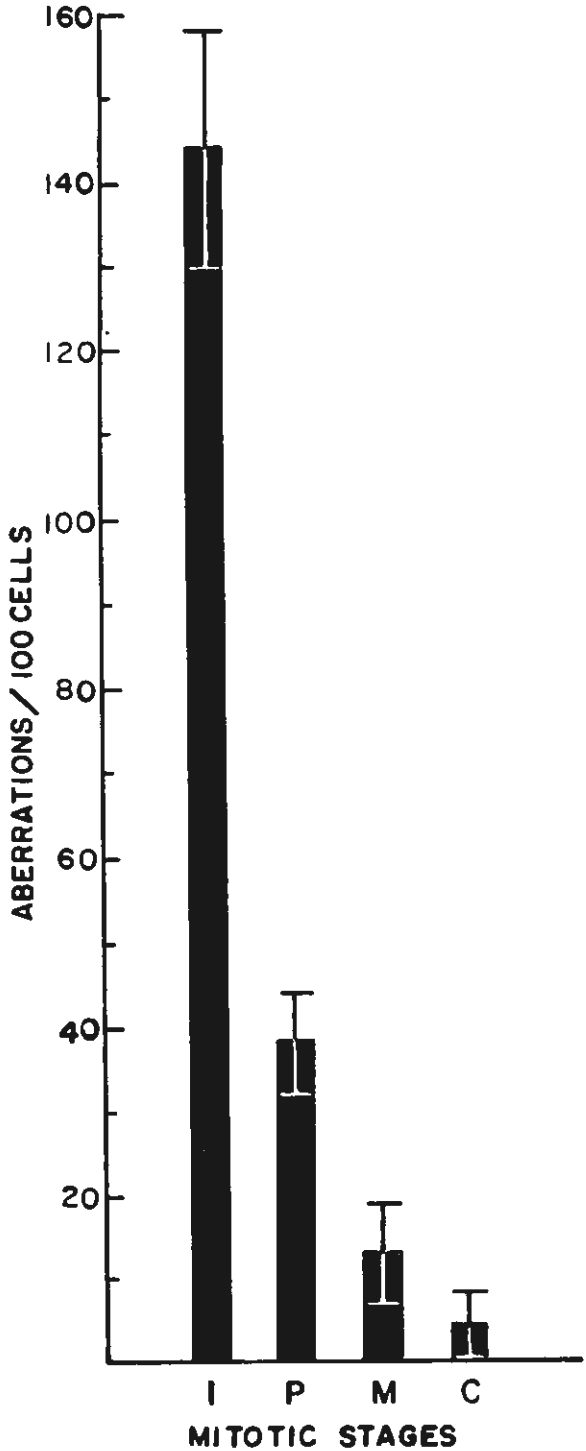

fig. 3. Histograms showing the difference in sensitivity at Interphase (I), Prophase (P), and Metaphase (M) to 310 R X-rays; and the Control (C).

The comparison of mutagenic efficiencies, expressed in terms of number of aberrations per unit dose of X-rays, of hydroxyurea, and of SO_2, in summer and in winter is shown in Table 1.

TABLE I

MUTAGENIC EFFICIENCIES OF X-RAYS, HYDROXYUREA AND SO_2 IN WINTER AND IN SUMMER

Mutagens	*X-rays (aberr./cell/100R)*		*Hydroxyurea* (aberr./cell/mM)*		*SO_2* (aberr./cell/0.1 ppm)*	
Seasons	*summer*	*winter*	*summer*	*winter*	*summer*	*winter*
Aberr. Freq.	1.53	0.44	0.431	0.080	0.82	0.05
Background	0.04	0.04	0.154	0.043	0.26	0.03
Net Freq.	1.49	0.40	0.277	0.037	0.56	0.02
Dosages	310 R	310 R	3 mM	3 mM	0.1 ppm	0.1 ppm
Mutag. Effic.	0.48	0.13	0.092	0.012	0.56	0.02
Folds of decrease	3.7		9		28	

* Data obtained from previous studies.

The importance of the decrease of mutagenic efficiencies in the winter season will be discussed later.

DISCUSSION AND CONCLUSIONS

The X-ray-induced chromatid aberration frequencies in cultured pollen tubes of *Tradescantia* demonstrated a distinct pattern of seasonal variation (Fig. 2) which resembles closely those observed in hydroxyurea (Khan y Ma, 1974) and SO_2 (Ma *et. al.*, 1973) studies previously reported. Present data indicate that fusion interference mechanism contributed to high frequency of aberration in summer and fall. A substantial amount of aberration in winter and spring could be generated through the excesion mechanism of X-rays. Whereas low level 10_2 seems to lack the ability totally in winter cultures.

Comparison of aberration frequencies induced during 3 different stages of mitosis (Fig. 3) in summer cultures indicates that the excesion mechanism of X-rays probably caused the relative low frequency in prophase and very low frequency in metaphase. The hingh frequency induced in interphase was caused by both fusion interference and excision mechanism, and fusion interference was only possible during sister chromatid crossingover in interphase.

The change in mutagenic efficiencies for these 3 agents from summer to winter was gradiently different (Table 1). X-ray showed the least decrease in mutagenic efficiency (3.7 fold) when compared with the other 2 agents. This indicates that X-rays induced a substantial amount of breakage through excision mechansim. Hydroxyurea showed a moderate decrease (9 fold). This could be attributed to hy-

droxyurea's inhibitory action upon DNA synthesis. The 28-fold decrease for SO_2 and the very low aberration frequency in winter could mean that most of the aberrations induced by SO_2 were of fusion interference origin.

If it is true that the fusion interference mechanism generated most of the chemical mutagen induced chromatid aberrations, there should be a reassessment of the danger of low dose environmental mutagens. When a persistent pollutant exists throughout the year or during the specific season of the year when certain plants or animals having their peak rate of growth, differentiation or gametogenesis, there will be a substantial amount of chromatid aberration and point mutation induced. Considering the long range genetic damage to living beings, any amount of pollutant is dangerous. Mere absence of immediate sign of damage in vegetation or detectable illness in human beings is insufficient to justify exposure to low dose radiation or chemical mutagens.

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