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Low-dose mutation-response relationships in Tradescantia; principles and comparison to mutagenesis following low-dose gaseous chemical mutagen exposure

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Abstract

Inflorescences of several clones of Tradescantia heterozygous for flower color have been treated with ionizing radiation and with the gaseous form of several known or suspected chemical mutagens. Pink somatic mutations were subsequently scored in the stamen hair cells of mature flowers and dose-/exposure-response curves constructed. Results indicate clearly that there is no evidence for a threshold for mutation response following x or neutron irradiation. Results so far obtained for gaseous chemical mutagens are less clear, but also suggest that there is no threshold for mutation response.

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Low-Dose Radiation Responses

Figure 1 demonstrates neutron and x-ray dose-response curves for pink mutations induced in stamen hairs of Tradescantia clone 02 (1). Following exposure to 0.43 MeV neutrons the mutation response is directly proportional to dose, forming a +1 slope on a log-log plot, from 10 millirad to the area of mutation saturation beginning at ca. 10 rad. The 250-kVp x-ray mutation-response curve is more complex, forming a +1 slope between doses of 0.25 and about 5 rad; a steeper slope may be fitted to the data for doses from about 5 rad to the area of saturation at about 100 rad. Clearly, an extrapolation from the high-dose portion of the x-ray curve to lower doses would have increasingly underestimated the mutagenic potential of doses lower than about 5 rad. In addition, a threshold effect would have been predicted. Similarly, extrapolation from the low-dose x-ray curve to higher doses would have underestimated the mutagenicity of doses higher than about 5 rad.

Since neutron and x-ray curves are converging above 5 rad of x rays, relative biological effectiveness (RBE) decreases as x-ray dose increases up to about 100 rad. However, since the curves are parallel below 5 rad of x rays RBE is constant and maximum in that region. Extrapolation from higher to lower x-ray doses would have predicted an ever increasing RBE. Dose-response curves for 3.9 GeV nitrogen ions also have +1 slopes on a double logarithmic plot; thus, the RBE of nitrogen ions relative to x rays also increases with decreasing dose and becomes maximal at an x-ray dose of about 5 rad (2).

Maximum RBE of monoenergetic neutrons of various energies, with reference to x rays, increases from 14.6 at 0.065 MeV up to a peak value of 47.6

at 0.43 MeV, and then decreases to a low of 10.4 at 13.4 MeV (3). In contrast, there is only a slight dependence of oxygen enhancement ratio (OER) on neutron energy, with values falling between 1.3 and 1.7 for neutron energies between 0.065 and 13.4 MeV (3). The OER for x rays is 3.2, at least for doses above 10 rad (3).

Departure of the x-ray dose-response curve above 5 rad to a slope steeper than +1 on a log-log plot indicates the presence of a quadratic component to the curve, which is usually taken to indicate the presence of a dose-rate effect. This prediction was confirmed when it was demonstrated that mutation frequency per rad decreased over 5-fold when the dose rate required to deliver 58-80 rad was decreased over five orders of magnitude, from 105 rad/min to 3.6 millirad/min (4). The dose-rate effect indicates that there is a relatively rapid component to the repair process, i.e., repair of premutational lesions during irradiation. Thus, the dose rate at which a radiation dose-response curve is determined can have a significant influence on any downward extrapolation. So far the data indicate that the lower the dose rate the closer the curve will approach a +1 slope; the closer the slope of the upper dose-response curve is to unity, the more accurately would downward extrapolation predict the mutagenicity of lower doses.

X-ray dose fractionation also demonstrated a sparing effect for pink mutation induction in clone 02. Compared to an unfractionated dose of 60 rad, fractionation intervals (between two 30 rad doses) up to ca. 15 min did not reduce the mutation frequency. Progressively longer intervals resulted in a reduction in mutation frequency, but only by about 20% for an interval of

24 hr (5). This slow and limited decline in mutation rate indicates a slower component to the repair process than that seen during continuous irradiation.

Comparison to Low-Dose Gaseous Chemical Mutagen Exposure

Inflorescences of three clones of Tradescantia have been exposed to the gaseous form of ethyl methanesulfonate (EMS) and 1,2-dibromoethane (DBE). EMS is a well-known and powerful alkylating mutagen; DBE is also an alkylating agent and has been found to be mutagenic in Salmonella (6), Neurospora (7), Hordeum (8) and Tradescantia (9, 10). DBE is used heavily in industry as a fumigant for soil and stored foods, as an intermediate in the production of dyes and pharmaceuticals and as a lead scavenger in gasoline.

The exposure-response curves that have been determined for Tradescantia clones 02, 0106 and 4430 following 6-hr exposures to EMS and DBE (10) are presented in Fig. 2. Included are the x-ray dose-response curves for the same three clones. The scale of the ordinate (pink events/100 hairs, minus spontaneous frequencies) applies to both chemical and radiation exposures so that direct comparisons of mutagenic efficiency can be made for the three clones. Several general relationships can be noted in this intercomparison: (A) EMS is more mutagenic than DBE in each clone. (B) The relative responses of these clones to chemicals and x rays do not parallel one another, e.g., while clone 4430 is 6-9 times more sensitive to EMS and DBE than clone 02, these two clones have essentially identical responses to x rays. (C) The mutagenic potential of EMS and DBE on the linear portion of the exposure-

response curve is equal to that of at least 10 rad of x rays in each case, and in one case the mutagenicity of EMS exceeds that of x rays: 250 parts per million (ppm) of EMS (for a 6-hr exposure) is over 4 times more mutagenic than 160 rad of acute x rays in clone 4430. (D) The radiation equivalence of any 6-hr chemical exposure can be determined easily. For example, an exposure of clone 4430 to 50 ppm DBE elicits a mutation response equal to that induced by 18 rad of x rays; an exposure of the same clone to 100 ppm EMS results in a mutation yield equal to the response to 160 rad of x rays.

Separate exposure-response curves have been constructed for DBE when duration of exposure of clone 4430 to 0.5 to 100 ppm was varied between 2 hr and 6 days (11). In general, slopes approximate +1 for 2- and 4-hr exposures, and +1.5 for exposures from 6 hr to 6 days. Data from all of these individual curves can be plotted to construct a single curve (Fig. 3) when the product of concentration (ppm) and duration of exposure (hr) is expressed as total exposure (ppm-hr) on the abscissa (11). This convention permits the plotting of data from many experiments with differing exposure concentrations and durations as a single relationship. The data which make up this curve must be considered to be preliminary, but the approximate radiation equivalence of any DBE exposure can be determined graphically from this relationship. For example, mutation frequencies induced by exposures of 70 and 1000 ppm-hr are equal to those induced by acute x-ray doses of ca. 10 and 100 rad, respectively.

The overall slope of the curve in Fig. 3 is +1.2. The data points in the low-exposure region of this curve are somewhat scattered; mutation

response to chemicals is usually more variable than that following radiation exposure, presumably due to nonuniform penetration of the chemical. However, there is some indication, especially when more recent 6-hr data are also considered, that the slope may be decreasing at low exposures. If this initial indication is generally correct, extrapolation of curves from high- to low-level exposure would give an underestimate of the danger of a low-level exposure to gaseous chemicals like DBE. Further investigation of the shape of the curve in the low-exposure region is in progress.

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FIGURE LEGENDS

1. Dose-response curves for pink mutations induced in clone 02 by 0.43-MeV neutrons and 250-kVp x rays, plotted from the data in Sparrow et al. (1). Dashed lines are extrapolations from the upper and lower portions of the x-ray dose-response curves.

2. Exposure- and dose-response curves for pink mutations induced in clones 02, 0106 and 4430 by EMS, DBE and 250-kVp x rays. Chemical exposures were of 6-hr duration in all cases. The scale of the ordinate is related to both chemical and x-ray exposures so that chemical- and radiation-induced mutation levels can be compared directly (from ref. 10).

3. Exposure-response curve for pink mutations induced in clone 4430 by DBE. Total exposure on the abscissa is expressed as the product of concentration (ppm) and time (hr). Mutation levels induced by various x-ray doses are included on the ordinate for reference (unpublished data of Sparrow and associates).

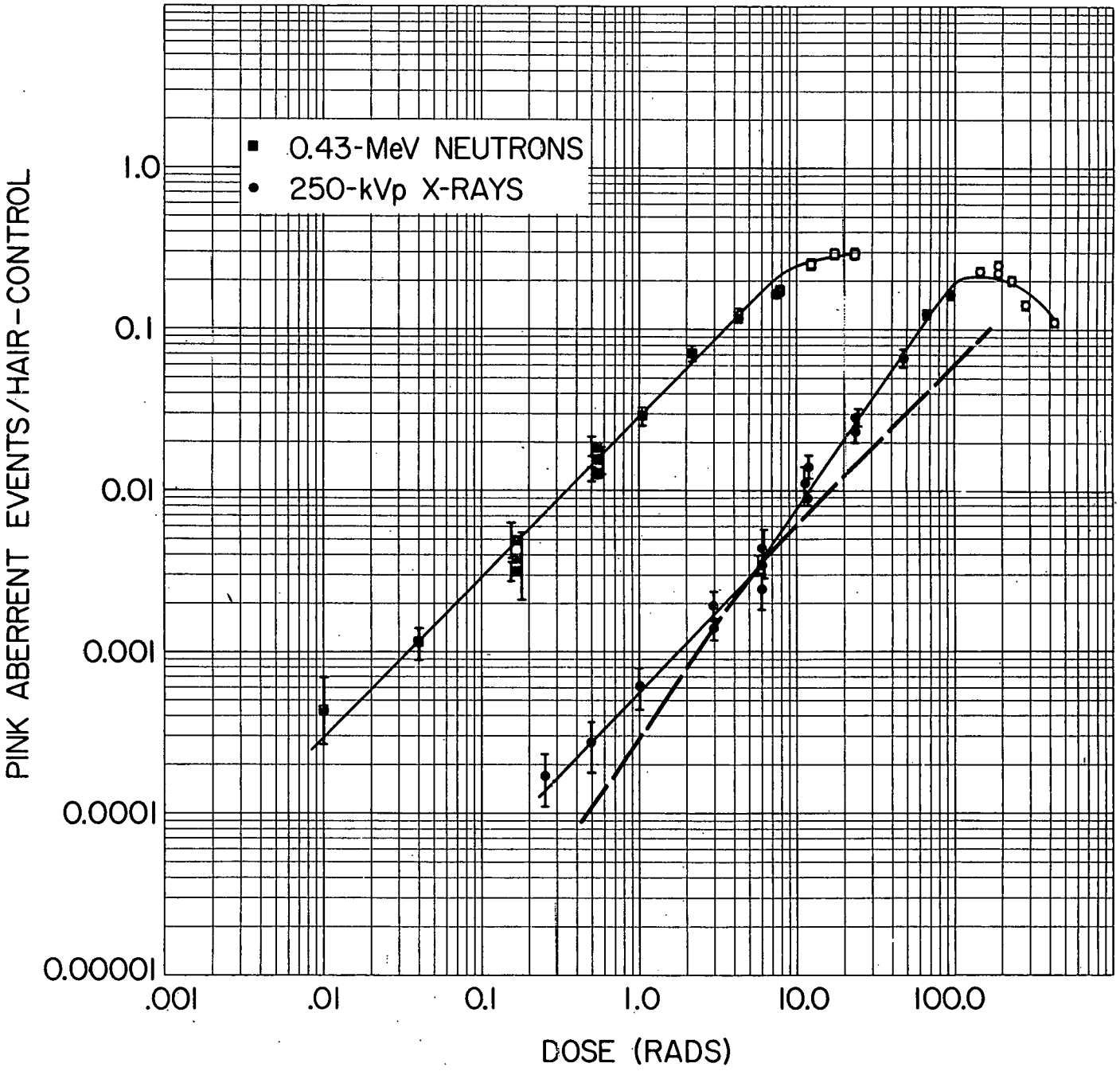
0.43-MeV NEUTRONS
250-kVp X-RAYS
PINK ABERRENT EVENTS/HAIR-CONTROL
1.0
0.1
0.01
0.001
0.0001
0.00001
.001
.01
0.1
1.0
10.0
100.0
DOSE (RADS)

FIGURE 1

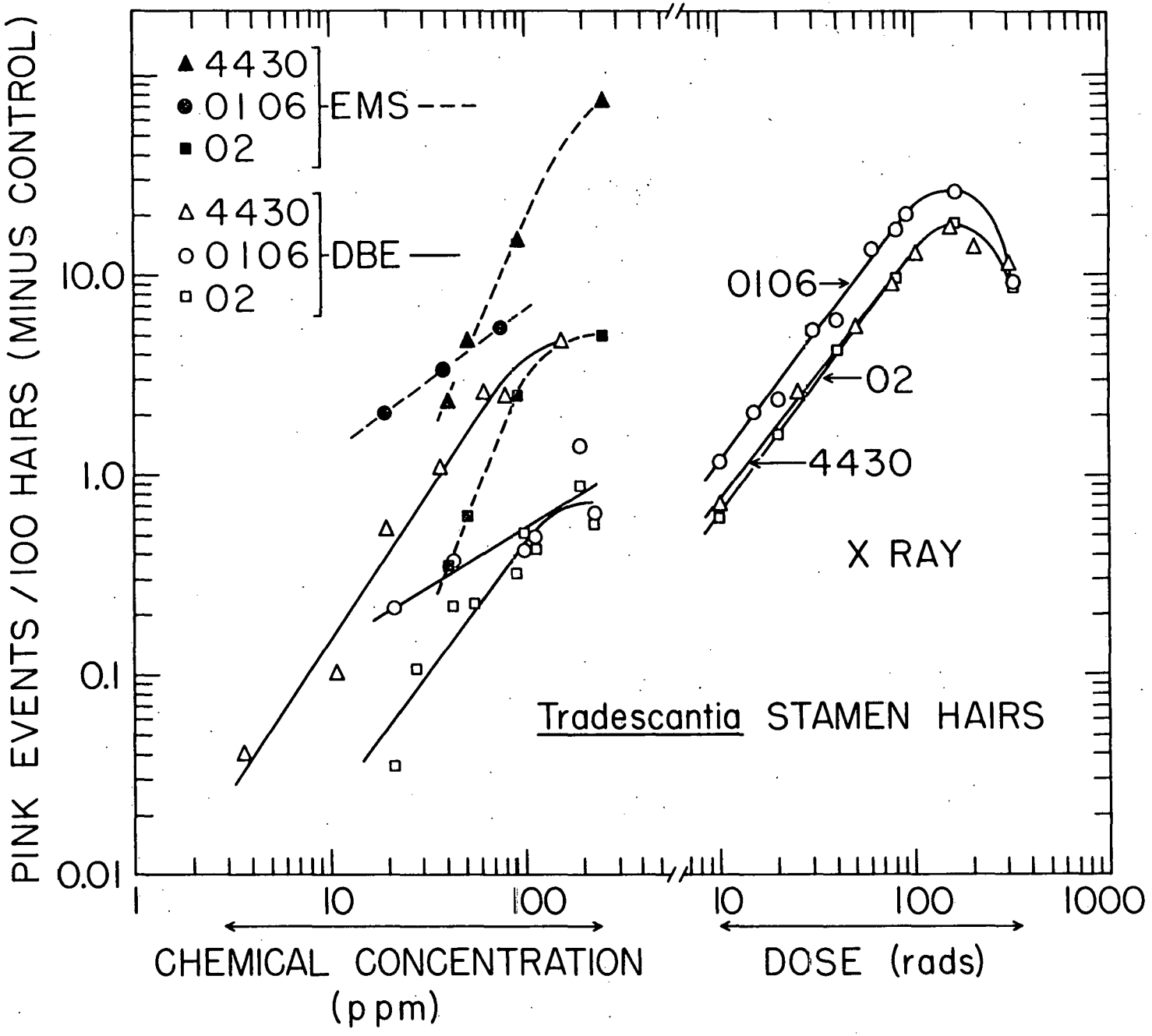
PINK EVENTS / 100 HAIRS (MINUS CONTROL)
10.0
1.0
0.1
0.01
▲ 4430
● 0106
■ 02
EMS ---
△ 4430
○ 0106
□ 02
DBE ——
0106
02
4430
X RAY
Tradescantia STAMEN HAIRS
1
10
100
10
100
1000
CHEMICAL CONCENTRATION
(ppm)
DOSE (rads)

FIGURE 2

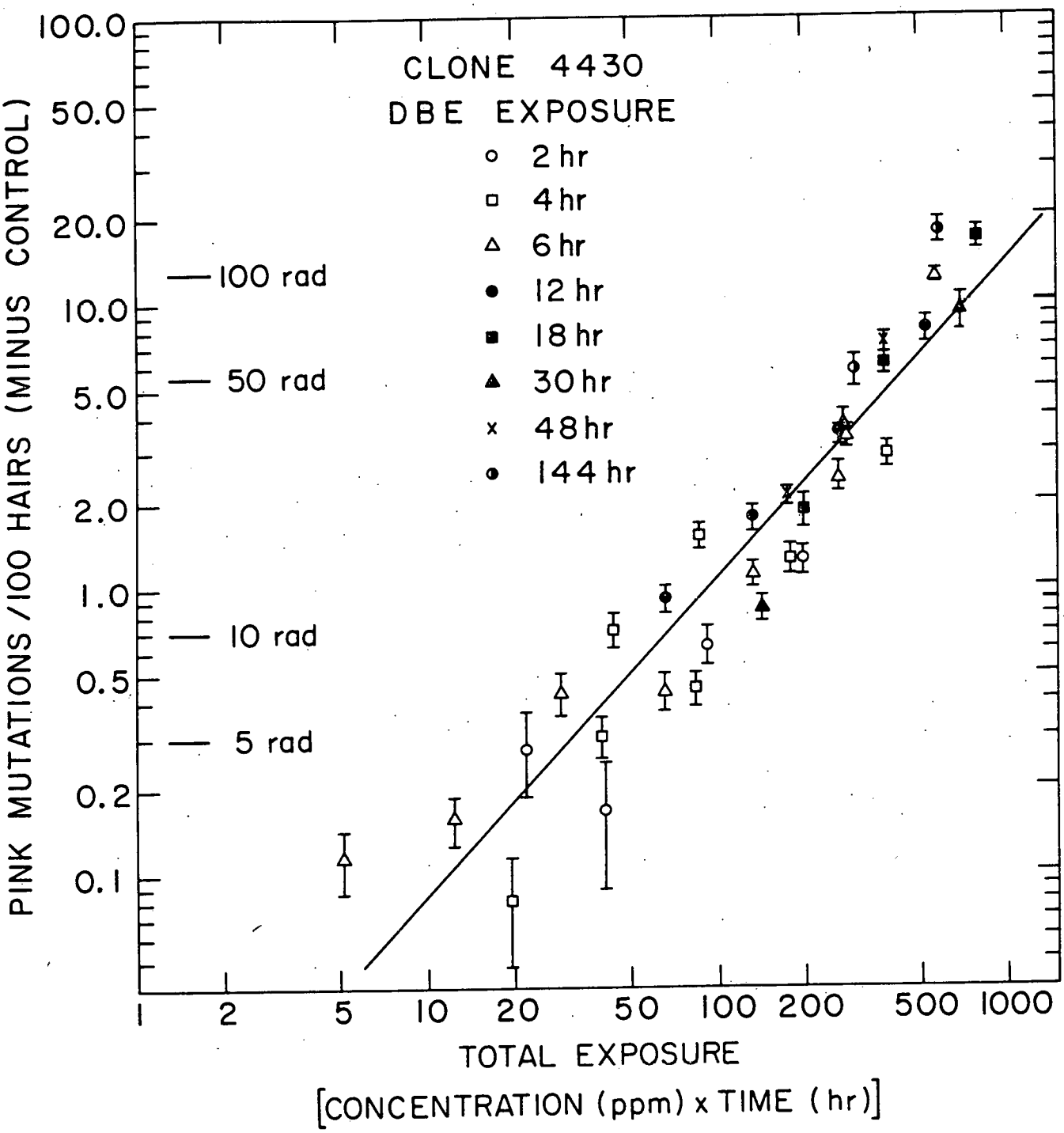
CLONE 4430
DBE EXPOSURE
2 hr
4 hr
6 hr
12 hr
18 hr
30 hr
48 hr
144 hr
100 rad
50 rad
10 rad
5 rad
PINK MUTATIONS / 100 HAIRS (MINUS CONTROL)
100.0
50.0
20.0
10.0
5.0
2.0
1.0
0.5
0.2
0.1
1
2
5
10
20
50
100
200
500
1000
TOTAL EXPOSURE
[CONCENTRATION (ppm) x TIME (hr)]

FIGURE 3