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Frequencies of Chromosomal Aberrations and Sister Chromatid Exchanges in the Benthic Worm *Neanthes arenaceodentata* Exposed to Ionizing Radiation

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FOREWORD

The objective of this study is to determine the feasibility of a cytogenetic approach to detect the effects of ionizing radiation in a marine worm Neanthes arenaceodentata. Such an approach may have applicability in evaluating the impact of the disposal of radioactive waste on marine ecosystems.

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FREQUENCIES OF CHROMOSOMAL ABERRATIONS AND SISTER CHROMATID EXCHANGES IN THE BENTHIC WORM *Neanthes arenaceodentata* EXPOSED TO IONIZING RADIATION

ABSTRACT

Traditional bioassays are unsuitable for assessing sublethal effects from ocean disposal of low-level radioactive waste because mortality and phenotypic responses are not anticipated. We compared the usefulness of chromosomal aberration and sister chromatid exchange (SCE) induction as measures of low-level radiation effects in a sediment-dwelling marine worm, *Neanthes arenaceodentata*. The SCEs, in contrast to chromosomal aberrations, do not alter the overall chromosome morphology and in mammalian cells appear to be a more sensitive indicator of DNA alterations caused by environmental mutagens.

Newly hatched larvae were exposed to two radiation-exposure regimes of either x rays at a high dose rate of 0.7 Gy (70 rad)/min for as long as 5.5 min or to ^{60}Co gamma rays at a low dose rate of from 4.8×10^{-5} to 1.2×10^{-1} Gy (0.0048 to 12 rad)/h for 24 h. After irradiation, the larvae were exposed to 3×10^{-5} M bromodeoxyuridine (BrdUrd) for 28 h (x-ray-irradiated larvae) or for 54 h (^{60}Co-irradiated larvae). Larval cells were examined for the proportion of cells in first, second, and third or greater division. Frequencies of chromosomal aberrations and SCEs were determined in first and second division cells, respectively.

Results from x-ray irradiation indicated that dose-related increases occur in chromosome and chromatid deletions, but a dose of $\geq$2 Gy ($\geq$200 rad) was required to observe a significant increase. Worm larvae receiving ^{60}Co irradiation showed elevated SCE frequencies with a significant increase at 0.6 Gy (60 rad).

We suggest that both SCEs and chromosomal aberrations may be useful for measuring effects on genetic material induced by radiation. However, more detailed studies on these responses and the factors affecting them are needed before either can be used to quantify the effects of the chronic exposure to low-level radiation that is received under field conditions.

INTRODUCTION

Since the onset of the nuclear age, radioactive wastes have been disposed on land and in the ocean. In addition, the testing of nuclear weapons has contributed measurable quantities of radionuclides to the oceans. In the U.S., low-level solid radioactive wastes were disposed in the coastal areas of both the Atlantic and Pacific Oceans (Joseph et al., 1971). Although these practices were discontinued by 1970, little effort was made until recently to determine the subsequent fate and distribution of radionuclides in these wastes. Information now available indicates that some man-made radionuclides from ocean disposal are present in bottom sediments but that there is little or no accumulation by organisms in man's food chain (Dyer, 1976; Noshkin et al., 1978).

Several countries have continued to dispose of radioactive wastes in the ocean. The use of oceanic waters for radioactive waste disposal is being considered currently in the U.S. because of special problems presented by land disposal of radioactive particulate waste (Meyer, 1979). Some opposition to oceanic disposal of nuclear waste has been based on fear of irreparable consequences to ocean ecosystems and on the continuing lack of empirical scientific data documenting the effects. This lack of data can be attributed in part to the absence of appropriate bioassays. Traditional bioassays that use mortality and phenotypic responses as end points are unsuitable for assessing the sublethal effects that may be expected from oceanic disposal of low-level radioactive waste.

The U.S. Marine Protection Research and Sanctuaries Act as amended requires that the Environmental Protection Agency (EPA) Administrator, in reviewing requests for permits, determine that ocean "dumping will not unreasonably degrade or endanger human health, welfare, or amenities, or the marine environment, ecological systems, or economic potentialities" (Marine Protection, Research, and Sanctuaries Act, 1972). This act requires the EPA to establish regulations and criteria to implement a permit program. One possible criterion would utilize a bioassay technique that requires a methodology for detecting the response of marine organisms to low levels of chronic irradiation. With such methodology, post-disposal monitoring could verify the assumptions regarding doses to marine organisms and evaluate ultimately the impact of radiation on the organisms. Such a monitoring scheme could theoretically serve as an early-warning system.

Deleterious effects of radiation on organisms are well documented (Templeton et al., 1976; U.S. National Academy of Science (NAS), 1980). Increased cell death and mutations have been related to increased radiation dose. Changes in genetic material include base damage, single-strand breaks, double-strand breaks, hydrogen-bond rupture, and cross-linking between DNA and proteins (Yu, 1976). Some lesions can be detected by

examining cells in metaphase for chromosomal aberrations and sister chromatid exchanges (SCEs). The yield of chromosomal aberrations in cells exposed to radiation (Blaylock and Trabelka, 1978) is much better documented than that for SCEs (Kato, 1979).

The chromosomal aberrations are caused by the breakage of chromosomes followed by either the subsequent rejoining of the broken ends to form new combinations or the failure of broken ends to rejoin. They are identified by changes in chromosomal structure that include deletions, translocations, and rings (Archer et al., 1981). It is well established that a substantial part of the changes in DNA induced by ionizing radiation consists of single- and double-strand breaks in the phospho-diester backbone of the DNA molecule. However, according to Evans (1977), all chromosomal aberrations do not result from one, two, or three specific lesions, but are caused by either a variety of changes in the DNA that lead to helix disruption, helix distortion, or interference with the normal replication process of the cell.

The SCEs represent the interchange of DNA replication products at apparently homologous loci (Latt et al., 1981). This exchange, which does not alter the overall chromosome morphology, was demonstrated first by autoradiographic techniques using tritiated thymidine (Taylor, 1958). Currently, these exchanges are distinguished by exposing cells to 5-bromodeoxyuridine (BrdUrd) for two rounds of replication and a combined staining with fluorochrome plus Giemsa (FPG) (Perry and Wolff, 1974). Data on SCE frequency in cells exposed to some physical and chemical agents indicate that in mammalian cell systems, SCEs are a sensitive indicator of DNA alterations caused by environmental mutagens and carcinogens.

The effects of ionizing radiation on the frequencies of SCEs have been studied in a number of cell systems exposed to either beta rays, beta plus x rays, x rays, or gamma rays (Table 1). Ionizing radiation resulted generally in increases in the baseline frequency of SCEs. However, for equivalent doses there was a greater increase in frequency of chromosomal aberrations than of SCEs. Very few data from these experiments are applicable to whole-animal, in vivo irradiation. Increases in SCEs were found following in vivo radiation of mice with x rays (Nakanishi and Schneider, 1979). In this study, as in the majority of those listed in Table 1, dose rates much greater than those expected at oceanic disposal sites were used.

Studies are required to further characterize the incidence of chromosomal aberrations and SCEs in organisms that have been irradiated with the doses and the long-term exposure regimes expected at radioactive waste-disposal sites. Nereidae worms are indigenous to marine disposal sites used by the U.S. in the past, and it is expected that they would be present in any future designated areas as well. Because they live in the benthos and do not migrate, they are well suited to studies of radionuclides and other contaminants that sorb to sediments.

Table 1. Irradiation conditions used in previous studies examining the frequencies of sister chromatid exchanges in cells exposed to different kinds of radiation.

Cell system	Radiation source	Total dose	Dose rate	Reference
Kangaroo rat cells (Pt-K1)	$\beta(^3H)$[a]	8-38 rad	0.3-1.4 rad/h	Gibson and Prescott (1972)
Chinese hamster cells (D-6)	$\beta(^3H)$	8-38 rad	0.3-1.4 rad/h	Kato (1974)
Chinese hamster cells (CHEF-125)	$\beta(^3H)$	27-3560 rad	0.8-99 rad/h	Marin and Prescott (1964)
	or $\beta(^3H)$	380-700 rad	11-19 rad/h	
	plus x ray	25-200 rad	50 rad/min	
Chinese hamster cells (CHEF-125)	$\beta(^3H)$	~80 & 400 rad	~3-14 rad/h	Gatti et al. (1974)
	plus x ray	175 rad	60 rad/min	
Chinese hamster cells (CHO)	x ray	50-80 rad	50 rad/min	Perry and Evans (1975)
Chinese hamster cells (CHO)	x ray	50-100 rad	100 rad/min	Yu (1976)
Human lymphocytes (normal & ataxia telangiectasia)	x ray	200 rad	50 rad/min	Galloway (1977)
Chinese hamster cells (CHO)	x ray	100-600 rad	450 rad/min	Livingston and Dethlefsen (1979)
Live mice	x ray	200-1500 rad	--[b]	Nakanishi and Schneider (1979)
Human lymphocytes	x ray	100-400 rad	--[b]	Morgan and Crossen (1980)
Syrian hamster embryo cells	x ray	300 R	32 R/min	Geard et al. (1981)
Mouse C3H/10T-1/2 cells	x ray	300 R	32 R/min	Geard et al. (1981)
Mouse 10T-1/2 cells	x ray	50-400 R	80 R/min	Nagasawa and Little (1981)
Syrian hamster embryo cells	x ray	200-500 R	126 R/min	Popescu et al. (1981)

Table 1. (Continued)

Cell system	Radiation source	Total dose	Dose rate	Reference
Chinese hamster cells (V79)	x ray	50-800 R	100 R/min	Renault et al. (1982)
Chinese hamster ovary cells	x ray	300 R	--[b]	Morgan et al. (1983)
Human lymphocytes	$\gamma(^{60}Co)$	50-150 R	300 R/min	Solomon and Bobrow (1975)
Human lymphocytes	$\gamma(^{60}Co)$	25-200 R	125 R/min	Abramovsky et al. (1978)
Human lymphocytes	$\gamma(^{60}Co)$	150-300 R	50 R/min	Littlefield et al. (1979)

[a] We estimated total doses and dose rates for $\beta(^{3}H)$ radiation from autoradiographic film grain-count data provided by the investigators. To calculate the β-radiation dose to a cell nucleus from ^{3}H incorporated into DNA, we assumed that there was 1.08 rad/ disintegration (Goodheart, 1961) and that 14 disintegrations were required to produce one grain count (Marin and Prescott, 1964).
[b] Dose rate not specified.

Pesch and Pesch (1980a) proposed that the marine polychaete Neanthes arenaceodentata be used as an in vivo cytogenetic model for marine genetic toxicology. N. arenaceodentata is very suitable for cytogenetic studies because it has 18 large chromosomes. This is in contrast to many invertebrates and fishes that have large numbers of small chromosomes. The effects of ionizing radiation on this species were assessed by quantifying the number of chromosomal aberrations induced by ^{60}Co radiation; at a dose rate of 7.5 R/h and a total dose of 180 R, an increase in chromosomal aberrations was found (Pesch et al, 1981). Also, a preliminary study on N. arenaceodentata was performed to determine the usefulness of SCE induction as a measure of low-level radiation effects (Harrison and Rice, 1981). Larvae exposed to ^{60}Co radiation at intermediate total doses of 10 to 60 R had SCE frequencies about two times that of the control larvae, but those exposed to higher total doses of 170 to 309 R had SCE frequencies that approximated those of the control larvae.

Our objective was to assess the feasibility of a cytogenetic approach to detect alterations from radiation in the genetic material of a marine organism. We evaluated the responses to irradiation by using the classical cytogenetic approach of quantifying the frequency of chromosomal aberrations, and by using the more recently developed technique of quantifying the frequency of SCEs. The responses of N. arenaceodentata to

radiation delivered at high dose rates (x rays) and low dose rates (^{60}Co) are evaluated as well as those to a known mutagen, mitomycin C (MMC). Results from these experiments will be used to determine what additional studies are required before cytogenetic changes in nereidae worms can be used to detect radiation effects at radioactive waste disposal sites. After establishing a dose-response relationship, we propose to validate assumptions regarding doses expected to be received by organisms in the field from potential disposal operations.

METHODS

WORM CULTURE AND HANDLING

Neanthes arenaceodentata were cultured following methods recommended by Dr. Donald Reish of the California State University at Long Beach (Reish, 1974). Mated pairs of adult worms were obtained from Dr. Reish and shipped through the U.S. mail in inflated plastic bags containing approximately 100 mL of seawater. The worms were shipped in the tubes they had constructed from the algae they were fed. Because shipping time seldom exceeded 3 d, worm mortality was low; only a [illegible] death occurred during all shipments.

On arrival at Lawrence Livermore National Laboratory (LLNL), each mated pair of adult worms was placed in a 4-L glass beaker. The adult worms that produced larvae used in experiments 1 through 6 were maintained using semistatic culture conditions; the water was aerated continuously and three-fourths of the volume in the beakers was exchanged weekly. Thereafter, adult worms were reared in 2-L beakers using flow-through conditions; flow rate through the beaker was 100 mL/min. Adult worms were maintained for 20 to 30 d in our laboratory before larvae were harvested. The mean culture temperature was 19.4 ± 1.4°C. The adult worms were fed frozen Enteromorpha sp. ad libitum and uneaten food was removed weekly.

The life cycle of this species is well known (Fig. 1) (Reish, 1957). Female worms die after laying eggs and the embryos are brooded by the surviving male. Hatching occurs 8 to 10 d following egg deposition. We harvested larvae 1 to 3 d after they hatched (3 to 5 setiger stage) by removing the intact worm tube containing the adult male and larvae from the beaker and gently aspirating the larvae from the walls of the tube with a large-bore plastic pipette. Harvested larvae were washed two times with seawater passed through a 0.45-μm- pore size Millipore filter.

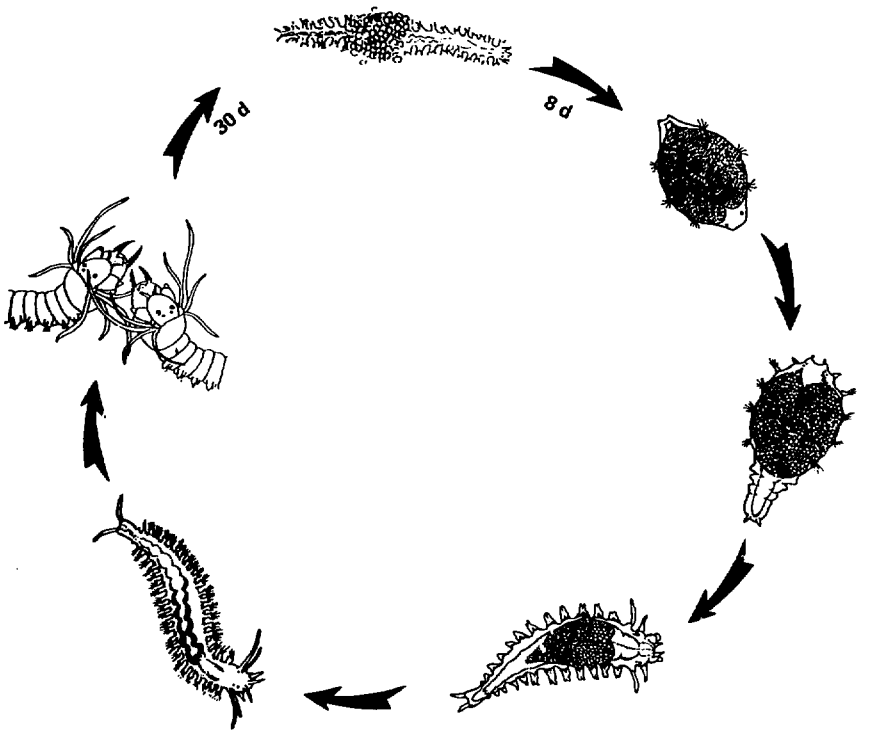

Figure 1. Life cycle Neanthes arenaceodentata (3 to 4 mo at 20 to 22°C).

The cleanliness of the glass and plastic ware was found to be an important factor in the success of experiments. Some disposable plastic ware is sterilized with ethylene oxide, which has been shown to increase SCEs in hospital workers (Garry et al., 1979). Because some larvae appeared to be sensitized by laboratory ware that was not rinsed or that contained residues of laboratory detergent, all containers used in the assay were rinsed 20 times in hot tap water and then air dried.

IRRADIATION

Larvae were irradiated with x rays generated in a 40-keV x-ray machine and delivered at 0.7 Gy (70 rad)/min for as long as 5.5 min and then examined for chromosomal aberration induction. The doses ranged from 0.08 to 3.8 Gy (8 to 380 rad) (Table 2). A 28-h BrdUrd exposure time was used to obtain the high proportion of first-division cells required for chromosomal aberration scoring. The irradiation was conducted in plastic 100- X 20-mm Petri dishes containing 10 mL of seawater (Fig. 2). Three thermoluminescent dosimeters (TLDs) were placed in the water along with the worms to determine the x-ray dose delivered.

Larvae were exposed to ^{60}Co for 24 h and total doses delivered were different (Table 3). Some larvae were examined for SCEs and chromosomal aberrations and others just for SCEs. All irradiations were conducted in our low-level radiation facility equipped with a 4.4×10^{10} Bq (1.25 Ci) ^{60}Co source. A 54-h BrdUrd exposure was used to obtain the high proportion of second-division cells required for scoring SCEs. For each exposure, 50 to 75 worm larvae harvested from 1 to 3 broods were placed in a cylindrical plastic chamber (2.5-cm diam) containing 30 mL of filtered seawater. A Plexiglas sheet (5 X 7 X 0.6 cm) was placed in front of each exposure chamber to ensure electron equilibrium (Fig. 2). Different dose rates and total doses were obtained by varying the distance between the chamber and the source. Delivered dose was determined from three TLDs placed behind each exposure chamber.

Two groups of control worms were tested during each experiment. Neither group was irradiated but one was treated with 5×10^{-7} M MMC, a drug known to increase the frequency of SCEs, and served as a positive control. Both controls were maintained in the exposure facility during the irradiation of the other groups of worms.

CYTOGENETIC PREPARATION AND SCORING OF WORM CHROMOSOMES

Immediately following irradiation for experiments 1 to 3, each treatment group of worm larvae was transferred under amber light to 100- X 20-mm plastic culture dishes; a large-bore plastic pipette was used to make the transfer. Each dish contained 30 mL of

Table 2. The x-ray doses delivered to Neanthes arenaceodentata larvae.

Experiment	X-ray dose (Gy)[a]								
	0.08	0.18 to 0.24	0.37	0.45 to 0.48	0.88 to 1.0	1.6 to 1.7	2.0 to 2.2	2.5 to 2.6	3.6 to 3.8
1				X	X				
2	X	X		X					
3								X	
4		X		X					
5		X	X						
6					X				
7							X		
8							X		
9						X			
10		X							
11									X
12					X	X		X	X

[a] Dose rate was 0.7 Gy /min. One gray (Gy) is equivalent to 100 rads.

3 X 10^{-5}M BrdUrd in filtered seawater. The same procedure was followed in experiments 4 to 30, except 50 mL of seawater in 100-mL glass beakers were used. Nonirradiated control groups were also transferred to the same concentration of BrdUrd or to BrdUrd plus 5 X 10^{-7}M MMC. The BrdUrd and MMC exposures were carried out in the dark, and colchicine (final concentration of 0.4 mg/mL) was added to the seawater 4 h before the termination of the BrdUrd exposure. Colchicine is a microtubule disruptor that results in the accumulation of cells in metaphase.

We generally followed the method of harvest of larvae and preparation of larval tissue developed by Pesch and Pesch (1980b). Larvae were transferred to 15-mL conical plastic tubes, the seawater decanted, and 10 mL of 0.075M potassium chloride added. After 12 min, this solution was decanted and the larvae were fixed in three changes of methanol plus acetic acid (3:1). The first fixative change was performed after 5 min, and the remaining changes after 15 min each. Fixed larvae (50 to 75) were placed in a depression of a ceramic spot dish and mashed twice with broad-tipped forceps. Next, 1 mL of 60% acetic acid was added, and the mixture was mashed continuously for an

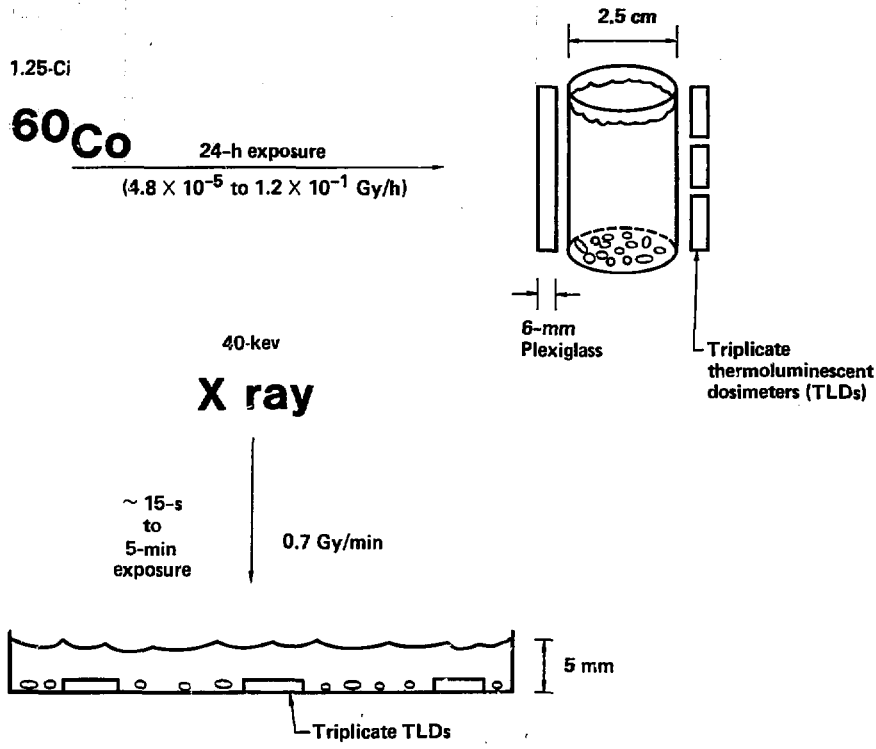

Figure 2. Exposing larvae to x rays and ^{60}Co irradiation.

additional minute. Two drops of the worm tissues suspended in acetic acid were deposited on the end of a clean microscope slide held at 45°C. Using a disposable plastic pipette, we made 10 to 15 successive transfers of the original drops of tissue suspension to clean areas of the slide. This process resulted in the deposition of cells in a series of rings along the length of the slide. The slides were dried at 45°C before staining. Generally, 4 slides could be made from the macerated tissues of 50 to 75 worm larvae. The best spreading of chromosomes was ensured by preparing the slides within 1 h of the start of fixation.

Differential staining of the sister chromatids was accomplished essentially according to the procedure described by Minkler et al. (1978) (Fig. 3). Preparations were first stained for 10 min in 5-µg/mL Hoechst 33258 solution (Aldrich Chemical Company, Inc., Milwaukee, WI) in 0.9% sodium chloride (pH 6). Hoechst-stained slides were rinsed for

Table 3. The ^{60}Co doses delivered to Neanthes arenaceodentata larvae.

	^{60}Co dose (Gy)[a]						
Experiment	0.001	0.01	0.1	0.3	0.6	1.7	3.0
13				X	X	X	X
14				X	X	X	X
15			X	X	X		
16				X			
17				X	X		
18				X	X		
19				X	X		
20[b]					X		
21[b]				X	X		
22	X						
23	X						
24	X						
25	X						
26		X					
27			X				

[a] One gray (Gy) is equivalent to 100 rads.
[b] Scored for both chromosomal aberrations and sister chromatid exchanges.

5 min in distilled water and air dried for at least 20 min. They were next placed in a shallow, clear plastic tray and covered with 0.067M phosphate buffer (pH 6.8) to a depth of 5 mm. Slides were then exposed to UV light in an M-99 printer (400-W General Electric mercury lamp from Colight, Inc., Minneapolis, MN) for 45 min. They then were transferred to 10% Giemsa stain in 0.067M phosphate buffer (pH 6.8) for 6 to 10 min, air dried, and mounted with Permount (Fisher Scientific Company, Fairlawn, NJ). Worm tissue fixation, slide preparation, and staining were all carried out under amber light.

Slides were scored by scanning the entire slide using a Zeiss Universal microscope (Carl Zeiss, Inc., Oberkochen, West Germany) equipped with a 10X objective, 63X objective, 1.25X optovar, and 12.5X oculars.

The proportions of metaphases identified as first, second, and third divisions after the beginning of BrdUrd exposure were recorded. First- and second-division metaphases were examined for the number of chromosomal aberrations and SCEs per metaphase,

Figure 3. Metaphase chromosomes from irradiated larvae of Neanthes arenaceodentata stained to visualize sister chromatid exchanges.

respectively. The number of chromosomes scored was recorded for all metaphases examined. Data for chromosomal aberrations were recorded only for cells that had 17 or 18 chromosomes that could be scored; data for SCEs were recorded for those that had 15 to 18 chromosomes that could be scored.

QUALITY ASSURANCE

All slides were scored blind, and four people performed the scoring. The results of the comparative scoring of experiments are summarized in Appendix A. In addition to a control population that received no irradiation, a positive control was run using larvae that received no irradiation but were exposed to a concentration of 5×10^{-7} M MMC for 54 h before they were harvested.

RESULTS

CHROMOSOMAL ABERRATIONS

Almost all chromosomal aberrations induced by x rays in cells of N. arenaceodentata larvae were chromosome and chromatid deletions and gaps. An aberration was classified as a deletion when the fragment was displaced and when all undisplaced fragments were separated by a non-staining region equal to or greater than one chromatid width. If the non-staining region was less than a chromatid width or did not extend across the chromatid and was not displaced, it was scored as a gap. However, only data on chromosome and chromatid deletions are included because the scoring of gaps is subjective and not generally reported in the literature.

The frequency of chromosome and chromatid deletions in cells that received no irradiation was low (Table 4). The mean chromosome and chromatid deletion per cell for the 14 control (zero dose) experiments was 0.06, and the individual means from the experiments ranged from 0.00 to 0.22. Differences in the frequencies of chromosome and chromatid deletions reported by different scorers and for different slides were tested for homogeneity of binomial proportions using Cochran's test (Snedecor and Cochran, 1967). There was excellent agreement between scorers when the same slides were scored independently; all differences were easily accounted for by binomial sampling variability. There was also good agreement in frequencies reported for different slides within the same experiment, with differences not exceeding those expected from binomial sampling. In contrast to these results, there was significant heterogeneity among the chromosome and chromatid deletion rates from different experiments ($p = 0.0016$ based on Cochran's test for homogeneous binomial proportions, Snedecor and Cochran, 1967, p. 240).

The frequency of chromosome and chromatid deletions was determined also in cells exposed to x rays (Table 5, Fig. 4). Weighted (by the number of cells scored) least-squares linear regression was used to determine if there was a linear relationship between dose and chromosome and chromatid deletion rate. The regression is highly significant ($F = 76.6$ with 1 and 14 degrees of freedom, $p < 0.01$), and the estimated slope is 0.094 chromosome and chromatid deletion per cell per gray of radiation (standard error = 0.017). However, a prediction based on the least-squares best-fit line is not very reliable because of the extreme heterogeneity of the responses at each dose level. Nevertheless, it appears that doses above 2.0 Gy lead to increased frequencies of chromosome and chromatid deletions.

In the experimental cells scored, the number of chromosome and chromatid deletions per individual cell ranged from one to greater than four. The percentage of the total chromosome and chromatid deletions that occurred singly (one per cell) and multiply

Table 4. Mean frequency per cell of chromosome and chromatid deletions in cells from Neanthes arenaceodentata larvae that received no radiation. Larvae were harvested after a 28-h exposure to BrdUrd.

Experiment	Number of scorers[a]	Cells scored[b]	Deletions scored[b]	Deletion per cell
1	3	74	0	0.00
2	3	33	0	0.00
3	3	62	2	0.032
4	3	28	6	0.21
5	1	30	1	0.033
6	3	64	5	0.078
7	3	50	3	0.060
8	2	51	11	0.22
9	1	9	0	0.00
10	3	11	2	0.18
11	2	41	2	0.049
12	1	89	5	0.056
20	1	16	1	0.062
21	3	95	1	0.010

[a] Four scorers were available for the experiments.
[b] For slides scored by more than one person for an individual experiment, the number of cells and chromosome and chromatid deletions scored were averaged.

(more than one per cell) was compared to the total dose delivered (Table 6). The fraction of the total chromosome and chromatid deletions scored that occurred as more than one chromosome and chromatid deletion per cell did not appear to be dose related.

Limited data are available on the frequencies of chromosome and chromatid deletions induced in worm larvae exposed to low dose rates of ^{60}Co (Table 7). All larvae were held in BrdUrd for 28 h after exposure to the different total doses. Over the range tested, no significant increases in chromosome and chromatid deletions were found.

Table 5. Mean frequency per cell of chromosome and chromatid deletions in cells from Neanthes arenaceodentata larvae exposed to x rays at 0.7 Gy (70 rad)/min. Larvae were harvested after a 28-h exposure to BrdUrd that followed the irradiation.

Dose (Gy)	Experiment	Number of scorers[a]	Cells scored[b]	Deletions scored[b]	Deletion per cell
0.08	2	1	41	2	0.049
0.18	5	1	30	2	0.067
0.19	4	1	54	2	0.037
0.22	10	1	29	3	0.10
0.24	2	1	40	8	0.20
0.37	5	1	109	4	0.037
0.45	4	2	22	1	0.045
0.47	2	2	28	2	0.071
0.48	1	2	112	12	0.11
0.88	6	2	66	5	0.076
0.88	12	2	95	7	0.074
1.0	1	2	57	10	0.18
1.6	8	3	12	2	0.17
1.7	12	3	52	7	0.13
2.0	7	3	21	12	0.57
2.2	8	3	61	16	0.26
2.5	3	3	48	36	0.75
2.6	12	2	51	11	0.22
3.6	11	3	34	12	0.35
3.8	12	2	56	14	0.25

[a] Four scorers were available for the experiments.
[b] For slides scored by more than one person for an individual experiment, the number of cells and chromosome and chromatid deletions scored were averaged.

SISTER CHROMATID EXCHANGES

The frequency of SCEs in cells from worm larvae exposed to BrdUrd for 54 h, but not to radiation, was determined in 18 different experiments. The mean SCE frequency per chromosome for the individual experiments ranged from 0.096 to 0.38 (Table 8). These experiments were performed over 17 mo, and occasionally, larvae were harvested after

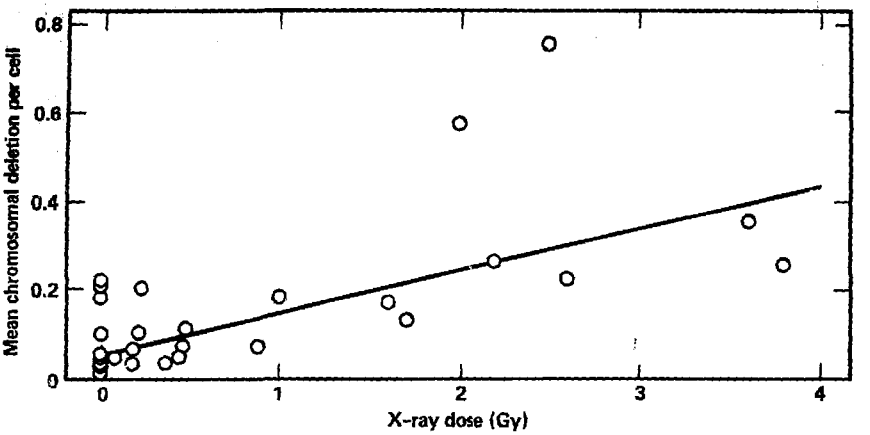

Figure 4. Mean frequencies per cell of chromosome and chromatid deletions in cells from Neanthes arenaceodentata larvae exposed to x rays at 0.7 Gy (70 rad)/min. Larvae were harvested after a 28-h exposure to BrdUrd that followed the radiation. The regression was highly significant ($p<0.01$) and the estimated slope of the regression line is 0.094 chromosome and chromatid deletion per gray (standard error = 0.017).

varying BrdUrd exposure times (including 54 h). Before the SCE frequency data on control cells were pooled to compile control baseline values for SCE frequencies, the effect of cell-cycle time on SCE frequency was assessed. Using data from larvae harvested at times ranging from 28 to 66 h, we determined that the cell-cycle time was about 28 h and had little effect on SCE frequency (Appendix B).

An analysis of individual results (not shown) revealed that at zero dose, the experiment-to-experiment variability was large compared with that between scorers and slides. The standard deviation from scorer to scorer was 0.033, from slide to slide it was 0.037, and from experiment to experiment it was 0.056.

Cellular SCEs tended to follow a skewed distribution with medians consistently lower than mean SCEs because of the presence of variable numbers of high-frequency cells (HFCs) (Fig. 5). The HFC is defined by pooling all SCEs from the controls (1059 cells from 18 experiments) and finding, in our case, the 90th percentile of this pooled distribution. To have 95% confidence that the estimated percentile will, in fact, contain

Table 6. Percentage of total chromosome and chromatid deletions occurring singly or multiply in cells of Neanthes arenaceodentata larvae exposed to x rays at 0.7 Gy (70 rad)/min. Larvae were harvested after a 28-h exposure to BrdUrd that followed the irradiation.

Dose (Gy)	Cells scored	Number of deletions	Occurance of deletions per cell (%)			
			1 del.	2 dels.	3 dels.	≥ 4 dels.
Control	643	39	63	21	16	0
0.08	41	2	100	0	0	0
0.18 to 0.24	153	15	60	40	0	0
0.37	109	4	100	0	0	0
0.45 to 0.48	164	15	60	13	0	27
0.88 to 1.0	228	22	60	24	0	16
1.6 to 1.7	62	9	57	0	0	43
2.0 to 2.2	82	28	63	15	22	0
2.5 to 2.6	99	47	42	20	38	0
3.6 to 3.8	90	26	56	25	0	19

90% of the cells, we used the nonparametric procedure described by Walsh (1962) and found the kth-largest SCE value from the pooled sample, where k is given by

$$k = 1059(1.0 - 0.90) + 0.5 - 1.645\sqrt{1059(0.90)(0.10)}$$

where 1059 is our sample size and 0.90 is the percentile expressed as a fraction. (The 1.645 comes from the 95th percentile of the standard normal distribution.) The 90th-largest SCE frequency in our sample is 0.44 SCE per chromosome. Thus, we define an HFC as a cell with more than 0.44 SCE per chromosome.

There is clearly variability in the number of HFCs in our control samples. Fortunately, the means of the SCEs are reasonably normally distributed with an overall mean (weighted by number of cells scored) of 0.19 SCE per chromosome and a weighted standard deviation of the means of 0.056. Normality of the means was tested using Filliben's order statistic correlation test; a value of 0.988 was obtained, which is well above the 5% critical value of 0.938 (Filliben, 1975).

Worm larvae exposed to different doses of ^{60}Co and then examined for SCE induction after a 54-h exposure to BrdUrd had mean frequencies of SCEs that varied with the total

Table 7. Frequency of chromosome and chromatid deletions in cells from Neanthes arenaceodentata larvae exposed to ^{60}Co for 24 h. Larvae were harvested after a 28-h exposure to BrdUrd that followed the irradiation.

Experiment	Dose (Gy)[a]	Cells scored	Deletion per cell
19	0.6	12	0.072
20	0.6	2	0.045
21	0.6	5	0.072
			0.054[b]
19	0.3	11	0.035
21	0.3	11	0.027
			0.030[b]
19	Control	18	0.022
20	Control	1	0.054
21	Control	12	0.033
			0.028[b]

[a] One gray (Gy) is equivalent to 100 rads.
[b] Mean weighted by the number of cells scored.

dose (Table 9). Analysis of results from individual experiments conducted at comparable doses revealed again that there was significant variability from experiment to experiment. This was caused mainly by significantly high proportions of HFCs in a few of the experiments.

The frequency of SCEs in cells from larvae receiving 0.6 Gy of ^{60}Co radiation is clearly different from those not receiving radiation (Fig. 6). The frequency distribution is similar to that of larvae exposed to MMC; both groups are characterized by having increased incidences of HFCs.

A least-squares linear regression of mean SCE on radiation dose was performed (Fig. 7). When weighted (by number of cells scored) linear regression is performed for all doses, the slope of the best-fit line is not significantly different from zero. This is caused by the low responses at the two highest doses. When these data pairs are omitted, a significant ($p = 0.025$) slope results (0.241 ± 0.008 increase in SCE per chromosome per gray). Again, the best-fit line is not very useful for predicting responses because of the large experiment-to-experiment variability. However, the significant regression indicates that over this range of radiation there is a general rise in SCE frequency with increased radiation dose. A significant increase in SCE frequency occurred at 0.6 but not at 0.3 Gy

Table 8. Mean and median sister chromatid exchanges per chromosome (SCEs/C) in cells from Neanthes arenaceodentata larvae that were not irradiated (control). Larvae were harvested after a 54-h exposure to BrdUrd.

Experiment	Cells scored	Mean SCEs/C	Standard deviation	Median SCEs/C	Percent HFCs[a]
13	24	0.096	0.08	0.094	0
14	18	0.20	0.22	0.14	16.7
15[b]	21	0.27	0.22	0.20	14.3
16	33	0.13	0.10	0.13	0
17	65	0.22	0.15	0.18	4.6
18	52	0.30	0.20	0.25	26.9
19	40	0.18	0.19	0.13	7.5
20	9	0.19	0.15	0.18	11.1
21	12	0.13	0.09	0.11	0
22	32	0.17	0.14	0.12	3.1
23	52	0.12	0.10	0.089	0
24	21	0.22	0.25	0.17	9.5
25	24	0.26	0.24	0.17	20.8
26	67	0.15	0.12	0.11	1.5
27	73	0.18	0.13	0.17	4.1
28	20	0.38	0.51	0.17	20
29	22	0.18	0.17	0.17	9.1
30	18	0.17	0.32	0.063	11.1

[a] Percentage of high-frequency cells (HFCs) (cells with more than 0.44 SCE per chromosome).
[b] Larvae harvested after a 48-h exposure to BrdUrd.

(Bonferroni t-test adjusted for seven multiple comparisons; p = 0.0003) (Miller, 1966). The absence of a significant difference from control at 0.3 Gy may be a false negative. A difference may have been detected if the sample were larger.

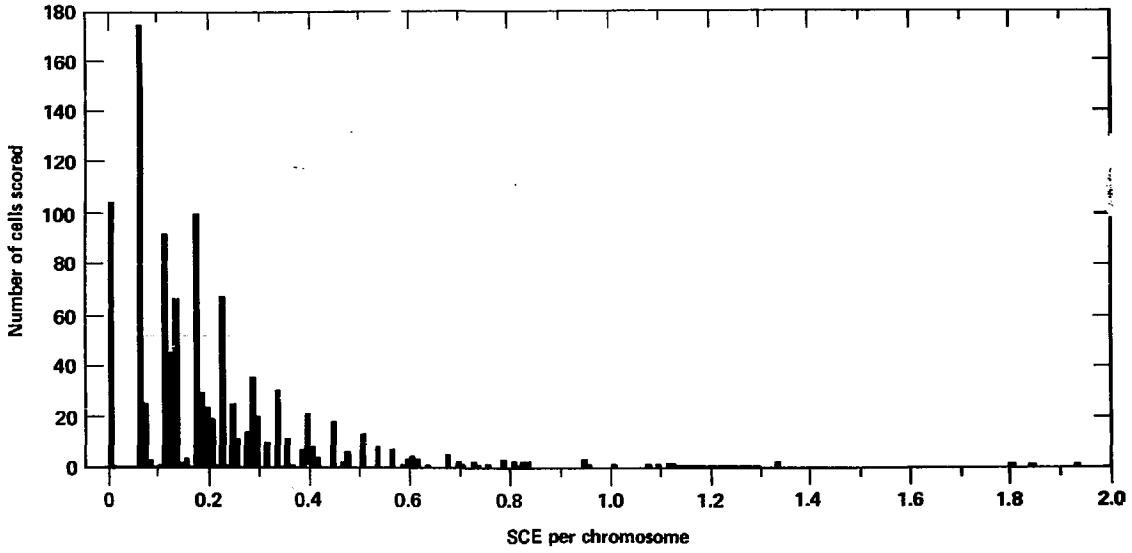

Figure 5. The SCE frequency distribution in Neanthes arenaceodentata larvae that were not irradiated (control). Larvae were harvested after a 54-h exposure to BrdUrd.

Table 9. Mean and median sister chromatid exchanges per chromosome (SCEs/C) in cells from Neanthes arenaceodentata larvae irradiated with ^{60}Co. Larvae were harvested after a 54-h exposure to BrdUrd that followed the irradiation.

Dose (Gy)[a]	Experiment	Mean SCEs/C	Standard deviation	Median SCEs/C	Cells scored	Percent HFCs[b]
0.001	22	0.22	0.22	0.17	81	12
0.001	23	0.17	0.13	0.13	42	2.4
0.001	24	0.16	0.15	0.11	29	6.3
0.001	25	0.17	0.14	0.12	10	0
0.01	26	0.17	0.14	0.17	152	5.3
0.1	15	0.42	0.34	0.44	25	32
0.1	27	0.17	0.19	0.12	140	5.7
0.3	13	0.55	0.49	0.47	24	50
0.3	14	0.16	0.18	0.11	15	13
0.3	15	0.34	0.31	0.19	25	32
0.3	16	0.12	0.11	0.11	49	2
0.3	17	0.23	0.23	0.12	19	10
0.3	18	0.30	0.27	0.26	12	25
0.3	19	0.17	0.22	0.13	32	3.1
0.3	21	0.20	0.22	0.12	22	14
0.6	13	0.41	0.54	0.13	25	24
0.6	14	0.20	0.28	0.06	22	18
0.6	15	0.47	0.39	0.33	32	41
0.6	17	0.37	0.22	0.39	11	36
0.6	18	0.32	0.36	0.22	17	24
0.6	19	0.15	0.09	0.12	23	0
0.6	20	0.46	0.60	0.24	15	33
0.6	21	0.25	0.22	0.20	6	17

Table 9. (Continued)

Dose (Gy)[a]	Experiment	Mean SCEs/C	Standard deviation	Median SCEs/C	Cells scored	Percent HFCs[b]
1.7	13	0.24	0.21	0.16	10	10
1.7	14	0.15	0.12	0.13	13	0
3.0	13	0.24	0.40	0.17	23	4.3
3.0	14	0.18	0.27	0.17	31	6.5

[a] One gray (Gy) is equivalent to 100 rads.
[b] Percentage of high-frequency cells (HFCs) (cells with more than 0.44 SCE per chromosome).

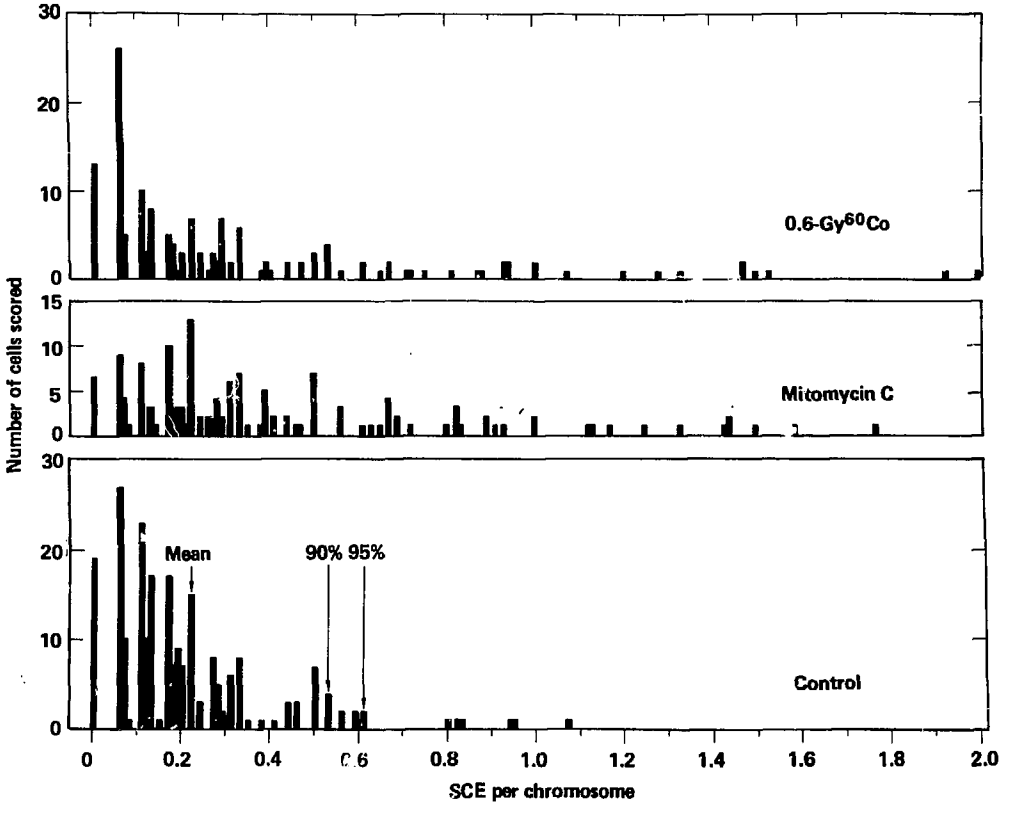

Figure 6. The SCE frequency distributions in Neanthes arenaceodentata larvae that received 0.6 Gy (60 rad) of ^{60}Co radiation, were treated with 5×10^{-7} M mitomycin C, or received no irradiation. Larvae were harvested after a 54-h exposure to BrdUrd that followed the treatment.

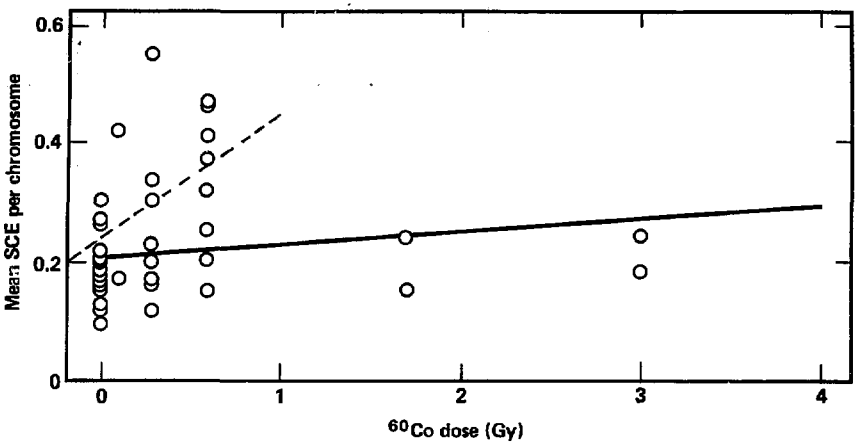

Figure 7. The mean SCE per chromosome in cells from Neanthes arenaceodentata larvae that had been irradiated with ^{60}Co. Larvae were harvested after a 54-h exposure to BrdUrd that followed the radiation. The solid line shows the regression of all mean SCE per chromosome on dose and the slope is not significantly different from zero. The dotted line shows the regression of mean SCE per chromosome on dose for doses <1 Gy and the slope is significant (P = 0.025) and is 0.241 ± 0.008 increase in SCE per chromosome per gray.

DISCUSSION

CHROMOSOMAL ABERRATIONS

The induction of chromosomal aberrations by irradiation has been demonstrated both in vivo and in vitro in many mammals and in mammalian cell systems (Gebhart, 1981). Although the response to radiation was dose related in mammalian systems, the rate of induction differed with the test system and the cell-cycle at the time of irradiation. In the worm larvae, we found that the response was dose related also. The chromosomal aberrations that we quantified, chromosome and chromatid deletions, are one-break aberrations that are considered to be induced linearly with dose. However, our studies on the background levels of chromosomal aberration induction indicate that in worm larvae, as in mammalian systems, the incidence of chromosomal aberrations is low. Neither the incidence of true point (intragenic) mutations, which are base-pair changes or frame shifts

in DNA, nor gross chromosomal (polygenic) mutations, which are brought about by the breakage of chromosomes, is easily quantified at low radiation doses. This is because very few of these incidents are found and it is difficult to get a significant increase over background values unless large numbers of cells are scored.

SISTER CHROMATID EXCHANGES

The induction of SCEs is a sensitive indicator of changes due to mutagenic chemicals. However, the data available on mammalian cells indicate that the response is more sensitive to chemicals than to ionizing radiation. Our results for worm larvae show that a response that was significantly different from controls was obtained at lower doses, using the SCE frequency, than by using the chromosomal aberration frequency as an endpoint. However, the SCE response to doses $\geq$1.7 Gy (170 rad) was not related to dose; the frequency of SCEs appeared to plateau or decline.

A plateau in the SCE induction rate in mammals was found for beta-radiation doses (Gibson and Prescott, 1972). More recently, however, Nagasawa and Little (1981) reported a dose-related response in SCE frequencies in the density-inhibited plateau phase of cultures of mouse 10T-1/2 cells irradiated with x rays, but commented that the relationship of the induction of SCE to total dose was more complex and very different from that for the production of chromosomal aberrations. They reported that the dose-response curve for SCEs increased linearly up to 100 rad (1 Gy), then declined with increasing doses. At the doses on the exponential portion of the survival curve (>200 rad, or 2 Gy), the frequency of induced SCEs declined rapidly. For 400 rad (4 Gy), the SCE frequency was only 20 to 30% higher than the baseline (spontaneous frequency) compared to the twofold increase induced by 100 rad (1 Gy).

Furthermore, the induction of SCE was also related to the repair interval; there was a rise in SCE with repair intervals up to 4 h, followed by a decline at later times. They noted also that the mean frequencies of SCEs and their distribution among cells showed little change during repair periods in the sublethal dose range (50 to 100 rad, or 0.5 to 1 Gy), but did change during the repair period at the higher doses to greater numbers of cells with high frequencies of SCEs. These investigators propose that these phenomena occur as a result of the kinetics of repair and of cell survival.

The existence of a plateau or a decline at high doses in SCE induction may limit the range of doses over which SCEs in N. arenaceodentata could be used as an indicator of in vivo environmental exposure to radiation. However, this would not be expected to be a problem at the doses expected at low-level waste disposal sites.

Factors that may affect SCE incidence in benthic worms include the following:

- cell-cycle stage at the time of irradiation,
- length of time induced SCEs are retained in cells, and
- life-history stage at the time of irradiation.

Some of these were shown to be important in mammalian cell culture systems and may also be important in our in vivo worm bioassay.

From data on synchronously dividing cultures of mammalian cells, it is known that the induction of SCEs and chromosomal aberrations by x rays is dependent on the cell-cycle stage during irradiation. For SCE induction, S is the most sensitive cell-cycle stage (Yu, 1976; Morgan and Crossen, 1980). For chromosomal aberration induction, G_2 is the most sensitive stage (Carrano, 1975; Yu, 1976). Because N. arenaceodentata larvae represent a nonsynchronously dividing complex population of cells and our irradiations were during several cell-cycle stages, the role that changes in radiosensitivity during cell-cycle or for differentially responsive subpopulations of cells might play in our observed SCE frequencies is unclear.

Another factor to be considered is the length of time that induced SCEs are retained in the cells. In vivo irradiated mice that received BrdUrd 26 h after irradiation still showed an increased SCE incidence (Nakanishi and Schneider, 1979). Experiments to examine changes in SCE incidence with time following the irradiation of worms would provide data relevant to possible influences conservation of SCEs might have on SCE incidence.

A final factor is the effect of life-history stage on observed SCE frequencies. Worm larvae contain populations of rapidly dividing cells, whereas adult worms may contain primarily slowly dividing cells. Nondividing human lymphocytes exposed to x rays showed no increase in SCE incidence (Galloway, 1977; Littlefield et al., 1979; Morgan and Crossen, 1980), while similar populations of cells induced to divide and then irradiated showed significant increases in SCE frequencies (Galloway, 1977; Morgan and Crossen, 1980). If measurements of SCE frequency in chronically exposed animals are conducted on adult rather than larvae worms, then dose response for adults must be established.

FEASIBILITY OF CYTOGENETIC METHODOLOGY

The feasibility of using cytogenetic endpoints to measure low-level radiation effects required (1) identification of an appropriate cytogenetic model and (2) obtaining a response with the model organism that is related to the dose delivered. Our results indicate that N. arenaceodentata is a good cytogenetics model. Both chromosome and

chromatid deletions and SCEs are induced by ionizing radiation delivered in vivo to N. arenaceodentata. The induction of chromosome and chromatid deletions by x rays delivered at 0.7 Gy (70 rad)/min was dose related, but doses $\geq$2 Gy (200 rad) were required to get a response that was significantly different from background levels. The induction of SCEs by ^{60}Co delivered at total doses of $\leq$0.6 Gy (60 rad) appeared to be dose related, but at higher doses it was not; only the frequency of SCEs induced by 0.6 Gy (60 rad) was significantly different from that of controls.

If variability in cytogenetic response is so high that a large amount of data is required to establish a difference between control animals and those at a radioactive dump site, this method will not be cost effective. Therefore, we made a concerted effort to identify variability sources in the bioassay as well as to understand the process of induction of chromosomal aberrations and SCEs. We investigated factors that could potentially alter their frequency and concentrated our efforts on SCE induction, because a response that was significantly different from controls was obtained at a lower dose for SCEs than for chromosomal aberrations.

Some of the factors we investigated were:

- baseline SCE frequency per chromosome,
- cell-cycle time in larvae,
- effect of cell-cycle time on SCE induction,
- among and between experiment variability, and
- between scorer variability.

The results on SCE were analyzed using standard statistical procedures as well as methods developed by LLNL personnel for application to SCE distribution.

Our data show that there is great variability in the baseline SCE frequency in worm larvae; the greatest variability was between experiments. Variability was reported also in mammalian systems (Carrano and Moore, 1982). Some of the variability in an unexposed reference population can be attributed to differences in incorporation of BrdUrd used to visualize the SCEs, to differences in the cell's repair capacities, and to other unknown inherent differences (Carrano et al., 1980). Increased sensitivity of our bioassay could be obtained by reducing the high variability in background SCE frequencies.

At the Windscale radioactive waste disposal site in the Irish Sea, the maximum possible dose rate is estimated to be about 45 mrem/h ($\sim 4.5 \times 10^{-4}$ Gy/h) (Woodhead, 1980). The lowest dose rate used in our study that gave a response statistically greater than that of the control was the induction of SCEs by 2.5×10^{-2} Gy (2.5 rad)/h of ^{60}Co for a total dose of 0.6 Gy (60 rad). Although a small increase in mean SCE frequency was observed at a dose rate of 5×10^{-4} Gy (0.05 rad)/h of ^{60}Co and a total dose of 0.01 Gy

(1.2 rad), no significant increase in the number of HFCs was found. The time over which the doses were delivered in our system was 24 h; in natural systems it may be over the life span of the organism. Greater sensitivity of our bioassay could be obtained by increasing the duration of the exposure to the low dose rates.

A reliable method for determining the exposure of irradiated people to ionizing radiation has been developed using peripheral lymphocytes. Because these cells are long-lived and nondividing, aberrations can persist rather than be converted to lethal events as a result of genetic imbalance in cell division. These cells have been shown to act as an integrating dosimeter for ionizing radiation.

The feasibility of using chromosomal aberrations in environmental monitoring appears to be related to the availability of an integrating dosimeter. We have identified several systems in worms comparable to that of the lymphocytes in mammals. One of these would be to utilize the changes that occur during regeneration. In adult organisms, the rate of division of somatic cells is generally low. However, it is well documented that at the site of regeneration there is increased rate of division. We propose that adult worms be exposed to radiation, regeneration be induced by excising part of the worm, and then the cells of the blastema be examined for increased incidence of chromosomal aberrations in somatic cells that have been stimulated to divide. This system would be amenable to field studies because regeneration could be induced in animals recovered from nuclear waste-disposal sites.

Another possibility would be to utilize the changes that occur during oogenesis. In nereidae worms, primary oocytes undergo maturation in the coelom, the mature gametes are released into seawater upon rupture of the body wall, and then are fertilized. The maturing oocytes may serve as an integrating system. The frequency of chromosomal aberrations could be examined either during meiosis or the first mitotic division after fertilization. This system would be amenable to field studies only in those situations where sexually mature adults were available from the field.

A third possibility would be to utilize blood cells of worms in tests similar to those that have been developed for human lymphocytes. This system could be used in the study of those sites that have worms of sufficient size for blood sampling.

The use of SCE frequency in tissues as an integrating dosimeter for chronic exposure of mammalian cells has not yet been established. For those chemical mutagens that are S-dependent, DNA repair can potentially remove adducts before the cells enter S phase, and result in increased variability of response. S-dependency means that the substance must be present during replicative DNA synthesis, or the lesions it produces in the chromatin require DNA synthesis to be translated into a structural change. Because the

induction of SCEs by radiation is also S-dependent, such increased variability can be expected also. We still, however, suggest considering the use of SCE induction in cells as a dosimeter while searching for an integrating system for chromosomal aberrations.

MECHANISMS OF CHROMOSOMAL ABERRATION AND SISTER CHROMATID EXCHANGE FORMATION

Much effort has been directed toward understanding the mechanisms of chromosomal aberration and SCE formation and their relationships in genetic material (Gebhart, 1981; Carrano and Moore, 1982). It has been shown that chemicals attach to DNA and produce a variety of lesions that can vary from chemical to chemical (Wolff, 1982). With several chemicals, both SCEs and aberrations increase linearly with the dose. Thus, for a given chemical, the ratio of SCEs to aberrations is constant over a large dose range (Carrano et al., 1978; 1980). Because this ratio changes for each chemical, it may indicate that of the multitude of lesions produced for a given chemical, some could lead to SCEs and others to aberrations, or that the lesions that lead to the induction of aberrations are a subset of those that produce SCEs (Carrano and Thompson, 1982). Wolff (1982) states that in any case, the induction of SCEs shows that DNA is being affected and that SCEs are an indicator of damage.

According to Wolff (1982),

> Most geneticists agree that induced mutations are detrimental and, therefore, that any general increase in the mutation rate will also be detrimental. The reasons for this are both empirical and theoretical. For instance, radiation-induced mutations in plants, fruit flies, or any other system that is favorable for genetic analysis usually lead to reduced fitness, i.e., are lethal or semilethal. This makes theoretical sense because all living organisms are the result of eons of evolution and have been selected to fit their particular ecological niche; mutations, which are random changes in the genetic constitution of the organisms, can upset the balance brought about by natural selection. There is, however, a problem in determining exactly how detrimental the effects of mutations will be and how much damage really will be done, especially after low doses.

Because of the uncertainties in our ability to predict the consequences of changes in chromosomes induced by low levels of irradiation, the presence of the changes should be used currently only to signal potential problems in a population. As more data on radiation effects at low dose levels become available, we may be able to relate changes in chromosomes to those in populations and, in turn, to those in communities.

CONCLUSIONS

Nereidae worms, because of their karyotype and life style, are a good cytogenetic model for studying radiation effects on benthic organisms. We used N. arenaceodentata larvae to characterize the rates of induction of chromosome and chromatid deletions and SCEs from exposure to ionizing radiation.

The induction of chromosome and chromatid deletions by x rays delivered at 0.7 Gy (70 rad)/min was dose related, but doses $\geq$2 Gy (200 rad) were required to obtain a response that was significantly different from background. At present we have data from organisms that were exposed for a maximum period of only 24 h. Because under field conditions organisms would be exposed to radiation over their entire lifetime, further studies are required to characterize the effects of chronic exposure. Also, before this bioassay can be applied to conditions that exist at low-level, radioactive-waste disposal sites, increased sensitivity is required. We suggest that increased sensitivity of the response in the worm be achieved by identifying a long-lived cell system, similar to that of the lymphocytes in mammals, that can be used as a integrating dosimeter for chromosomal aberrations.

The induction of SCEs by ^{60}Co delivered at total doses of 0.001 to 0.6 Gy (0.1 to 60 rad) appeared to be dose related, but at higher doses it was not. A response that was significantly different from controls was obtained at 0.6 Gy (60 rad). This dose is considerably lower than that needed to obtain a significant difference for chromosome and chromatid deletion frequencies, but higher than that required to monitor most radioactive-waste disposal sites. Also, the dose over which SCE induction may be used as an indicator of environmental exposure is limited because of the decline in the dose response at higher doses. Consequently, this bioassay could be applied only to field situations where no doses higher than 0.6 Gy (60 rad) are expected, unless factors producing the decline at high doses are identified. Further, we suggest that the required increased sensitivity of this response be obtained by either decreasing the variability in the response at low dose levels or by identifying a cell system that can be used as an integrating dosimeter for SCEs.

APPENDIX A.
QUALITY ASSURANCE

Most of the slides that were scored for chromosomal aberrations (specifically, chromosome and chromatid deletions) and SCEs were examined by more than one person. The results of the multiple scoring of chromosome and chromatid deletions are presented in Table A1 and those of SCEs in Table A2.

Table A1. Chromosomal and chromatid deletions (Del) detected by different individuals scoring the same slide.

Experiment	Slide	Scorer 1 Cells scored	Scorer 1 Del	Scorer 1 Del/ cell	Scorer 2 Cells scored	Scorer 2 Del	Scorer 2 Del/ cell	Scorer 3 Cells scored	Scorer 3 Del	Scorer 3 Del/ cell
		Control larvae								
1	a	28	1	0.036	24	0	0	24	0	0
	b	21	0	0	21	0	0	21	0	0
	c	28	0	0	--	--	--	--	--	--
2	a	14	0	0	15	0	0	15	0	0
	b	9	0	0	14	0	0	14	0	0
	c	6	0	0	--	--	--	--	--	--
3	a	40	1	0.025	43	3	0.070	43	2	0.047
	b	20	0	0	--	--	--	--	--	--
4	a	13	2	0.15	14	2	0.14	14	2	0.14
	b	3	0	0	3	0	0	3	0	0
	c	12	6	0.50	10	3	0.30	--	--	--
5	a	11	0	0	12	0	0	11	0	0
	b	7	1	0.14	--	--	--	--	--	--
	c	12	0	0	--	--	--	--	--	--
6	a	19	1	0.053	25	1	0.040	25	1	0.040
	b	25	1	0.040	30	1	0.033	16	0	0
	c	17	3	0.12	--	--	--	--	--	--
7	a	17	2	0.12	19	2	0.10	10	1	0.056
	b	22	1	0.045	24	1	0.042	23	1	0.043
	c	12	0	0	--	--	--	--	--	--
8	a	32	5	0.16	38	7	0.18	37	6	0.16
	b	15	5	0.33	15	5	0.33	--	--	--

Table A1. (Continued)

Experiment		Scorer 1			Scorer 2			Scorer 3		
	Slide	Cells scored	Del	Del/ cell	Cells scored	Del	Del/ cell	Cells scored	Del	Del/ cell
9	a	5	0	0	--	--	--	--	--	--
	b	3	0	0	--	--	--	--	--	--
	c	1	0	0	--	--	--	--	--	--
10	a	12	1	0.083	12	2	0.17	10	2	0.20
11	a	19	1	0.053	30	2	0.067	--	--	--
	b	17	0	0	--	--	--	--	--	--
12	a	34	3	0.088	34	3	0.088	--	--	--
	b	24	1	0.042	--	--	--	--	--	--
	c	31	1	0.032	--	--	--	--	--	--
20	a	6	0	0	--	--	--	--	--	--
	b	10	1	0	--	--	--	--	--	--
21	a	46	1	0	49	1	0.20	27	1	0.21
	b	27	0	0	27	0	0	21	0	0
	c	21	1	0.022	21	0	0	47	0	0
		Larvae irradiated with x rays								
3.6 to 3.8 Gy[a]										
11	a	10	1	0.10	15	2	0.13	15	2	0.13
	b	7	4	0.57	7	4	0.57	7	4	0.57
	c	13	4	0.31	15	7	0.47	--	--	--
12	a	15	4	0.27	22	4	0.23	--	--	--
	b	20	3	0.15	32	11	0.34	--	--	--
	c	12	3	0.25	--	--	--	--	--	--

Table A1. (Continued)

Experiment		Scorer 1			Scorer 2			Scorer 3		
	Slide	Cells scored	Del	Del/ cell	Cells scored	Del	Del/ cell	Cells scored	Del	Del/ cell
2.5 to 2.6 Gy										
3	a	27	30	1.11	28	25	0.89	28	19	0.68
	b	7	2	0.29	7	4	0.57	7	3	0.43
	c	12	5	0.42	14	11	0.71	13	8	0.62
12	a	22	3	0.14	20	4	0.20	--	--	--
	b	16	3	0.19	16	4	0.25	--	--	--
	c	18	4	0.22	10	2	0.20	--	--	--
2.0 to 2.2 Gy										
7	a	10	5	0.50	1	0	0	7	2	0.29
	b	3	1	0.33	6	6	1	8	3	0.38
	c	15	7	0.47	3	5	1.67	9	9	1
8	a	13	0	0	4	4	1	6	0	0
	b	4	1	0.25	1	0	0	5	1	0.20
	c	22	1	0.045	14	0	0	17	2	0.12
	d	10	0	0	5	0	0	4	1	0.25
	e	15	5	0.33	15	7	0.47	14	4	0.29
	f	13	6	0.46	8	13	1.62	11	5	0.46
1.6 to 1.7 Gy										
8	a	2	0	0	6	1	0.17	4	1	0.25
	b	3	0	0	1	0	0	0	0	0
	c	7	1	0.14	7	1	0.14	7	1	0.14
12	a	18	7	0.39	18	7	0.39	16	5	0.31
	b	16	0	0	22	0	0	23	0	0
	c	19	1	0.053	14	1	0.071	12	0	0

Table A1. (Continued)

Experiment		Scorer 1			Scorer 2			Scorer 3		
	Slide	Cells scored	Del	Del/ cell	Cells scored	Del	Del/ cell	Cells scored	Del	Del/ cell
0.88 to 1.0 Gy										
1	a	12	2	0.17	12	3	0.25	12	3	0.25
	b	28	4	0.14	27	4	0.15	--	--	--
	c	19	3	0.16	15	3	0.20	--	--	--
6	a	25	3	0.12	24	1	0.042	--	--	--
	b	16	0	0	15	2	0.13	15	2	0.13
	c	24	1	0.042	30	3	0.10	--	--	--
12	a	26	1	0.038	28	1	0.036	28	1	0.036
	b	30	1	0.033	49	6	0.14	--	--	--
	c	29	3	0.10	28	2	0.071	--	--	--
0.45 to 0.48 Gy										
1	a	31	3	0.097	31	7	0.21	30	5	0.17
	b	30	5	0.17	28	4	0.14	--	--	--
	c	47	2	0.043	56	4	0.07	--	--	--
2	a	4	0	0	4	0	0	4	0	0
	b	10	0	0	10	0	0	--	--	--
	c	15	2	0.13	14	1	0.071	--	--	--
4	a	8	0	0	--	--	--	--	--	--
	b	15	1	0.067	14	1	0.071	--	--	--
0.37 Gy										
5	a	37	1	0.027	--	--	--	--	--	--
	b	44	3	0.068	--	--	--	--	--	--
	c	28	0	0	--	--	--	--	--	--

Table A1. (Continued)

Experiment	Slide	Scorer 1 Cells scored	Scorer 1 Del	Scorer 1 Del/ cell	Scorer 2 Cells scored	Scorer 2 Del	Scorer 2 Del/ cell	Scorer 3 Cells scored	Scorer 3 Del	Scorer 3 Del/ cell
0.18 to 0.24 Gy										
2	a	17	4	0.24	--	--	--	--	--	--
	b	12	2	0.17	--	--	--	--	--	--
	c	11	2	0.18	--	--	--	--	--	--
4	a	17	2	0.12	--	--	--	--	--	--
	b	16	0	0	--	--	--	--	--	--
	c	21	0	0	--	--	--	--	--	--
5	a	7	1	0.14	--	--	--	--	--	--
	b	23	1	0.043	--	--	--	--	--	--
10	a	4	0	0	--	--	--	--	--	--
	b	14	0	0	--	--	--	--	--	--
	c	11	3	0.27	--	--	--	--	--	--
0.08 Gy										
2	a	19	2	0.105	--	--	--	--	--	--
	b	14	0	0	--	--	--	--	--	--
	c	8	0	0	--	--	--	--	--	--

[a] One gray (Gy) is equivalent to 100 rads.

Table A2. Mean SCE frequencies determined by different individuals scoring the same slide.

Experiment		Scorer 1			Scorer 2			Scorer 3		
	Slide	Cells scored	Mean SCEs	SD[a]	Cells scored	Mean SCEs	SD	Cells scored	Mean SCEs	SD
		Control larvae								
13	a	16	0.10	0.083	15	0.15	0.10	--	--	--
	b	8	0.083	0.084	10	0.14	0.10	--	--	--
14	a	6	0.18	0.14	6	0.20	0.31	--	--	--
	b	5	0.14	0.089	--	--	--	--	--	--
	c	7	0.23	0.21	--	--	--	--	--	--
15[b]	a	7	0.38	0.30	--	--	--	--	--	--
	b	11	0.22	0.14	14	0.22	0.14	14	0.22	0.14
16	a	17	0.13	0.077	20	0.11	0.089	20	0.12	0.085
	b	13	0.16	0.12	--	--	--	--	--	--
17	a	19	0.28	0.24	13	0.30	0.25	13	0.32	0.24
	b	16	0.36	0.33	13	0.22	0.12	12	0.24	0.13
	c	15	0.23	0.14	--	--	--	--	--	--
	d	24	0.17	0.077	--	--	--	--	--	--
18	a	8	0.34	0.35	7	0.32	0.26	7	0.32	0.26
	b	7	0.32	0.24	--	--	--	--	--	--
	c	16	0.30	0.22	--	--	--	--	--	--
	d	14	0.24	0.15	--	--	--	--	--	--
	e	8	0.32	0.20	--	--	--	--	--	--
19	a	19	0.16	0.12	--	--	--	--	--	--
	b	28	0.13	0.12	21	0.20	0.24	--	--	--
20	a	1	0.059	--	1	0.11	--	1	0.11	--
	b	9	0.20	0.16	8	0.20	0.16	8	0.20	0.16

Table A2. (Continued)

Experiment	Slide	Scorer 1			Scorer 2			Scorer 3		
		Cells scored	Mean SCEs	SD[a]	Cells scored	Mean SCEs	SD	Cells scored	Mean SCEs	SD
21	a	12	0.11	0.097	7	0.13	0.09	--	--	--
	b	6	0.12	0.072	5	0.12	0.10	5	0.14	0.099
22	a	17	0.19	0.16	--	--	--	--	--	--
	b	4	0.06	0.051	--	--	--	--	--	--
	c	11	0.17	0.12	--	--	--	--	--	--
23	a	9	0.11	0.12	--	--	--	--	--	--
	b	18	0.10	0.12	--	--	--	--	--	--
	c	13	0.17	0.09	--	--	--	--	--	--
	d	12	0.08	0.074	--	--	--	--	--	--
24	a	3	0.14	0.08	--	--	--	--	--	--
	b	10	0.20	0.18	--	--	--	--	--	--
	c	8	0.28	0.36	--	--	--	--	--	--
25	a	6	0.28	0.24	--	--	--	--	--	--
	b	14	0.25	0.24	--	--	--	--	--	--
	c	4	0.25	0.28	--	--	--	--	--	--
26	a	22	0.14	0.093	--	--	--	--	--	--
	b	24	0.18	0.14	--	--	--	--	--	--
	c	13	0.09	0.092	--	--	--	--	--	--
	d	8	0.16	0.10	--	--	--	--	--	--
27	a	42	0.18	0.11	32	0.17	0.10	--	--	--
	b	13	0.18	0.12	13	0.18	0.11	13	0.18	0.14
	c	10	0.20	0.13	11	0.25	0.20	--	--	--
	d	9	0.15	0.070	14	0.177	0.088	--	--	--
	e	3	0.11	0.10	3	0.15	0.11	--	--	--
28	a	15	0.46	0.57	--	--	--	--	--	--
	b	5	0.12	0.094	--	--	--	--	--	--

Table A2. (Continued)

Experiment	Slide	Scorer 1 Cells scored	Scorer 1 Mean SCEs	Scorer 1 SD[a]	Scorer 2 Cells scored	Scorer 2 Mean SCEs	Scorer 2 SD	Scorer 3 Cells scored	Scorer 3 Mean SCEs	Scorer 3 SD
29	a	15	0.18	0.20	--	--	--	--	--	--
	b	7	0.20	0.12	--	--	--	--	--	--
30	a	11	0.077	0.059	--	--	--	--	--	--
	b	7	0.31	0.49	--	--	--	--	--	--
		Larvae irradiated with ^{60}Co								
3.0 Gy[c]										
13	a	23	0.235	0.401	--	--	--	--	--	--
14	a	21	0.183	0.148	--	--	--	--	--	--
	b	10	0.195	0.227	--	--	--	--	--	--
1.7 Gy										
13	a	5	0.26	0.12	4	0.28	0.16	4	0.28	0.14
	b	12	0.65	0.79	6	0.16	0.16	6	0.22	0.26
14	a	14	0.17	0.18	6	0.12	0.12	6	0.12	0.12
	b	12	0.12	0.10	7	0.17	0.12	8	0.21	0.20
0.6 Gy										
13	a	19	0.43	0.45	12	0.24	0.34	20	0.39	0.46
	b	10	0.48	0.82	13	0.26	0.33	5	0.62	1.21
14	a	4	0.20	0.029	4	0.078	0.097	3	0.037	0.064
	b	10	0.36	0.38	6	0.068	0.058	8	0.32	0.38
	c	13	0.22	0.28	11	0.14	0.24	10	0.15	0.22
15	a	16	0.50	0.34	13	0.40	0.27	12	0.40	0.28
	b	17	0.62	0.60	20	0.52	0.44	18	0.48	0.40

Table A2. (Continued)

Experiment	Slide	Scorer 1			Scorer 2			Scorer 3		
		Cells scored	Mean SCEs	SD[a]	Cells scored	Mean SCEs	SD	Cells scored	Mean SCEs	SD
17	a	4	0.46	0.089	5	0.40	0.13	4	0.42	0.070
	b	10	0.30	0.27	7	0.34	0.27	7	0.40	0.28
18	a	12	0.32	0.22	12	0.28	0.20	9	0.29	0.18
	b	9	0.22	0.32	6	0.36	0.56	8	0.35	0.50
19	a	13	0.12	0.078	11	0.16	0.076	--	--	--
	b	13	0.14	0.099	12	0.15	0.10	11	0.16	0.092
20	a	7	0.67	0.52	8	0.54	0.52	6	0.44	0.27
	b	7	0.37	0.64	7	0.35	0.70	7	0.38	0.68
21	a	3	0.17	0.14	4	0.13	0.10	4	0.13	0.10
	b	2	0.46	0.34	3	0.41	0.29	2	0.47	0.25
0.3 Gy										
13	a	11	0.97	1.59	9	0.84	0.32	19	0.55	0.94
	b	15	0.53	0.41	15	0.51	0.13	6	0.48	0.32
14	a	11	0.22	0.14	11	0.16	0.16	10	0.16	0.16
	b	11	0.26	0.29	6	0.10	0.22	5	0.13	0.24
15	a	23	0.45	0.38	25	0.34	0.31	22	0.34	0.32
16	a	13	0.14	0.13	24	0.074	0.067	24	0.081	0.014
	b	26	0.14	0.13	25	0.14	0.10	25	0.16	0.13
17	a	10	0.19	0.15	11	0.16	0.12	8	0.15	0.12
	b	11	0.46	0.41	9	0.30	0.24	8	0.32	0.30
18	a	5	0.23	0.10	5	0.22	0.094	4	0.18	0.099
	b	5	0.54	0.34	6	0.26	0.22	7	0.36	0.34

Table A2. (Continued)

Experiment	Slide	Scorer 1			Scorer 2			Scorer 3		
		Cells scored	Mean SCEs	SD[a]	Cells scored	Mean SCEs	SD	Cells scored	Mean SCEs	SD
19	a	15	0.23	0.30	23	0.13	0.096	22	0.18	0.25
	b	12	0.16	0.20	10	0.12	0.10	8	0.10	0.099
21	a	12	0.20	0.17	14	0.19	0.24	12	0.16	0.25
	b	10	0.22	0.16	8	0.23	0.18	8	0.24	0.20
0.1 Gy										
15	a	25	0.42	0.34	--	--	--	--	--	--
27	a	29	0.30	0.30	28	0.16	0.18	22	0.17	0.18
	b	18	0.281	0.22	18	0.24	0.22	18	0.24	0.23
	c	10	0.30	0.48	14	0.22	0.45	12	0.22	0.42
	d	10	0.12	0.10	13	0.14	0.092	11	0.10	0.08
	e	6	0.30	0.33	14	0.22	0.20	14	0.22	0.21
	f	18	0.13	0.10	22	0.12	0.10	20	0.12	0.11
	g	16	0.093	0.073	22	0.12	0.083	--	--	--
	h	2	0.056	0.000	11	0.15	0.05	10	0.15	0.094
	i	6	0.17	0.17	12	0.19	0.14	11	0.18	0.15
0.01 Gy										
26	a	26	0.19	0.14	--	--	--	--	--	--
	b	27	0.20	0.15	--	--	--	--	--	--
	c	13	0.21	0.20	--	--	--	--	--	--
	d	11	0.18	0.14	--	--	--	--	--	--
	e	14	0.17	0.13	--	--	--	--	--	--
	f	21	0.18	0.13	--	--	--	--	--	--
	g	14	0.11	0.097	--	--	--	--	--	--
	h	14	0.16	0.10	--	--	--	--	--	--
	i	12	0.10	0.10	--	--	--	--	--	--

Table A2. (Continued)

Experiment	Slide	Scorer 1			Scorer 2			Scorer 3		
		Cells scored	Mean SCEs	SD[a]	Cells scored	Mean SCEs	SD	Cells scored	Mean SCEs	SD
0.001 Gy										
22	a	9	0.16	0.09	--	--	--	--	--	--
	b	17	0.16	0.23	--	--	--	--	--	--
	c	12	0.37	0.27	--	--	--	--	--	--
	d	14	0.19	0.22	--	--	--	--	--	--
	e	17	0.22	0.22	--	--	--	--	--	--
	f	12	0.21	0.14	--	--	--	--	--	--
23	a	17	0.21	0.17	--	--	--	--	--	--
	b	10	0.14	0.087	--	--	--	--	--	--
	c	13	0.14	0.079	--	--	--	--	--	--
	d	2	0.17	0.16	--	--	--	--	--	--
24	a	11	0.083	0.040	--	--	--	--	--	--
	b	8	0.22	0.19	--	--	--	--	--	--
	c	10	0.18	0.17	--	--	--	--	--	--
25	a	4	0.15	0.15	--	--	--	--	--	--
	b	4	0.18	0.18	--	--	--	--	--	--
	c	2	0.20	0.12	--	--	--	--	--	--
Larvae treated with Mitomycin C										
13	a	14	0.51	0.56	9	0.38	0.55	--	--	--
14	a	11	0.25	0.23	--	--	--	--	--	--
	b	6	0.45	0.36	--	--	--	--	--	--
	c	7	0.37	0.32	--	--	--	--	--	--
15	a	20	0.78	0.46	16	0.62	0.41	--	--	--

Table A2. (Continued)

Experiment	Slide	Scorer 1			Scorer 2			Scorer 3		
		Cells scored	Mean SCEs	SD[a]	Cells scored	Mean SCEs	SD	Cells scored	Mean SCEs	SD
17	a	16	0.31	0.23	14	0.29	0.12	--	--	--
	b	13	0.48	0.38	--	--	--	--	--	--
18	a	10	0.44	0.40	9	0.42	0.36	--	--	--
	b	5	0.16	0.063	--	--	--	--	--	--
19	a	11	0.30	0.18	12	0.25	0.077	--	--	--
	b	10	0.21	0.11	--	--	--	--	--	--
20	a	1	0.067	--	1	0.000	--	--	--	--
	b	4	0.17	0.13	--	--	--	--	--	--
21	a	8	0.36	0.17	8	0.35	0.21	--	--	--
	b	7	0.19	0.17	--	--	--	--	--	--
22	a	4	0.38	0.30	--	--	--	--	--	--
	b	14	0.19	0.16	--	--	--	--	--	--
	c	7	0.29	0.24	--	--	--	--	--	--
23	a	3	0.15	0.12	--	--	--	--	--	--
	b	14	0.34	0.19	--	--	--	--	--	--
24	a	10	0.14	0.097	--	--	--	--	--	--
	b	10	0.27	0.29	--	--	--	--	--	--
	c	3	0.075	0.031	--	--	--	--	--	--
25	a	8	0.29	0.19	--	--	--	--	--	--
	b	2	0.063	0.006	--	--	--	--	--	--
	c	6	0.087	0.081	--	--	--	--	--	--

Table A2. (Continued)

Experiment	Slide	Scorer 1			Scorer 2			Scorer 3		
		Cells scored	Mean SCEs	SD[a]	Cells scored	Mean SCEs	SD	Cells scored	Mean SCEs	SD
26	a	25	0.32	0.28	--	--	--	--	--	--
	b	17	0.21	0.18	--	--	--	--	--	--
	c	21	0.18	0.13	--	--	--	--	--	--
	d	8	0.23	0.10	11	0.28	0.14	--	--	--
27	a	9	0.33	0.32	7	0.24	0.22	--	--	--

[a] Standard deviation.
[b] Cells harvested at 48 h; all others harvested at 54 h..
[c] One gray (Gy) is equivalent to 100 rads.

APPENDIX B.
EFFECTS OF CELL-CYCLE TIME ON SISTER CHROMATID EXCHANGE FREQUENCY

Control larvae were exposed to 3 X 10^{-5}M BrdUrd for 28, 36, 42, 48, 54, 60, and 66 h. In a given experiment, either single or multiple harvest times were used (Table B1). The cells of N. arenaceodentata may not all divide at the same rate. If some cells are dividing more rapidly than others, they will be found at a given division (first, second, or third) at an earlier time than those that are dividing more slowly. That is, second division cells harvested at 28 h represent a faster dividing population of cells than those harvested at later times. The proportion of cells observed in first, second, or third division varied with the BrdUrd harvest time (Fig. B1). The percent of cells in first division is high at 28 h and low at 66 h; the converse is true for cells in third division.

The number of cells scored and the mean and median SCE frequency obtained for each experiment at differing harvest times were compiled (Table B2, Fig. B2). Because the number of cells scored for each experiment was different, we used the Kruskal-Wallis (K-W) nonparametric test (Conover, 1971) to compare the means of each experiment to the number of cells scored. No significant bias of the mean by the number of cells scored was found; p was 0.80 (Table B3). Next, the significance of differences in mean and median SCE frequencies at different BrdUrd harvest times was examined using the K-W test. The probability that all of the data from different BrdUrd harvest times were drawn from a homogeneous pool was 0.43 for means and 0.39 for medians. This is in agreement with the cell culture literature (Leonard and Decat, 1979; Giulotto et al., 1980).

Table B1. *Times at which cells were harvested after initiation of the BrdUrd exposure.*

Experiment	Harvest time (h)						
	28	36	42	48	54	60	66
1	X						
2	X						
3	X						
4	X						
5	X						
6	X						
7	X						
8	X						
9	X						
11	X						
12	X						
13					X		
14					X		
15				X			
16			X	X	X	X	X
17					X		
18					X		
19					X		
20	X				X		
21	X				X		
22					X		
23					X		
24					X		
25					X		
26					X		
27					X		
28			X	X	X		
29		X	X	X	X	X	
30					X		

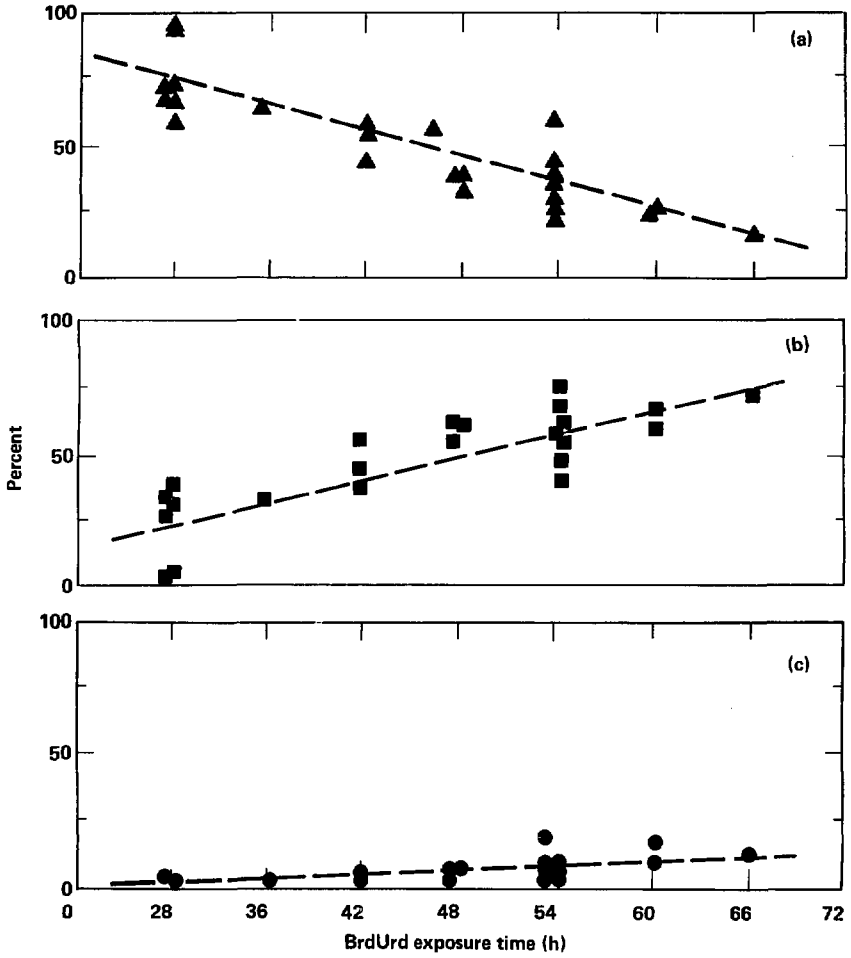

Figure B1. Percent (a) first, (b) second, and (c) third division cells observed in control Neanthes arenaceodentata larvae at differing times following initiation of BrdUrd exposure.

Table B2. Mean and median sister chromatid exchanges per chromosome (SCEs/C) observed in control cell populations harvested at differing times following initiation of BrdUrd exposure. The fraction of cells in first, second, and third division for each experiment harvest time is also given.

BrdUrd harvest time(h)	Experiment	Cells scored[a]	Mean SCEs/C	Median SCEs/C	Fraction of cells in specific division			N[b]
					First	Second	Third	
28	1	3	0.30	0.24	0.97	0.03	0	174
	2	11	0.37	0.28	0.66	0.33	0.01	169
	3	7	0.28	0.29	0.83	0.17	0	408
	4	34	0.24	0.22	0.59	0.39	0.02	568
	5	19	0.16	0.17	0.47	0.44	0.09	182
	6	48	0.24	0.17	0.57	0.42	0.01	282
	7	6	0.18	0.17	0.85	0.15	0	163
	8	3	0.45	0.44	0.85	0.15	0	137
	9	17	0.12	0.12	0.42	0.47	0.11	126
	11	2	0.51	0.51	0.75	0.25	0	206
	12	8	0.18	0.13	0.80	0.20	0	82
	20	20	0.31	0.23	0.60	0.39	0.01	82
	21	23	0.11	0.07	0.94	0.04	0.01	163
	--[c]	201	0.22	0.17	0.72	0.26	0.20	--
					± 0.18	± 0.15	± 0.04	
36	29	23	0.22	0.18	0.64	0.34	0.02	126
	--[c]	23	0.22	0.18	0.64	0.34	0.02	--
42	16	39	0.56	0.11	0.45	0.55	0.01	207
	28	28	0.24	0.17	0.58	0.37	0.05	148
	29	17	0.20	0.18	0.55	0.44	0.01	107
	--[c]	84	0.19	0.16	0.53	0.45	0.02	--
				± 0.07	± 0.09	± 0.02		

Table B2. (Continued).

BrdUrd harvest time(h)	Experiment	Cells scored[a]	Mean SCEs/C	Median SCEs/C	Fraction of cells in specific division			N[b]
					First	Second	Third	
48	15	21	0.27	0.20	0.28	0.44	0.28	307
	16	26	0.12	0.11	0.38	0.60	0.02	100
	28	22	0.30	0.21	0.39	0.54	0.08	13
	29	21	0.35	0.27	0.32	0.60	0.09	111
	--[c]	90	0.25	0.18 ± 0.05	0.34 ± 0.08	0.55 ± 0.11	0.12	--
54	13	24	0.10	0.09	0.19	0.58	0.23	218
	14	18	0.20	0.14	0.42	0.50	0.08	208
	16	33	0.13	0.13	0.35	0.62	0.03	146
	17	65	0.22	0.18	0.35	0.61	0.04	685
	18	52	0.30	0.25	0.37	0.55	0.08	883
	19	40	0.18	0.13	0.39	0.56	0.06	205
	20	9	0.19	0.18	0.44	0.47	0.08	727
	21	12	0.13	0.12	0.60	0.39	0.01	117
	22	32	0.17	0.12	0.35	0.61	0.04	137
	23	52	0.12	0.09	0.35	0.63	0.02	210
	24	21	0.22	0.17	0.43	0.48	0.09	148
	25	24	0.26	0.27	0.48	0.48	0.04	118
	26	67	0.50	0.11	0.53	0.44	0.03	359
	27	73	0.19	0.17	0.32	0.62	0.06	293
	28	20	0.38	0.17	0.29	0.53	0.19	70
	29	22	0.19	0.17	0.21	0.74	0.05	87
	30	18	0.17	0.06	0.25	0.65	0.10	83
	--[c]	582	0.19	0.14	0.37 ± 0.11	0.56 ± 0.09	0.07 ± 0.06	--

Table B2. (Continued).

BrdUrd harvest time(h)	Experiment	Cells scored[a]	Mean SCEs/C	Median SCEs/C	Fraction of cells in specific division			N[b]
					First	Second	Third	
60	16	25	0.16	0.17	0.26	0.57	0.17	81
	29	33	0.17	0.15	0.25	0.66	0.09	124
	--[c]	58	0.17	0.16	0.26	0.62	0.13	--
				± 0.01	± 0.06	± 0.06		
66	16	21	0.13	0.11	0.16	0.71	0.13	85
	--[c]	21	0.134	0.11	0.16	0.71	0.13	--

[a] Data from experiments with less than 10 cells scored were pooled and treated as a single experiment with n = 29, mean = 0.269, and median = 0.222.
[b] N, total number of cells examined.
[c] Values for this BrdUrd harvest time.

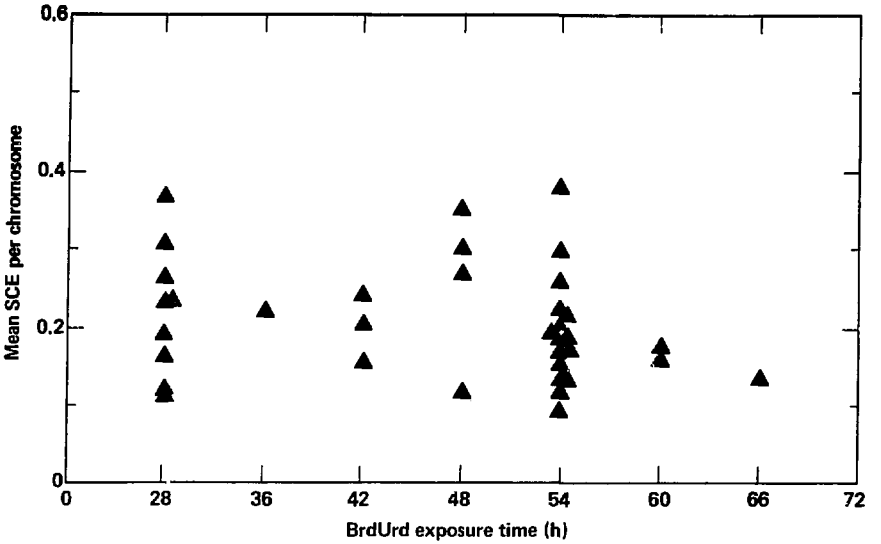

Figure B2. Mean SCE frequencies observed in Neanthes arenaceodentata larvae control cells harvested at differing times following initiation of 3×10^{-5}M BrdUrd exposure.

Table B3. Distribution of the means of sister chromatid exchanges per chromosome (SCEs/C) in control cells in relation to the number of cells scored.

Cells scored	Mean SCEs/C								
0-10	0.18	0.18	0.19	0.28	0.30	0.45	0.51	--	--
11-20	0.12	0.13	0.16	0.17	0.20	0.20	0.31	0.37	0.38
21-30	0.096	0.11	0.11	0.13	0.16	0.19	0.22	0.22	0.24
	0.26	0.27	0.30	0.35	--	--	--	--	--
31-40	0.31	0.16	0.17	0.17	0.18	0.24	--	--	--
41-50	0.24	--	--	--	--	--	--	--	--
51-60	0.12	0.30	--	--	--	--	--	--	--
61-70	0.15	0.22	--	--	--	--	--	--	--
>71	0.18								

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