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Effect of Low ^{60}Co Dose Rates on Sister Chromatid Exchange Incidence in the Benthic Worm *Neanthes arenaceodentata*

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FOREWORD

The objective of this study is to determine the feasibility of using 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.

This investigation was funded by the Environmental Protection Agency (EPA), Office of Radiation Programs, under FY 80 Environmental Protection Agency Agreement Number EPA-AD89F00070. The project officer for EPA is Marilyn Varela.

The authors thank Jay Minkler, Irene Jones, and Tony Carrano for their technical advice during this study and Rose Carrillo for her assistance in scoring slides.

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ABSTRACT

The usefulness of sister chromatid exchange (SCE) induction as a measure of low-level radiation effect was examined in a benthic marine worm, Neanthes arenaceodentata. Larvae were exposed to ^{60}Co radiation for 12 to 24 h at total doses ranging from 0.5 to 309 R and at dose rates from 0.04 to 13 R/h. Animals exposed at intermediate dose rates (0.5, 0.6, 1.25, 2.0, and 2.5 R/h) had SCE frequencies per chromosome about twice that of those receiving no radiation (controls), whereas those exposed at the higher dose rates (7.0 and 13 R/h) had SCE frequencies lower than the controls. Animals exposed at the lower dose rates (0.04 and 0.1 R/h) had lower SCE frequencies than those exposed at intermediate dose rates (and higher SCE frequencies than controls).

The length of chromosome pair number one differed among metaphase spreads and was used as an index of chromosome condensation in a given metaphase. Because there is a possibility that chromosome morphology may affect the ability to resolve SCEs, morphology will be monitored in future studies.

A preliminary experiment was performed to assess the effects of 2.2 and 11.5 R/h for 24 h on growth and development. Larvae observed at 6 and 17 d after irradiation did not have significantly different numbers of abnormal larvae or survival rates.

RECOMMENDATIONS FOR FURTHER STUDY

DOSE RATE

Extension and confirmation of preliminary data assessing the effects of ^{60}Co dose rate on sister chromatid exchange (SCE) frequency in Neanthes arenaceodentata.

TOTAL DOSE

Extension and confirmation of preliminary data assessing the effects of total ^{60}Co dose on SCE frequency in Neanthes arenaceodentata.

THE SCE CHANGES WITH TIME

Determination and assessment of the changes in SCE frequency with time following ^{60}Co irradiation. The SCE frequency should be observed at 4 to 8 h intervals following ^{60}Co irradiation using dose rates and total doses indicated from the results of previous experiments.

THE SCE LIFE-HISTORY STUDIES

Comparison of the induction of SCEs in larvae, juveniles, and adult Neanthes arenaceodentata following ^{60}Co exposure. The dose rates and total doses used for these experiments should be decided from the results of previous experiments.

LYSOSOMAL LATENCY

Determination and assessment of the effect of ^{60}Co radiation on lysosomal latency. The feasibility of using lysosomal latency as a measure of radiation should be evaluated using a range of either total doses or dose rates, as indicated by the results of previous experiments.

INTRODUCTION

Disposal of low-level solid radioactive wastes in marine environments occurred on both the east and west coasts of the United States (Joseph et al., 1971). Although these practices were discontinued by 1970, little effort was made until recently to determine the fate and distribution of the radionuclides released from these wastes. Information now available indicates that some leakage of radioactivity occurred (Dyer, 1976; Noshkin et al., 1978), but the total impact of the releases on the ecosystems is not known.

The United States Environmental Protection Agency (EPA) is authorized under the Marine Research and Sanctuaries Act of 1972 (PL-92-532) to regulate the ocean disposal of radioactive material. This Act requires the EPA to establish regulations and criteria to implement a permit program. To develop valid criteria, the EPA needs information on the effects on the environment of radioactive waste disposal at sea.

Deleterious effects of radioactivity on marine organisms are well documented (Templeton et al., 1976; Blaylock and Trabelka, 1978; Egami, 1980). Damage to genetic material includes 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 metaphase chromosomes for 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 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). More recently, these exchanges are distinguished by exposing cells to 5-bromo-deoxyuridine (BrdU) 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 SCEs are a sensitive indicator of DNA damage caused by environmental mutagens and carcinogens.

The effects of ionizing radiation on the frequencies of SCE were studied in a number of cell systems exposed to either beta-rays, beta plus x-rays, x-rays, or gamma-rays (Table 1). Some studies used ^{3}H-thymidine incorporated into DNA to autoradiographically demonstrate the SCEs produced by x-rays (Marin and Prescott, 1964; Gatti et al., 197[illegible]. Other studies, also using autoradiography, indicated that the high beta-ray dose from the incorporation of H^{3}-thymidine into DNA resulted in induced SCEs (Gibson and Prescott, 1972; Kato, 1974).

After the FPG technique was developed, several studies were undertaken to determine the effect of ionizing radiation on SCE frequencies in mammalian chromosomes. However, few data from these experiments are applicable to whole-animal in vivo irradiation. Results from studies in which the irradiation of the cells took place in the presence of BrdU (Perry and Evans, 1975; Solomon and Bobrow, 1975; Abramovsky et al., 1978; Livingston and Dethlefsen, 1979) may be confounded by BrdU-induced changes in radiosensitivity (Yu, 1976; Galloway, 1977; Morgan and Crossen, 1980). Studies using human peripheral blood lymphocytes that were irradiated and then labeled with BrdU show conflicting results. Cells irradiated at the initiation of culture, when the cells were in the G_0 stage of their cycle, showed no increase in SCEs (Galloway, 1977; Littlefield et al., 1979), whereas cells irradiated 42 h after initiation of culture, when the cells were in synthesis (S) stage, showed significant increase in SCEs (Morgan and Crossen, 1980).

Increases in SCEs were found following in vivo x-irradiation of mice (Nakaniski and Schneider, 1979). In this study, like the majority of those listed in Table 1, dose rates much greater than those expected at ocean disposal sites were used. Studies are required that characterize the incidence of SCEs in whole animals irradiated with dose rates expected at ocean radioactive waste disposal sites.

TABLE I. Irradiation conditions used in previous studies examining the frequencies of SCEs 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)	$\beta(^3H)$[a]	8-38 rad	0.3-1.4 rad/h	Kato, 1974
Chinese hamster cells (CHEF-125)	$\beta(^3H)$[a]	27-3560 rad	0.8-99 rad/h	Marin and Prescott, 1964
	or $\beta(^3H)$[a]	380-700 rad	11-19 rad/h	
	plus x-rays	25-200 rad	50 rad/min	
Chinese hamster cells (CHEF-125)	$\beta(^3H)$[a]	~80 & 400 rad	~3-14 rad/h	Gatti et al., 1974
	plus x-rays	175 rad	60 rad/min	
Chinese hamster cells (CHO)	x-rays	50-80 rad	50 rad/min	Perry and Evans, 1975
Chinese hamster cells (CHO)	x-rays	50-100 rad	100 rad/min	Yu, 1976
Human lymphocytes (normal & ataxia telangiectasia)	x-rays	200 rad	50 rad/min	Galloway, 1977
Chinese hamster cells (CHO)	x-rays	100-600 rad	450 rad/min	Livingston and Dethlefsen, 1979
Live mice	x-rays	200-1500 rad	--[b]	Nakanishi and Schneider, 1979
Human lymphocytes	x-rays	100-400 rad	--[b]	Morgan and Crossen, 1980
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 $\beta(^3H)$ radiation from autoradiographic film grain counts. The β-radiation dose to a cell nucleus from ^3H incorporated into DNA was estimated to be 1.08 rad/disintegration (Goodheart, 1961) and 14 disintegrations were estimated to produce one grain count (Marin and Prescott, 1964).
[b]Dose delivered was not specified.

Nereid 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 these worms live in bottom sediments that are known to concentrate many radionuclides, they could therefore receive both an internal and external radiation dose. Pesch and Pesch (1980a) proposed that the marine polychaete Neanthes arenaceodentata be used as an in vivo cytogenetic model for marine genetic toxicology. In a baseline study, 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). However, the radiation doses used were higher than expected in most field situations. It is envisioned that a signal system could be developed for use as the first approach in monitoring potential disposal sites. If an elevated number of SCEs were seen in the sentinel organism in any future disposal site as compared to a control area, it could indicate that (1) investigation of gross chromosome anomalies should be made to indicate if genetic change may be sustained and affect localized populations and (2) that levels of radiation are becoming such that a reevaluation of the limiting capacity for sites should be made.

The objective of this preliminary study was to characterize the response of this worm to lower levels of radiation (from 0.5 R). We exposed Neanthes arenaceodentata to different dose rates and total doses to determine the relationship between SCE frequency and ^{60}Co radiation. We also performed a preliminary study of the effect of radiation on growth and development.

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 were 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 with which they were fed. Because shipping times seldom exceeded 3 d, worm mortality was low; only a single death occurred during all shipments and none occurred after arrival at Lawrence Livermore National Laboratory (LLNL).

On arrival at LLNL, mated pairs of adult worms were placed in 4-liter glass beakers. The adult worms that produced larvae used in the experiments were reared using semistatic culture conditions; the water was aerated continuously and three quarters of the volume in the beakers exchanged weekly. Adult worms were maintained 20 to 30 d in our laboratory before larvae were harvested. The mean culture temperature was 19.4°C ± 1.4°C. The adult worms were fed freeze-dried Enteromorpha sp. ad lib. Uneaten food was removed weekly.

The life cycle of this species is well known (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 larvae) 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 0.4-μm-pore size filter.

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 ethlyene 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.

THE ^{60}Co IRRADIATION

Exposures to ^{60}Co were either 12 or 24 h, and were delivered at different dose rates and total doses (Table 2). All irradiations were conducted in our low-level radiation facility equipped with a 1.25 Ci ^{60}Co source. For each exposure, 50 to 75 worm larvae harvested from 2 to 3 broods were placed in a cylindrical plastic chamber (2.5 cm in diameter) containing 30 ml of filtered seawater. A plexiglass sheet (5 x 7 x 0.6 cm) was placed in front of each exposure chamber to ensure electron equilibrium. Different dose rates were obtained by varying the distance between the chamber and the source, and different total doses by varying the duration of irradiation at a given distance from the source. Delivered dose was determined from triplicate thermoluminescent dosimeters placed behind each exposure chamber.

Two groups of control worms were run with each experiment. Neither group was irradiated, but one was treated with mitomycin C (MMC) and served as a positive control. Both controls were maintained in the exposure facility during the irradiation of the other groups of worms.

TABLE 2. The ^{60}Co dose rates and doses used during irradiations of Neanthes arenaceodentata larvae.[a]

Dose rate, R/h	Dose, R	Experiments 1	2	3	4	5	6	Preliminary growth and development experiment
13.0	309	X	X	--	--	--	--	--
11.5	280	--	--	--	--	--	--	X
7.0	172	X	X	--	--	--	--	--
5.9	65	--	--	--	X	X	X	--
2.5	70	--	--	X	--	--	--	--
2.5	60	X	X	--	--	--	--	--
2.2	52	--	--	--	--	--	--	X
2.0	25	--	--	--	X	X	X	--
1.25	30	X	X	X	--	--	--	--
0.6	7-8	--	--	--	X	X	X	--
0.5	12	--	--	X	--	--	--	--
0.1	1-2	--	--	--	X	--	X	--
0.04	0.5	--	--	--	X	X	X	--
Irradiation period (h):								
24		X	X	X	--	--	--	X
12		--	--	--	X	X	X	--
Brdu exposure period (h):								
52		X	X	X	--	--	--	--
40		--	--	--	X	X	X	--

[a]Total dose was determined by triplicate thermoluminescent dosimeters placed at the rear of each exposure chamber. Control and MMC triplicate thermoluminescent dosimeters showed no radiation exposure.

CYTOGENETIC PREPARATION AND SCORING OF WORM CHROMOSOMES

Immediately following ^{60}Co exposure, each treatment group of worm larvae was transferred under amber light, using a large-bore plastic pipette, to 100- x 20-mm plastic culture dishes, each containing 30 ml of 3×10^{-5} M BrdU in filtered seawater. Nonirradiated control groups were also transferred to the same concentration of BrdU or to BrdU plus 5×10^{-7} M MMC. The BrdU and MMC exposures were carried out in the dark, and colchicine (0.4 mg/ml) was added to the seawater for the last 4 h of the BrdU exposure. Two different BrdU exposure times were used (Table 2).

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

Differential staining of the sister chromatids was accomplished by first staining for 10 min in 5-µg/ml Hoechst 33258 solution made up in 0.9% sodium chloride (pH 6). Hoechst-stained slides were rinsed for 5 min in distilled water; air dried for at least 20 min; placed in a shallow, clear plastic tray; and covered with 1/15 M Sorensen's 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) for 45 min, transferred for 6 to 10 min to 10% Giemsa stain in 1/15 M Sorensen's buffer, air dried, and mounted with permount. 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 equipped with a 10x objective, 63x objective, 1.2 optovar, and 12.5x oculars. Photographs were taken with Kodak 35-mm, 2415 technical pan film.

For each treatment, the proportion of metaphases identified as first, second, and third divisions after the beginning of BrdU exposure was recorded (see Fig. 1). Second-division metaphases were examined for the number of chromosomes that could be scored and the number of SCEs per metaphase. In addition, chromosome length was evaluated for each metaphase that was scored. Chromosomes were measured using an ocular micrometer and the degree of chromatin condensation was evaluated based on the length of chromosome pair number one (Pesch and Pesch, 1980b).

STATISTICAL ANALYSIS

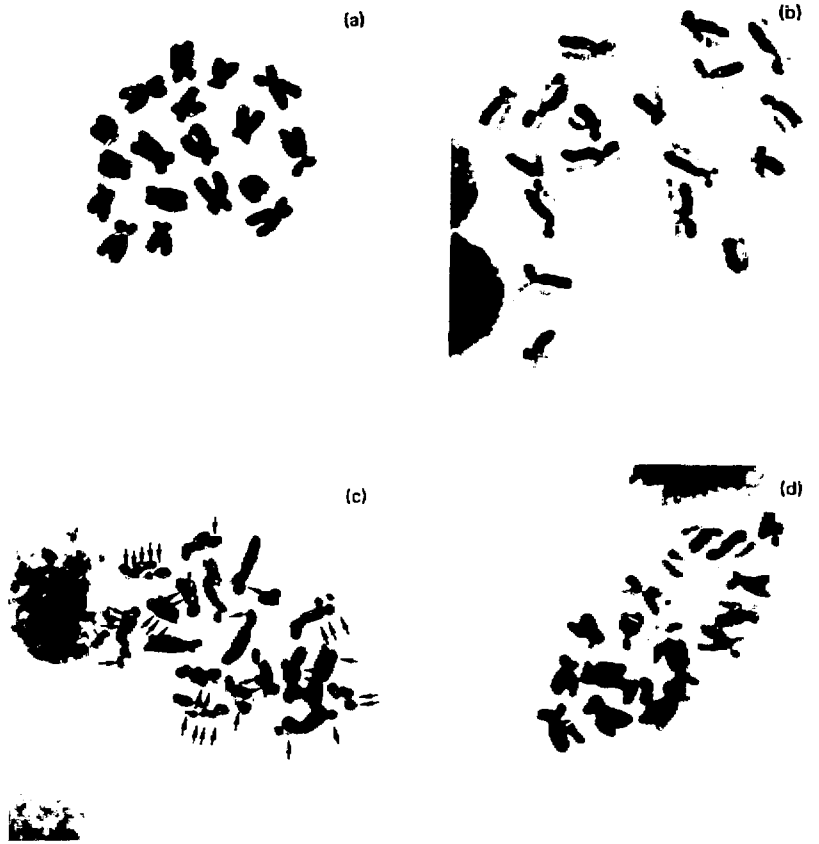
Statistical methods dealing with the complex populations of cells and events that whole-animal SCE data represent are not well developed. The mean of each treatment SCE frequency distribution ($\bar{X}$SCE) plus or minus the standard error of the mean was calculated. A simple, single-tailed test was also used to make initial comparisons between the SCE frequency distribution of control and those of treated larvae. For each exposure, we calculated the percentage of cells scored with a SCE per chromosome frequency greater in value than that found in 90% of the control cells (SCE >90%). The total number of control cells scored was 111. Of these, 10% (11 cells) had a SCE frequency per chromosome $\geq$0.58. For MMC (the positive control) a total of 92 cells was scored and, of these, 39 (42%) had a frequency $\geq$0.58. For each exposure, the percentage of scored cells that had a SCE frequency $\geq$0.58 was calculated and compared to the control and positive control.

QUALITY ASSURANCE

All slides were scored blind, and the same slides from the first experiment were scored randomly by Dr. G. Pesch (EPA, Narragansett) and David Rice and Rose Carrillo (LLNL). The results of the comparative scoring of the first experiment are summarized in the appendix. The XSCE observed by the EPA, Narragansett, and LLNL personnel were in good agreement except for the 13.0-R/h treatment.

RADIATION EFFECTS ON GROWTH AND DEVELOPMENT

Approximately 250 newly hatched larvae were exposed to 52 R (2.2 R/h) and 250 R (11.5 R/h) and examined for abnormalities and survival at 6 d (5 to 6 setiger larvae) and 17 d (27 to 30 setiger larvae) following irradiation.



IG. 1. Neanthes arenaceodentata metaphase chromosomes from cells that have undergone different number of replications after the beginning of BrdU exposure: (a) cell identified in first division, (b) cell identified in second division, (c) cell identified in second division and scored as an outlier (SCE >2.0 per chromosome), and (d) cell identified in third division. Image scale: 7 mm = 5 µm.

RESULTS

RADIATION EFFECTS ON SCE FREQUENCY

The frequency of SCEs in cells from worms irradiated with ^{60}Co varied with the dose rate and total exposure dose (Fig. 2, Table 3). For a given treatment, both the XSCE and the percentage of scored cells that had a SCE frequency per chromosome greater in value than that found in 90% of the control cells scored (SCE >90%) were determined. Among all the cells scored, six had very high SCE frequencies (SCEs >2.0 per chromosome) and were designated as outliers (Table 3, Fig. 1-c). These outliers seemed to be randomly distributed and not related to dose rate or total dose. Even though the number of outliers was low, the frequencies of SCEs in these cells were sufficiently high in some cases to substantially alter the XSCE values.

At the lower dose rates (0.04 and 0.1 R/h), the XSCE and SCE >90% were lower than those at intermediate dose rates and higher than those of the controls. At intermediate dose rates (0.5, 0.6, 1.25, 2.0, and 2.5 R/h), the XSCE and SCE >90% were all about twice the control values; at the high dose rates (7 and 13 R/h) the XSCE and SCE >90% were lower than the control values. These results demonstrate an increase in SCEs with certain ^{60}Co radiation regimes: a doubling of the control SCE frequency was detected at dose rates as low as 0.5 R/h and a total dose of 7 to 8 R. However, we cannot explain the decrease in SCEs at the higher dose rates and the similarities in XSCE and SCE >90% at the intermediate dose rates.

At a total dose of about 65 R, the SCE frequency in cells was much higher in worms irradiated at a dose rate of 2.5 R/h than in those irradiated at 5.0 R/h. In contrast, no apparent difference was seen between cells irradiated at 1.25 and 2.0 R/h, when the total dose delivered was about 25 R. These data are insufficient to distinguish between the effects of the total dose and dose rate.

Two different times of exposure to BrdU were used. In worms exposed to BrdU for 40 h, the percentage of cells in second division was 56 ± 8 and in those exposed for 52 h it was 54 ± 8. Because this difference of 12 h in BrdU exposure did not change the percentage of the cells found in second division, data from the 40- and 52-h exposure were combined. Our preliminary data on the number of first, second, and third divisions found in cells from larvae exposed to BrdU for increasing times indicate that the majority of the worm cells have a cell-cycle time of approximately 25 h.

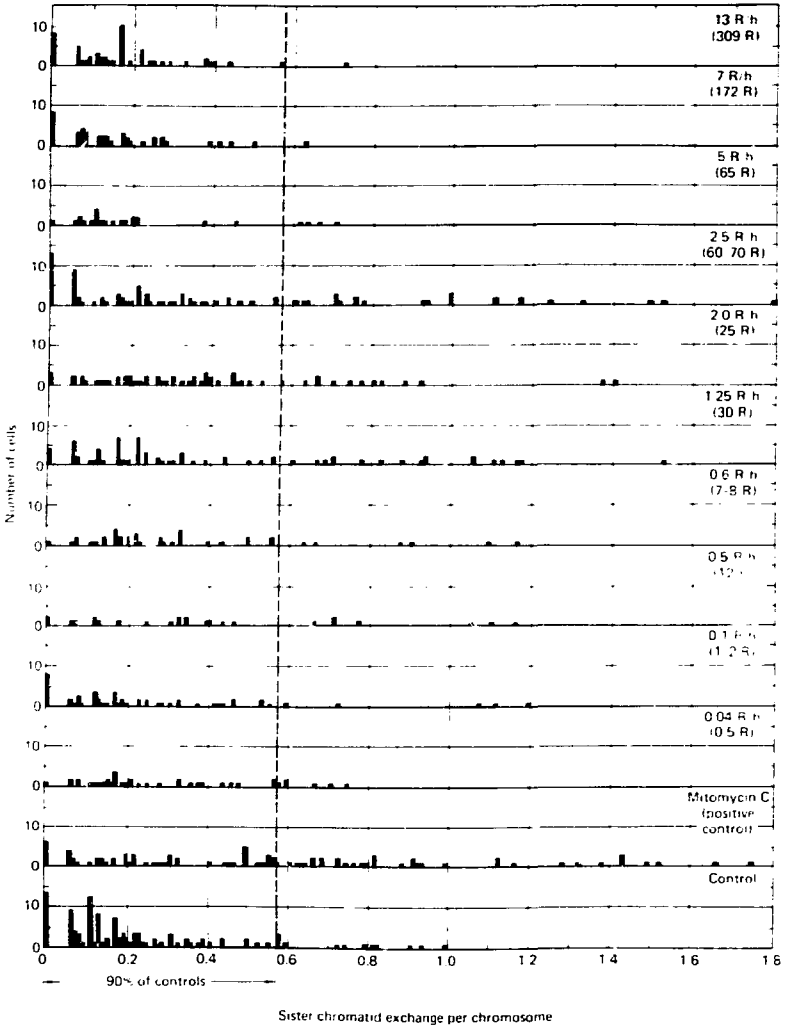


FIG. 2. The SCE frequency distributions observed in Neanthes arenaceodentata larvae following ^{60}Co irradiation. The ^{60}Co dose rate is shown in R/h. The total dose in R is given in parenthesis.

TABLE 3. The SCE frequencies in cells from Neanthes arenaceodentata larvae exposed to ^{60}Co radiation.

Dose rate, R/h	Exposure time, h	Dose, R	Number of cells scored	$\bar{X}SCE$[a]	XSCE[b]	SCE >99%[c]	Experiments pooled	SCE per chromosome of embryos XSCE
13.0	24	309	94	0.17 ± 0.02	0.21 ± 0.03	4	1	0.12
7.3	24	172	45	0.16 ± 0.02	0.13 ± 0.01	2	1	0.11
5.3	12	63	29	0.24 ± 0.04	0.24 ± 0.04	10	1	-
2.3	24	55-70	59	0.41 ± 0.03	0.43 ± 0.03	20	1	0.28
2.5	12	29	61	0.39 ± 0.04	0.39 ± 0.04	21	1	--
1.25	24	30	74	0.39 ± 0.04	0.40 ± 0.05	24	1	0.32
0.6	12	7-8	42	0.53 ± 0.06	0.49 ± 0.06	17	1	0.33
0.3	24	7	29	0.42 ± 0.07	0.42 ± 0.07	28	1	--
0.1	12	1-2	54	0.27 ± 0.04	0.27 ± 0.04	9	1	--
0.04	12	0.5	38	0.32 ± 0.03	0.32 ± 0.03	16	1	--
Control	--	--	111	0.22 ± 0.02	0.23 ± 0.03	12	1	0.13
H2O2 (0.1%)	--	--	92	0.56 ± 0.03	0.56 ± 0.03	42	1	--

[a]Mean SCE per chromosome ± standard error of the mean (SEM). Cells with > 2.0 SCE per chromosome were not included in XSCE.

[b]Mean SCE per chromosome ± SEM. Cells with > 2.0 SCE per chromosome were included in XSCE.

[c]Percent of scored cells with a SCE per chromosome frequency greater in value than that found in 99% of control cells scored, that is, > 0.58 SCE per chromosome.

EFFECTS OF CHROMOSOME LENGTH ON SCORING

The length of chromosome pair number one differed among metaphase spreads. The SCE control and MMC-treated cells containing chromosome pair number one having lengths >6.0 μm were compared with those having lengths ≤6.0 μm (Figs. 3 and 4 and Table 4). In cells from control worms that have low SCE incidence, there was little difference in SCE frequency distribution and XSCE between those designated as >6.0 μm and ≤6.0 μm. In cells from MMC-exposed worms, the XSCE for metaphase spreads with chromosome pair number one scored as >6.0 μm was higher than in those scored as ≤6.0 μm. Also, the frequency distribution of the SCEs in the MMC cells was not the same: in cells where the length of chromosome pair number one was scored as >6.0 μm, the incidence of metaphases with less than 0.2 SCE per chromosome was low. Because the total number of cells scored was low, this difference may not be significant.

RADIATION EFFECTS ON GROWTH AND DEVELOPMENT

The larvae that had been exposed to 52 and 280 R survived well and had few abnormalities (Table 5). At the observation times used, no differences between control and irradiated larvae were detected, indicating that study of growth and development is not a sensitive tool for measuring low-level radiation effects.

FIG. 3. Neanthes arenaceodentata metaphase chromosomes from cells in second division: (a) chromosome pair number one length scored as >6.0 μm and (b) chromosome number one length scored as ≤6.0 μm. Image scale: 7 mm = 5 μm.

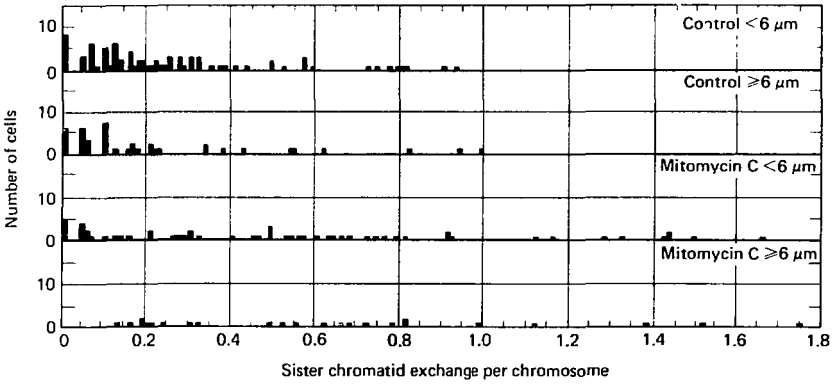


FIG. 4. The SCE frequency distributions in control and MMC-treated Neanthes arenaceodentata larval cells having chromosome pair number one lengths of >6.0 μm and of ≤6.0 μm. Data pooled from experiments 1 through 6.

TABLE 4. The SCE frequency distribution in control and MMC-treated Neanthes arenaceodentata larval cells having chromosome pair number one lengths of >6.0 μm and ≤6.0.[a]

Dose	Chromosome length	$\bar{X}$SCE	Number of cells scored
Control	>6.0 μm	0.21	40
	≤6.0 μm	0.26[b]	80
Mitomycin C	>6.0 μm	0.65	25
	≤6.0 μm	0.53	57

[a]Data pooled from experiments one through six.

[b]Outlier of 2.43 SCE per chromosome not included in mean.

TABLE 5. Percentage of abnormal larvae and of larval survival after exposure to ^{60}Co radiation.

	Days after ^{60}Co irradiation		
	6		17
Dose	% survival	% abnormal	% survival[a]
Control	99.2	5.4	84.0
52 R	97.4	4.9	89.0
280 R	99.0	1.0	87.5

[a]All larvae appeared normal.

DISCUSSION

The lowest dose rate used in this study (0.04 R/h) was similar to the estimated maximum possible dose rate of about 45 mrem/h at the Windscale radioactive waste disposal site in the Irish sea (Woodhead, 1980). Furthermore, the IAEA (1976) concluded that the lowest dose rate at which minor radiation-induced disturbances of physiology or metabolism might be detectable in fish is on the order of 40 mrem/h.

Our results indicate that SCEs are induced by low dose rates of ionizing radiation delivered in vivo to Neanthes arenaceodentata. Beta radiation delivered at dose rates comparable to those used in this study resulted in increased SCE frequencies in mammalian cells. Chinese hamster chromosomes exposed for 28 h to ^{3}H-thymidine (0.5 rad/h beta radiation) had XSCE frequencies per chromosome of 0.35 (Gibson and Prescott, 1972) and 0.42 (Kato, 1974). In worm larvae irradiated at a dose rate of 0.5 R/h and 0.6 R/h we found that the increase in XSCE frequency per chromosome over control values was 0.25 and 0.18, respectively.

Available data, both from this and other studies, are insufficient to determine the roles that dose rate and total dose play in SCE induction. Our preliminary data indicate that dose rates and total doses requiring further examination lie between 0.04 and 5.0 R/h and 0.5 and 70 R, respectively.

A plateau in the SCE induction rate was found for beta-radiation dose rates between 1.0 and 1.4 rad/h (Gibson and Prescott, 1972; Kato, 1974). We found little change in the SCE induction rate for gamma-radiation dose rates between 0.5 and 2.5 R/h. The existence of a plateau may limit the range of dose rates over which SCEs in Neanthes arenaceodentata could be used as an indicator of in vivo environmental exposure to radiation. Our data from preliminary experiments indicate that the useful range of dose rates may be between background and 0.5 R/h. Detection of changes in SCE frequencies greater than the controls will require large data bases and accurate statistical models.

To validate the usefulness of SCE frequency as a measure of radiation effect in benthic worms, more information is needed on factors that may affect SCE incidence and the ability to score them. The following need to be evaluated:

- effect of cell-cycle stage at the time of irradiation,
- effect of life-history stage at the time of irradiation,
- length of time induced SCEs are retained in cells,
- effect of chromosome length on ability to score, and
- effect of sampling time on SCE frequency.

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 SCE 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), whereas for chromosomal aberration induction, G_2 is the most sensitive stage (Carrano, 1975; Yu, 1976). Because Neanthes 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 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 BrdU after a 26 h delay following 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.

There is a possibility that chromosome length may affect the ability to resolve SCEs. Longer chromosomes may allow better resolution of SCEs very close together. Another possibility is that the longer chromosomes may represent a population of cells that enter metaphase late in the colchicine exposure and this population of cells may have a higher SCE frequency distribution. Future experiments to examine changes in SCE incidence with increasing time following irradiation should continue to monitor the effect chromosome morphology has on observed SCE frequency distributions.

A final factor deals with 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 must be established for adults.

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APPENDIX

TABLE A-1. Comparative scoring of experiment number one by LLNL and the EPA, Narragansett Laboratory.[a]

Dose rate, R/h	LLNL		EPA, Narragansett	
	Number of metaphases scored	$\bar{X}$SCE per chromosome[b]	Number of metaphases scored	$\bar{X}$SCE per chromosome[b]
13.0	22	0.16[c]	24	0.32
7.0	16	0.17	24	0.21
2.5	28	0.36	24	0.34
1.25	28	0.46	24	0.38
Control	24	0.10	25	0.15
Mitomycin C	14	0.51	25	0.59

[a]Mean number of chromosomes scored per metaphase by LLNL and the EPA was 16.3 and 17.6 respectively.

[b]Metaphases >2.0 SCE per chromosome not included in XSCE.

[c]This treatment was rescored by LLNL. The XSCE per chromosome for the rescored treatment was 0.14.