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Considerations for Gamma Irradiation of Aquatic Organisms

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Abstract.—Ionizing radiation has been used for decades in studies involving aquatic organisms to produce androgenesis and gynogenesis and in attempts to produce reproductively sterile individuals. In these studies, gametes or organisms were exposed to a broad range of doses of ionizing radiation in various containers to obtain the desired results. However, there are many factors that affect irradiation levels and cause target doses and actual absorbed doses to be different. Our goal in this study was to use intensive dosimetry to determine the variation in dosage possible in irradiation of aquatic organisms. Using the Fricke dosimetry technique, we found suprisingly high levels of vertical and horizontal variation in dose rates inside of a small (but typical) container (14.5 cm high, 16.7 cm wide, and 2.8 L in volume). The presence or absence of container rotation further affected these variations. When the container was rotated, vertical variation ranged between 1% and 21%, and horizontal variation ranged between 10% and 19%. When the container was not rotated, vertical variation ranged between 6% and 28%, and horizontal variation ranged between 20% and 72%. Comparisons between air and water as a surrounding medium showed that the dose rate in air could be 42% higher than in water when the container rotated and as much as 218% higher in air when the container did not rotate. These variations would cause samples irradiated in water to receive dosages lower than desired. It is therefore crucial that careful dosimetry be performed before carrying out experiments and that they be performed in the same medium in which specimens are to be irradiated (presumably water for aquatic organisms). Such measurements are typically not reported or are not performed in irradiation studies of aquatic organisms. Experimental errors such as these would complicate comparisons among studies and organisms and hinder development and application of valuable techniques such as radiation-induced sterilization of genetically modified organisms.

Ionizing radiation is commonly used to eliminate chromosome sets in fish and other aquatic organisms to produce androgenesis (all-paternal inheritance; Thorgaard et al. 1990; Nagoya et al. 1996) and gynogenesis (all-maternal inheritance; Dai et al. 1993). Androgenesis and gynogenesis have numerous potential applications, including the generation of homozygosity, in which the remaining chromosome set is duplicated by suppression of the first cleavage, and recovery of genotypes from cryopreserved sperm (Thorgaard 1986). Ionizing radiation is routinely used to sterilize pest insect species (Steiner et al. 1965, 1970). It has also been used to induce sterility in some fish species (Nelson et al. 1976; Konno and Tashiro 1982; Hanson 1990). Reproductively sterile individuals reduce the potential hazards caused by the introduction of exotic species or genetically modified organisms.

Radiation affects cells primarily by damage to nuclear DNA through base damage, single strand breaks, or double strand breaks. These breaks may result in chromosomal aberrations that prevent proper distribution and replication of the chromosomes and their transmission to daughter cells during cell division. For example, the frequency of DNA alterations detected in DNA fingerprinting analysis increased with increasing dose in embryos of the F_1 progeny of male Japanese medaka *Oryzias latipes* exposed to 950 and 1,900 radiation absorbed doses (rad = 0.01 gray) of gamma rays (Kubota et al. 1992, 1995). Chromosome fragments, including ring chromosomes, were reported in adult rainbow trout *Oncorhynchus mykiss* and their offspring as a result of exposing sperm to

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60,000 rad of gamma rays to produce gynogenetic individuals (Disney et al. 1987, 1988).

In such studies, electromagnetic radiation that could be used to treat gametes includes ultraviolet light, X rays, and gamma rays. However, gamma rays are the most commonly used form of ionizing radiation. Among the radioisotopes, cobalt 60 is the preferred radiation source because it is a high-energy gamma emitter (Sinclair 1969) and has a usefully long half-life of more than 5 years. In determining the dose rate of gamma radiation, measurements need to address the surrounding medium. Doses obtained from dosimetry using air as a surrounding medium are accurate if the sample to be irradiated will be surrounded by air. However, in irradiation of aquatic animals, samples frequently need to be in water, and doses obtained in air will not indicate energy delivery in water. This is because gamma radiation generates an electron flux when passing through materials, and changes in the flux occur at boundaries of different materials. A change in flux produces a change in dose rate for a given emission (Jefferson 1964).

Because aquatic animals have a high water content and are normally in water during irradiation, dosimetry should be performed in water and should use the container that will be used in the irradiation of samples. We chose to study dose variation because many published works on aquatic species do not indicate performance of dosimetry before irradiation or indicate that dosimetry was performed in air.

Fricke and Morse (1927) reported a dosimetric method that measured the conversion of ferrous iron (nonionic) to ferric iron (ionic) by ionizing radiation. This method allows measurement of energy deposition from radiation by a chemical change that is a direct function of the dose received. This procedure has been widely accepted as one of the most reliable techniques available for routine dosimetry.

In this paper, we discuss irradiation protocols for aquatic animals and some of the factors that need to be considered when water is used as a surrounding medium. The objectives of this study were to (1) determine the dose rates at various positions inside an irradiation container, (2) compare the dose rates with and without rotation of the container on a turntable, and (3) compare the dose rates when water or air were in the container. We found considerable variation in radiation dose in this study (>200%), indicating the need for careful standardization of dosimetry in irradiation studies.

Methods

Fricke solution (1 L) was prepared by dissolving 0.40 g of $FeSO_4 \cdot 7H_2O$, 0.06 g of NaCl, and 22 mL of concentrated (95.8%) sulfuric acid (H_2SO_4) in deionized water (Shalek et al. 1962). The solution was placed in glass tubes (Vacutainer VT6434, Becton Dickinson, New Jersey) with airtight and watertight closures. The tubes were 7.5 cm long and 1.3 cm wide and contained 5.5 mL of Fricke solution.

A Shepherd irradiator (model 484R), which is shielded by metal, was used for all irradiations. This irradiator has a high rate of gamma emission (which reduces the experimental time and stress on aquatic animals), a digital timer with time in minutes that is adjustable to 2 decimal places (ensuring accurate doses), and a turntable that rotates the samples (to ensure a uniform exposure within the container). This irradiator has a dual-source system and uses cobalt-60; it contains two 1,500-curie sources and one 5-curie source. The source transport system is electropneumatic, providing a reproducible geometry and travel time (Shepherd 1996). The central turntable rotates at 6 revolutions/min. All samples were placed in the center of the turntable during irradiation. The chamber of the irradiator was 25 cm high × 25 cm wide × 30 cm deep.

A cylindrical plastic container (Rubbermaid, Inc., Wooster, Ohio), which had a height of 14.5 cm, a diameter of 16.7 cm, and a volume of 2.8 L, was chosen as a model irradiation container for aquatic species. This container was chosen because it fit well inside the irradiator chamber, was inexpensive and easily obtained, had a watertight cap, was of a suitable size for irradiation of different species and life stages of aquatic animals, and was made of plastic, which did not affect the penetrating ability of gamma rays. The glass tubes containing Fricke solution were placed at three levels within the plastic container (Figure 1). Level 1 was 0.4 cm above the bottom, level 2 was 3.9 cm above level 1, and level 3 was 3.9 cm above level 2 (2.4 cm below the top of the container). At each level, four tubes were placed at equal distances from each other on the inner surface circumference of the container, and one glass tube was placed at the axial center (Figure 1). Duct tape (Duck Brand, Manco, Inc., Avon, Ohio) was used to attach the tubes to the sides of the container. The container was filled with tank water from a recirculating-water system until the level was 0.5 cm above the tubes at level 3. The water quality

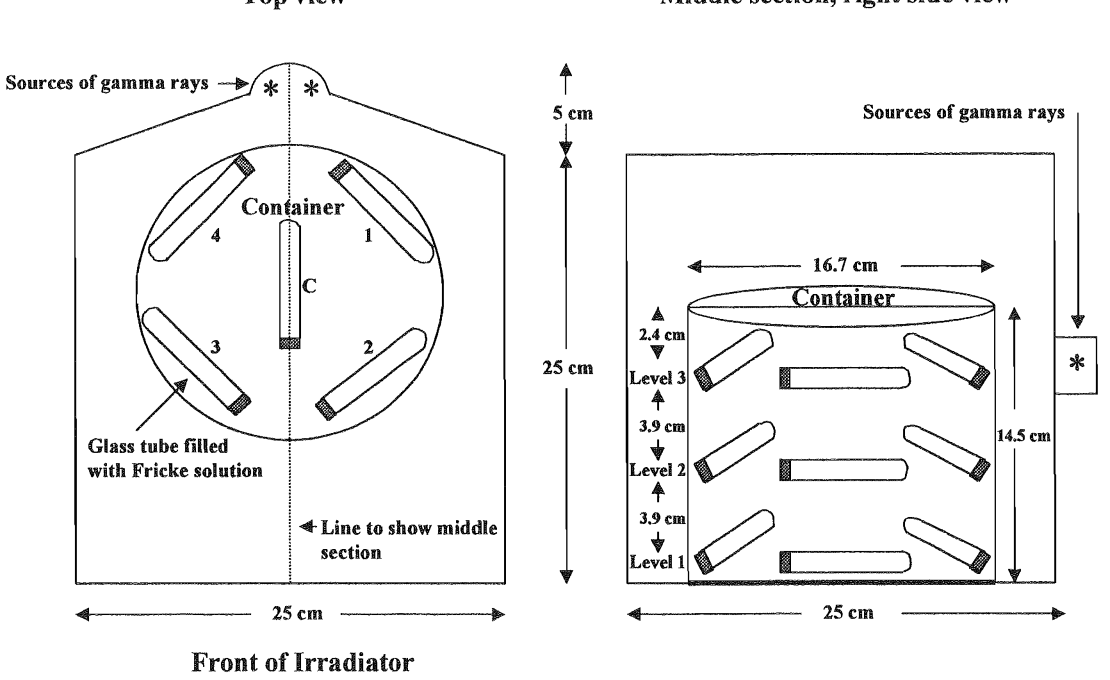

FIGURE 1.—The position of the irradiation container inside the chamber of the Shepherd irradiator. The top view shows the position of the dosimetry tubes at level 1 relative to the sources of gamma rays (C refers to center). The right side view shows the position of the three levels inside the container. In all cases, the tubes were positioned horizontally in a single plane.

values (mean ± SD) were 27 ± 1°C, 7.8 ± 0.2 mg/L for dissolved oxygen, 132.8 ± 18.5 mg/L for alkalinity, 7.6 ± 0.4 for pH, 0.025 ± 0.005 mg/L for nitrites; ammonia was not detectable (<0.05 mg/L).

Irradiations were carried out with the two 1,500-curie cobalt 60 sources, which totaled 3,000 curies, as calibrated by the manufacturer on 16 December 1997, 9 March 1998, and 30 March 1998. The turntable was rotated during irradiation of these samples. In all, 270 dosimeters were exposed at different exposure times at the three levels in the container. The exposure times were 1.75 min, 3.50 min, 5.20 min, 8.67 min, 13.83 min, and 16.42 min. There were two replicates for each of these exposure times. On 9 March 1998, irradiation was carried out for 16.42 min as described above without turntable rotation.

The absorbance values of the irradiated solutions and the blanks were determined with a spectrophotometer (Spectronic 1201; Milton Roy, Rochester, New York) at a wavelength of 304 nm. Unirradiated Fricke solution was used as a blank. The dose in rad was calculated from the formula

$$\text{rad} = \frac{10^9(\text{Ai} - \text{An})}{EbG} = 2.95 \times 10^4(\text{Ai}),$$

where Ai = absorbance of irradiated solution; An = absorbance of blank (set as zero); E = molar extinction coefficient (2,174 L · mol^{-1} · cm^{-1} for ferric ion); b = cell thickness of cuvette (1.0 cm); and G = yield term in ions/100 eV (15.6 for Fe^{+++}). A rad is defined as 100 ergs of energy imparted by any ionizing radiation that is dissipated in 1 g of any irradiated material (Wang et al. 1975).

Comparisons were also made between the dose rate calculated by the supplier of the irradiator with air as the surrounding medium and the values obtained in this study using water. The values in rad obtained at different dates in this study and those provided by the supplier were corrected for radioactive decay to 30 March 1998, using the formula

$$A_t = A_0 e^{-\lambda t},$$

where A_t = activity at time t; A_0 = activity at time 0; t = elapsed time in years; e = base of natural

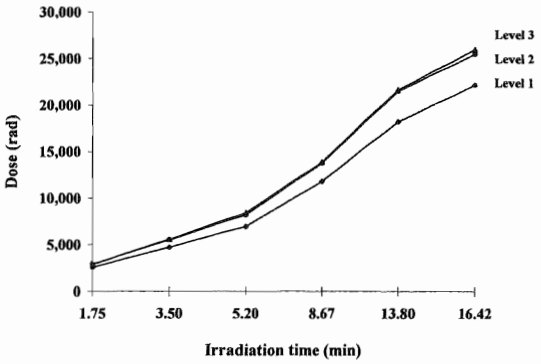

FIGURE 2.—The relationship between dose (radiation absorbed doses [rad] = 0.01 gray) and irradiation time for the three levels of the irradiation container. Data include the middle value for each level.

log; λ = decay constant = 0.693/half-life of cobalt 60 = 0.693/5.26 years.

Differences in dose rates between the side and middle of the container at each level and among levels within the side and middle of the container when the turntable was not rotated were calculated from the values of the mean. The differences in dose rates among the positions and levels when the turntable was not rotated were calculated in the same way. Percent differences were calculated as the difference in dose rate divided by the larger of the two values and multiplying by 100.

Statistical analyses were performed using SAS statistical analysis software for PC (version 6.10; SAS Institute, Cary, North Carolina). The null hypothesis (no interaction between duration of exposure, level, and position) was tested using split-plot design followed by Bonferroni *t*-tests. A value of $P \leq 0.01$ was chosen as the level for significance. Differences in dosage between air and water as the medium were determined by one-way factorial analysis (analysis of variance, ANOVA). Means were separated with Duncan's multiple-range test and were considered significant when $P \leq 0.01$.

Results

Cobalt 60 has a half-life of 5.26 years. On 30 March 1998, the dose rate in the irradiator (with air as the surrounding medium) was 1,824 rad/min.

In all, 270 dose measurements were made within the container with the turntable rotating. As expected, the doses at the three levels in the container increased with irradiation time (Figure 2). There was a significant interaction among the duration of exposure, level, and position in the container ($P = 0.0001$). Significant differences in dose were also found among the levels ($P < 0.01$). Doses recorded at level 1 were significantly lower than those at levels 2 and 3 for all irradiation times. There was no significant difference in dose between levels 2 and 3 for all irradiation times.

Vertical Variation When the Turntable Was Rotated

Significant differences in dose rate were found among the three levels at the sides of the container ($P = 0.0001$). The dose rate at level 1 (1,414 rad/min) was lower than at level 2 (1,652 rad/min) and level 3 (1,635 rad/min), but no significant difference was detected between levels 2 and 3 (Table 1). There was a 14% (237 rad/min) difference in dose rate between levels 1 and 2, and a 14% difference (220 rad/min) between levels 1 and 3 (Table 2).

Significant differences were also found among the dose rates at the middle of the container ($P = 0.0001$). The dose rate at level 1 (1,153 rad/min) was significantly lower than at level 2 (1,317 rad/min) and level 3 (1,465 rad/min). The dose rate at level 2 was significantly lower than the dose rate at level 3 (Table 1). The differences in dose rates were 13% (165 rad/min) between levels 1 and 2,

TABLE 1.—Dose rates (mean ± SD) at three levels with or without rotation of the turntable. The position of the glass dosimetry tubes is shown in Figure 1 ("rear" refers to positions 1 and 4, "front" refers to positions 2 and 3). Means within a column followed by a common letter were not significantly different ($P > 0.05$). Radiation absorbed dose (rad) = 0.01 gray.

	Dose rate (rad/min), turntable rotated		Dose rate (rad/min), turntable not rotated		
Level	Side ($N = 72$)	Middle ($N = 18$)	Rear ($N = 2$)	Middle ($N = 1$)	Front ($N = 2$)
1	1,414.9 ± 76.0 y	1,153.4 ± 38.6 x	2,008.6 ± 84.4	1,228.5	588.4 ± 25.2
2	1,652.1 ± 60.0 z	1,317.9 ± 31.3 y	2,324.9 ± 57.9	1,394.3	660.6 ± 18.8
3	1,635.1 ± 42.7 z	1,465.5 ± 46.8 z	2,135.1 ± 92.0	1,715.2	766.7 ± 37.8

TABLE 2.—Dose rate differences and percent differences in dose rates among the different levels and positions inside the container when the turntable was rotated. Radiation absorbed doses (rad) = 0.01 gray.

Comparison	Difference	
	rad/min	Percent[a]
Level 1 (side versus middle)	261.5	18.5
Level 2 (side versus middle)	334.2	20.2
Level 3 (side versus middle)	169.6	10.4
Side (level 1 versus level 2)	237.2	14.4
Side (level 1 versus level 3)	220.2	13.5
Side (level 2 versus level 3)	17.0	1.0
Middle (level 1 versus level 2)	164.5	12.5
Middle (level 1 versus level 3)	312.1	21.3
Middle (level 2 versus level 3)	147.6	10.1

[a] Calculated as the difference in dose rate divided by the larger of the two values and multiplied by 100.

21% (312 rad/min) between levels 1 and 3, and 10% (148 rad/min) between levels 2 and 3 (Table 2).

Vertical Variation When the Turntable Was Not Rotated

The dose rate at the three levels located at the rear of the container ranged between 2,008 rad/min at level 1 and 2,324 rad/min at level 2 (Table 1). There was a difference of 14% (316 rad/min) between levels 1 and 2, 6% (127 rad/min) between levels 1 and 3, and 8% (190 rad/min) between levels 2 and 3 (Table 3).

At the middle of the container, the dose rate ranged between 1,228 rad/min at level 1 and 1,715 rad/min at level 3 (Table 1). There was a difference of 12% (166 rad/min) between levels 1 and 2, 28% (487 rad/min) between levels 1 and 3, and 19% (321 rad/min) between levels 2 and 3 (Table 3).

Dose rate at the front of the container ranged between 588 rad/min for level 1 and 766 rad/min for level 3 (Table 1). The differences in dose rate were 11% (72 rad/min) between levels 1 and 2, 23% (178 rad/min) between levels 1 and 3, and 14% (106 rad/min) between levels 2 and 3.

Horizontal Variation When the Turntable Was Rotated

The sides of the container had a higher dose rate than did the middle at all three levels (Table 1). The difference in dose rate between the sides and middle due to shielding of the middle by the water was 19% (262 rad/min) at level 1, 20% (334 rad/min) at level 2, and 10% (170 rad/min) at level 3 (Table 3).

TABLE 3.—Dose rate differences and percent differences in dose rates among the different levels and positions inside the container when the turntable was not rotated. Radiation absorbed dose (rad) = 0.01 gray.

Comparison	Difference	
	rad/min	Percent[a]
Level 1 (front versus rear)	1,420.2	70.7
Level 2 (front versus rear)	1,664.3	71.6
Level 3 (front versus rear)	1,368.4	64.1
Level 1 (front versus middle)	640.1	38.8
Level 2 (front versus middle)	733.7	40.0
Level 3 (front versus middle)	948.5	19.7
Level 1 (rear versus middle)	780.1	47.9
Level 2 (rear versus middle)	930.6	52.6
Level 3 (rear versus middle)	419.9	55.3
Front (level 1 versus level 2)	72.2	10.9
Front (level 1 versus level 3)	178.3	23.3
Front (level 2 versus level 3)	106.1	13.8
Middle (level 1 versus level 2)	165.8	11.9
Middle (level 1 versus level 3)	486.7	28.4
Middle (level 2 versus level 3)	320.9	18.7
Rear (level 1 versus level 2)	316.3	13.6
Rear (level 1 versus level 3)	126.5	5.9
Rear (level 2 versus level 3)	189.8	8.2

[a] Calculated as the difference in dose rate divided by the larger of the two values and multiplied by 100.

Horizontal Variation When the Turntable Was Not Rotated

The distance from the radiation sources affected the dose rate received when the turntable was not rotated. At all three levels, the rear (closest to the source) received more radiation than did the middle or front (Table 1). The difference in dose rate between the front and rear was 71% (1,420 rad/min) at level 1, 72% (1,664 rad/min) at level 2, and 64% (1,368 rad/min) at level 3 (Table 3). The middle received 39% (640 rad/min) more radiation than did the front at level 1, 40% more (734 rad/min) at level 2, and 20% more (949 rad/min) at level 3. The rear received 48% (780 rad/min) more radiation than did the middle at level 1, 53% more (931 rad/min) at level 2, and 55% more (420 rad/min) at level 3 (Table 3).

Air or Water as Medium

The dose rate in air was always higher at all levels compared with the rate when water was used as a surrounding medium (Figure 3). This variation was affected by whether or not the turntable was rotating during irradiation. When the turntable was rotated, the mean dose rate in air was significantly higher than in water ($P = 0.0001$). No significant difference in mean dose rate between the two media was detected when the turntable was not rotated ($P = 0.05$).

At the sides of level 1, the dose rate in air was

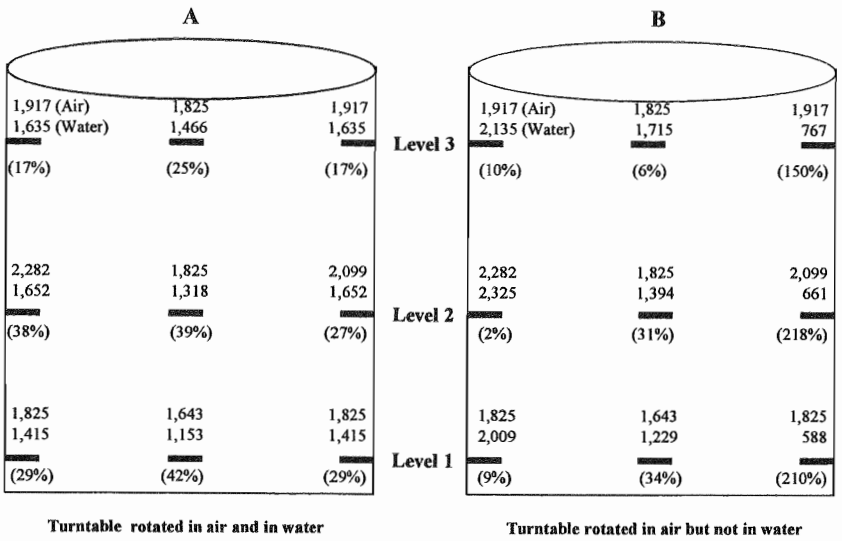

FIGURE 3.—The dose rate (radiation absorbed doses [rad] = 0.01 gray) at the three levels of the irradiation container for two different surrounding media. The top value is for samples irradiated in air and the lower value is for samples irradiated in water. In **(A)**, the turntable was rotated in air and water; in **(B)** the turntable was rotated in air but not in water. The percent differences in dose rate between air and water is given in parentheses.

29% higher (410 rad/min) than in water when the turntable was rotated. In the middle, the dose rate was 42% higher (490 rad/min) in air (Figure 3A). The difference at level 2 was 38% (630 rad/min) at the sides and 39% (507 rad/min) in the middle of the container. At level 3, the difference in dose rate was 17% (282 rad/min) at the sides and 25% (359 rad/min) in the middle.

There were greater variations in dose rates inside the container when the turntable was not rotated. At the back of the container at level 1, the dose rate was 10% lower (183 rad/min) in air than in water, but it increased to 34% higher (414 rad/min) in the middle and to 210% higher (1,237 rad/min) at the front of the container (Figure 3B). At the back of level 2, the dose rate was 2% lower (43 rad/min) in air than in water, 31% higher (431 rad/min) in the middle, and 218% higher (1,439 rad/min) at the front. At level 3, the dose rate at the back was 11% lower (219 rad/min) in air than water, 6% higher (110 rad/min) in the middle, and 150% higher (1,150 rad/min) at the front.

Discussion

In the irradiation of gametes, aquatic organisms, and other biological materials, it is desirable that samples receive equal and uniformly distributed doses. However, we found vertical and horizontal variations in dose rate inside the container. In addition, the presence or absence of turntable rotation affected the dose rate at various locations in the container. When the turntable was rotated, a 4-cm difference in height resulted in as much as a 14% difference in dose rate. Similarly a 4-cm difference in width resulted in as much as a 19% difference in dose rate. When the turntable was not rotated, a 4-cm difference in height resulted in as much as a 19% difference in dose rate, and a 4-cm difference in width resulted in a 55% difference in dose rate. In one of the few studies that reports dosimetry, Hanson (1990) found during irradiation of sea lampreys *Petromyzon marinus,* that the front of the container (30 cm × 30 cm × 30 cm, filled with 30 cm of water), which was nearer the source of radiation, received more than five times as much radiation as the back.

We also found that when the turntable was rotated, the dose rate in air could be as much as 42% higher than the rate in water. When the turntable was not rotated, the dose rate in air could be as little as 2% lower than the rate in water for areas nearest the source but as much as 218% higher for areas farthest from the source. This was a result of the specific linear energy transfer in air, which is nearly three orders of magnitude lower than that of water (less energy is transferred to the air than water making more energy available for the sample).

The rotation of the turntable reduced the vertical and horizontal variations in dose rate. When the

turntable was rotated, the sides of the container at the same level received similar doses of radiation, and there was also less difference in dose rate between the middle and sides of the container at the same level. In the absence of turntable rotation, a large horizontal variation (as much as 1,664 rad/min) occurred between the sides nearest and farthest from the radiation sources and as much as 949 rad/min between the sides and middle. The position of the source of radiation and the size and volume of the container influenced the dose rate at various positions inside the container. In this study, when the turntable was rotated, the lowest vertical and horizontal variations were recorded between levels 2 and 3. These two levels were nearest to the sources of radiation. When the turntable was not rotated, areas nearest the source received the highest dose rates, whereas those farthest from the source received the lowest rates due to the shielding effect of the water. A container of different size or shape could yield a different dose rate at the various levels. Similarly, an irradiator with a source of radiation emitting from a different angle would yield a different result.

It is difficult to expose a specimen to an exact required absorbed dose but it is possible to reduce the error. Various positions inside of containers could be used to irradiate different types of specimens. If, for example, a whole-body exposure was required (using the container we examined) for a larger-sized organism, it could be contained within levels 2 and 3 with rotation of the turntable. For the type of irradiator we used, it would not be possible to irradiate large specimens without variations in the absorbed dose in the absence of turntable rotation. If eggs or spermatozoa were to be irradiated, they could be held in test tubes and positioned at specific levels depending on the sample volume. Specimens such as these could be irradiated with or without rotation of the turntable.

Although there are many factors that could affect the dose rate, variations could be reduced if published reports and protocols used in the irradiation of aquatic organisms were standardized. Such standardization would seek to identify and correct common sources of dosage error (Table 4).

TABLE 4.—Recommendations for the irradiation of aquatic organisms.

Recommendation	Alternative
1. Perform dosimetry in water in the same container to be used for experiments	1. Calculate approximate dose rate in water from values obtained in air
2. Use turntable rotation during the irradiation process	2. If turntable is not present use the center position or a restricted area
3. Standardize vertical position of samples in container	3. None
4. Recalculate dose rate for radioactive decay to the actual experimental date	4. None
5. Use timer installed on irradiator for the required time exposure	5. If irradiator has no timer, time exposure as accurately as possible
6. Beware of the possible sources of variation when comparing among studies	6. None
7. Report these sources of variation whenever results are published	7. None

Implications and Practical Considerations

In the irradiation of aquatic organisms, it is important that careful dosimetry be carried out before experimentation and that the same medium be used that will surround the specimen. It is also important to standardize position, even inside a small container. Changes in the output of the source of radiation also require that the dose rate obtained by dosimetry be corrected for radioactive decay to the date when the experimentation was performed to avoid month-to-month errors in dose rates. Our study shows that the sources of variation included position within the container, surrounding medium, and whether or not the container was rotated. Within air or water, there were vertical and horizontal variations in dose rate inside the irradiation container.

In practical terms, substantial experimental error could be produced by these variations. For example, in our studies on the effects of gamma ray irradiation of the freshwater prawn *Macrobrachium rosenbergii,* eggs were irradiated at target doses ranging from 1 krad to 30 krad (unpublished data). Assuming the values obtained from dosimetry performed in air were used as the dose rates of the source, with rotation of the turntable and placement of eggs at the bottom and in the middle of the container, the eggs would have received only 70% of the desired doses (e.g., 0.7 krad instead of 1 krad and 21 krad instead of 30 krad). If eggs were placed at the sides of the container, they would have received 77% of the desired doses (0.8 krad instead of 1 krad and 23 krad instead of 30 krad). If the turntable was not rotated, they eggs would have received 110% of the desired doses if they were placed at the back (1.1 krad instead of 1 krad and 33 krad instead of 30 krad), 75% of the desired doses if they were placed in the middle of the container (0.8 krad instead of 1 krad and 22 krad instead of 30 krad), and 32% of the desired

doses if they were placed at the front (0.3 krad instead of 1 krad and 10 krad instead of 30 krad).

Future studies should evaluate the potential for experimental error in radiation doses in previous studies. Undocumented error in dose would complicate comparisons among studies and organisms. This is especially important in the delivery of radiation where differences in spatial variation can be multiplied by long exposure times. These results and conclusions would also hold true for irradiation in aquatic species by use of other forms of ionizing radiation, such as X rays.

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