**GUIDANCE DOCUMENT FOR PREPERMIT BIOASSAY TESTING
OF LOW-LEVEL RADIOACTIVE WASTE**

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FOREWORD

In response to the mandate of Public Law 92-532, the Marine Protection, Research, and Sanctuaries Act (MPRSA) of 1972, as amended, the Environmental Protection Agency (EPA) has developed a program to promulgate regulations and criteria to control the ocean disposal of radioactive wastes. The EPA seeks to understand the mechanisms for biological response of marine organisms to the low levels of radioactivity that may arise from the release of these wastes as a result of ocean-disposal practices. Such information will play an important role in determining the adequacy of environmental assessments provided to the EPA in support of any disposal permit application. Although the EPA requires packaging of low-level radioactive waste to prevent release during radiodecay of the materials, some release of radioactive material into the deep-sea environment may occur when a package deteriorates. Therefore, methods for evaluating the impact on biota are being evaluated.

Mortality and phenotypic responses are not anticipated at the expected low environmental levels that might occur if radioactive materials were released from the low-level waste packages. Therefore, traditional bioassay systems are unsuitable for assessing sublethal effects on biota in the marine environment. The EPA Office of Radiation Programs (ORP) has had an ongoing program to examine sublethal responses to radiation at the cellular level, using cytogenetic end points. This technical guidance report represents prepermit bioassay procedures that potentially may be applicable to the assessment of effects from a mixture of radionuclides that could be released from a point source at the ocean bottom. Methodologies along with rationale and a discussion of uncertainty are presented for the sediment benthic bioassay protocols identified in this report.

The Agency invites all readers of this report to send any comments or suggestions to Martin P. Halper, Director, Analysis and Support Division, Office of Radiation Programs (ANR-461), U.S. Environmental Protection Agency, Washington, DC 20460.

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EXECUTIVE SUMMARY

Past and potential future disposal of low-level radioactive waste in the ocean has long been a subject of international studies. We provide an assessment of the potential risks to aquatic life from the ocean disposal of low-level radioactive waste and present suggestions for long-term risk management. Our approach is to focus on current topics of concern to the U.S. Environmental Protection Agency (EPA) in the management of potential future disposal of packaged low-level radioactive waste. We also place emphasis on approaches to prepermit biomonitoring. We characterize potential risk to aquatic life in three steps. First, we describe the unique characteristics of deep-sea ecosystems. Second, we evaluate the types of biological effects that are significant; and third, we provide analyses of the potential for detrimental biological effects in the deep sea using two exposure scenarios. The first scenario is a generalized estimate of effects for the case in which packaging remains intact. The second scenario is a generalized estimate for the case in which packaging fails. Risk management suggestions include appropriate use of numerical limits and toxicity-based limits under the two exposure scenarios. An approach to implementing a prepermit toxicity testing program is described, and it includes proposed species and protocols.

The deep sea is a potential repository of low-level radioactive waste from activities in research, industry, and government. To predict risk to aquatic life in the deep sea, we summarize the characteristics that distinguish deep-sea ecosystems from more frequently studied coastal ecosystems. It is now understood that deep-sea ecosystems are more dynamic than was previously believed and that varying adaptations to life in the deep sea exist. Two traits of deep-sea species are of particular concern with respect to exposure to radiation. First, many deep-sea species have reproductive strategies that are characterized by low fecundity, brooding of larvae during development, and potential for developmental arrest. These characteristics may make deep-sea species more vulnerable to cumulative DNA damage in gametes and embryos. Secondly, it is not known whether unique biochemical adaptations to life at cold temperatures and high pressures include adaptations in DNA repair enzymes. If a lower capacity for DNA repair is exhibited at low temperatures and high pressures, deep-sea species could be more vulnerable than coastal species to the effects of ionizing radiation exposure.

Effects of exposure to ionizing radiation have been exceptionally well characterized, and similar responses have been documented in mammals and aquatic species. Significant effects at low doses include genotoxic responses, reproductive impairment, and developmental abnormalities. Dose rates of 0.005 Gy/d are the lowest at which detrimental effects have been observed in aquatic organisms. Full life-cycle reproductive experiments on fishes and invertebrates have provided the most sensitive estimates of effect. Uncertainty in estimates of effects of ionizing radiation are relatively few, but the most significant source of uncertainty is the potential error in extrapolating between results on coastal species to deep-sea species. In general, however, mechanisms of effect and effect-level estimates are better characterized for ionizing radiation than for any other toxic substance.

Two exposure scenarios are described, in general terms, for our assessment of potential risk to aquatic life. First, we analyze potential risk to aquatic life from exposure to gamma radiation at the surface of the waste package. This first exposure scenario is based on the assumption that packaging will remain intact. The second exposure scenario is based on the extremely conservative assumption that all the packaging will fail. Using the second scenario, we evaluate risks to aquatic life via sediment exposures. With respect to the first exposure scenario, we conclude that exposures on the package surfaces are not likely to produce acute effects. Chronic effects could be observed if exposures at the anticipated rate of 0.048 Gy/d occurred for several months to organisms on the surface of the packages. Organisms that are not in direct contact with the packages would probably exhibit no significant detrimental effects. For the second exposure scenario, potential effects of exposure in sediment are more difficult to predict. We conclude that chronic effects may be possible only in the immediate vicinity of breached containers, if the organisms are exposed constantly for several months or years.

Potential risk management approaches include the use of both toxicity-based and numerical limits on specific radionuclides. We propose that appropriate use of these two approaches may vary between the two exposure scenarios. For the scenario in which all the packaging is assumed to remain intact, numerical limits for gamma activity should be emphasized and toxicity-based limits should be de-emphasized. There is no rationale for prepermit bioassay testing in this case because the organisms will not be exposed to complex wastes. For the scenario in which we assume that the packaging fails, both numerical limits and toxicity-based limits should be considered. The latter would be advisable because organisms could be exposed to complex wastes comprised of multiple radionuclides and other toxic substances. The toxicity of all of these substances in combination cannot adequately be controlled, if only numerical limits are employed.

Comprehensive suggestions for risk management approaches are described, but we emphasize recommendations for prepermit toxicity monitoring under the second exposure scenario. Toxicity tests to be used for predisposal testing were selected for review if they met these criteria: (1) sublethal effects are measured, (2) protocols can be used for sediment exposures or exposures to prepared waste blocks, (3) marine species are used, and (4) protocols are amenable to standardization.

Three tests were recommended for exposures to sediment or prepared waste blocks: a test evaluating reproduction of the polychaete worm Neanthes arenaceodentata; a reproductive test using the amphipod Ampelisca abdita; and a reproductive test using the shrimp Palaemonetes pugio. Tests using fish embryos are described for possible use, but it is recommended that a new protocol be developed for an epibenthic fish species. Short-term genotoxicity tests could also be applied, practically, if additional research is directed toward development of methods that provide estimates of cumulative effects.

Chapter 1

INTRODUCTION

1.1 BACKGROUND

The disposal of low-level radioactive waste into the deep sea is authorized under the terms specified in the Marine Protection, Research, and Sanctuaries Act of 1972 (Public Law 92-532). In 1983, Section 424 of the Surface Transportation Act, an amendment to PL92-532, specified that low-level radioactive waste will be disposed of in the ocean for research purposes only (Public Law 97-424 1983). The regulations for ocean dumping are currently being revised by the U.S. Environmental Protection Agency. It is anticipated that low-level radioactive waste will be considered for disposal and that new regulations will be applied to protect aquatic life. However, before the disposal of low-level radioactive waste is permitted, requirements for prepermit testing of the wastes must be considered. Guidance is needed to ensure that future disposal will have minimum impact on the ecological health and economic potential of the deep sea.

The disposal of radioactive waste in the ocean has always been of international concern, and extensive investigations have been conducted to identify suitable dumpsites and dumping practices. Currently, the International Atomic Energy Agency (IAEA) is responsible for defining radioactive wastes and materials unsuitable for dumping into the ocean and for making recommendations about the issue of permits for the dumping of these wastes and materials (IAEA 1986). To protect the potential future uses of the ocean floor, the IAEA has proposed that the area of any given dumping site and the number of sites should be small. Models are used to define radioactive wastes unsuitable for dumping, and the assumptions used in the models are that dumping takes place in a single-ocean basin of volume 10^{17} m^3 , at a site where the average water depth is 4,000 m, at a rate of 10^8 kg/year , and continues for 1,000 yr. For this discussion, we assumed that any future dumping by the United States will take place at such a site and that dumping would proceed at the rate described.

A quantitative definition of the kinds of waste unsuitable for dumping at sea has been derived using models recommended by a joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP 1983) and data bases assembled by the IAEA (IAEA 1986). The definition is based on an annual dose limit of 1 mSv for individuals; this limit is applied to the critical group of the population. It was further assumed that the radionuclides are released instantaneously as soon as the waste reaches the ocean floor.

Existing and proposed international and U.S. regulations require all low-level radioactive waste destined for ocean disposal to be packaged (IAEA 1986 and EPA Ocean Dumping Regulations of 1972). Waste packaging was reviewed under the direction of the Organization for Economic Cooperation and Development (OECD) of the Nuclear Energy Agency (NEA 1985). Waste packages are to consist of a container and waste form in an integrated design and are to be constructed to provide shielding and packaging of waste during handling, transport, and disposal.

Waste packages are produced in two basic designs, ones with air spaces (voids) and ones without (monolithic); and both kinds are provided with an outer steel drum or concrete container. Void-containing packages incorporate a means of pressure equalization to ensure that implosion and destruction of the package does not occur as it descends into the deep ocean. In monolithic packages, the waste is incorporated in a matrix that may be cement, bitumen, or a polymer. Materials that may be incorporated include sludges, resins, and evaporator concentrates. In many cases, wastes will be reduced by incineration before packaging. The United States may choose to be more stringent than the international community in terms of packaging requirements. Low-level radioactive waste include such diverse materials as waste generated in biomedical testing and other laboratory uses of radionuclides as well as by-products of plutonium mining and weapons research. Considerable information is available on kinds and quantities of wastes that have been disposed of in the past (NEA 1985).

In this report we will consider two scenarios that will lead to exposure of deep-sea organisms to radioactivity: (1) there is no packaging failure and (2) packaging failure occurs and there is immediate or gradual release of radionuclides into the water. In the latter scenario, it is expected that subsequent to the release into the water there would be sorption of radionuclides to particulate material in the water and sediments.

1.2 PURPOSE

The purpose of this document is twofold. The first goal is to assess risk to aquatic life from ocean disposal of low-level radioactive waste. The second goal is to provide a conceptual framework for suggested risk management approaches, including predisposal bioassay testing requirements.

1.3 APPROACH

Risk assessment approaches are used in this document to evaluate the need for prepermit testing. The overall approach is described in Figure 1. First, some characteristics of the deep-sea ecosystem are described to characterize the unique ecological aspects of the disposal sites (Section 2.2). Second, risk to aquatic organisms from selected disposal scenarios is evaluated (Sections 2.3, 2.4, and 2.5) by (1) reviewing the available knowledge on the biological effects of ionizing radiation on aquatic organisms, (2) describing the exposure assessments based on two general scenarios, and (3) predicting risk to aquatic organisms based on overlap between the dose rates at which detrimental biological effects are observed in the laboratory and the expected exposures.

Third, an analysis of the available toxicity data and bioassay protocols that could be used to establish water-quality based controls is provided (Chapter 3). Potential approaches to the development of numerical criteria and toxicity criteria are discussed in general terms.

Finally, recommendations for predisposal testing are summarized, and the proposed risk management approaches are described for two exposure scenarios (Chapter 4).

Figure 1. Summary of risk assessment and proposed risk management approaches and their order of presentation in this report.

Source characterization and exposure assessments have been performed previously at an international level. Waste-package performance and the fate of radionuclides, should packaging fail, were considered. Assessments were performed for the North-East Atlantic dump site. These assessments were conducted under the auspices of the OECD, which established a "Multilateral Consultation and Surveillance Mechanism for Sea Dumping of Radioactive Waste," and requested that the Nuclear Energy Agency (NEA) assess every 5 years the suitability of the continued use of the North-East Atlantic dump site. The suitability of this dump site was reviewed in 1985 (NEA 1985). Included in the reviews are evaluations of assessment methodologies for radiological risk to man and biota, model development, bioaccumulation, sediment partition coefficients, and radiation regimes. Detailed discussion of these investigations is beyond the scope of this document. As such, exposure assessment will be discussed in broad terms.

1.4 SCOPE

The scope of this report is limited to development of a conceptual approach for protection of aquatic life from detrimental effects due to low-level radioactive waste disposal. As such, exposure and effects assessments are discussed in general terms. Risk to human health, which was considered extensively in the 1985 site suitability review, and beneficial uses of the ocean, other than its designated use as habitat for aquatic life, are considered only briefly here. This analysis is made with consideration of the unique spatial and temporal scales associated with this disposal activity.

Chapter 2

RISK CHARACTERIZATION AND ASSESSMENT

2.1 GENERAL PRINCIPLES OF HAZARD ASSESSMENTS APPLIED TO AQUATIC ECOSYSTEMS AND THE ECOLOGICAL SIGNIFICANCE OF SINGLE-SPECIES BIOASSAYS

To date, hazard assessments applied to aquatic ecosystems emphasize somewhat different approaches than do those developed to assess hazard to human health. Hazard assessments for aquatic ecosystems are based either on exceedances of EPA water-quality criteria for specific contaminants or exceedances of toxicity criteria (EPA 1985; Gentile et al. 1988). Hazard assessment, therefore, is based on extrapolations between the results of selected bioassays and the potential detrimental effects to aquatic organisms at the population and community levels. The bioassays used to develop water-quality criteria generally include assays of mortality, reproductive impairment, growth impairment, and detriment to embryological development. Exposures typically range between 2 and approximately 120 days.

Assessment of hazard to human health frequently emphasizes a more mechanistic approach than do assessments of hazard to aquatic life. The mechanistic approach is based on knowledge of the mechanism and types of effect anticipated from exposure to a specific contaminant. This knowledge is rarely available for aquatic communities because less is known about mechanisms of contaminant effect in aquatic organisms.

In a mechanistic approach to hazard assessment for humans, a distinction is made between stochastic and nonstochastic effects. These are defined as (ICRP 1979):

"Stochastic" effects are those for which the probability of a effect occurring, rather than its severity, is regarded as a function of dose, without threshold. "Nonstochastic" effects are those for which the severity of the effect varies with the dose, and for which a threshold may therefore occur. At the dose range involved in radiation protection, hereditary effects are regarded as being stochastic. Some somatic effects are stochastic; of these, carcinogenesis is considered to be the chief somatic risk of irradiation at low doses and therefore the main problem in radiation protection."

The goals of human radiation protection are (1) to prevent nonstochastic effects by setting limits at sufficiently low values so that no threshold dose would be reached and (2) to limit the probability of stochastic effects by keeping all justifiable exposures as low as is reasonably achievable.

Effects on human health may be caused by stochastic and nonstochastic effects in exposed individuals and stochastic effects in subsequent generations. The detriment

in a population is the expectation of harm based on the probability and severity of the effect. The detriment to health, G , in a group of persons, P_i , is given by

$$G = P \sum p_i g_i ,$$

where p_i is the probability of suffering the effect i and g_i is a weighting factor for the severity of the effect (ICRP 1979).

For predicting the probability of suffering a stochastic effect, linear extrapolation is used, based on a cautious assumption of proportionality. For nonstochastic effects, the probability of suffering a given effect is determined by the shapes of dose-response curves and threshold doses. The fact that many effects are age and tissue specific is also considered, as are the characteristics of the exposure.

In aquatic toxicology, little is known about frequencies and significance of stochastic effects, such as cancer induction and hereditary change. Information to protect aquatic ecosystems is obtained by examining primarily nonstochastic effects. Bioassays are performed to assess short-term changes in mortality rate in different life-history stages, changes in reproductive success, or other selected parameters. In most cases, the shapes of dose-response curves for specific effects have not been characterized well.

Another difference between hazard assessments for mammals and aquatic animals is that protection of aquatic life does not focus on the health of individuals but on the health of populations and communities. As such, great emphasis is placed on extrapolations between: (1) data obtained using indicator species and potential effects on other species in an aquatic community and (2) the levels at which acute lethal effects are observed relative to the level at which chronic effects are observed (EPA 1985; Le Blanc 1984; Sloof et al. 1983; Kimerle et al. 1985; Kenaga 1982).

The ecological significance of using predictive bioassays is dependant upon the validity of the extrapolations described above. In other words, the tests should predict the potential for harm to sensitive species, they should predict subtle chronic effects, and they should predict the types of effects that might be realized at higher levels of biological organization. The latter includes decreased population size or alterations in the functional diversity of communities.

At this time, there is no definitive consensus regarding the extent to which single-species bioassays can be predictive of significant environmental harm. However, a strong burden of proof does exist; and at a policy level, extensive regulatory requirements are now based on the premise that single-species bioassays are valuable tools (EPA 1985). From a more technical level, strong evidence that single-species bioassays are predictive of change at higher levels of biological organization is contained in several reports (e.g., Anderson and Harrison 1990; EPA 1985). Important and unique sources of uncertainty do exist, however, in predicting effects of ionizing radiation on deep-sea species from existing bioassay techniques. This topic is addressed in detail elsewhere in this report. In particular, we address the

unique responses caused by ionizing radiation and the unique characteristics of deep-sea species and ecosystems.

In this context, the importance of the differences between health- and aquatic-hazard assessment is that hazard assessment for exposure of aquatic organisms to ionizing radiation must incorporate some of each approach. The mechanism of action of ionizing radiation is well understood, so a more mechanistic emphasis is appropriate. However, because the goal is to predict detrimental effects on populations rather than individuals, many of the extrapolations used in aquatic toxicology must be considered.

2.2 REVIEW OF THE BIOLOGY OF DEEP-SEA ORGANISMS

2.2.1 Deep-Sea Ecosystems

For this discussion, it is assumed that any future dumping occurring under the auspices of the United States will occur in already designated areas. The areas of the deep sea where the disposal of radioactive waste has been considered by the international community are those that are between latitudes 50°N and 50°S; that have an average water depth greater than 4,000 m; that are located clear of continental margins, open sea islands, etc.; and that do not interfere with other legitimate uses of the seas (IAEA 1988).

There have been several recent reviews on the deep sea (NOAA 1986; Sibuet and Juniper 1986; IAEA 1988). Information from these has been summarized in the material that follows. In general, the areas considered for disposal sites of radioactive waste are the abyssal plains (4,000 to 6,000 m), which are poorly characterized. However, large areas of the abyssal plains appear to be physically quiescent and physically homogeneous (horizontal scales of from tens of meters to even hundreds of kilometers), to have low temperatures (<5°C), and to be devoid of sunlight. The substrate, in general, consists of fine-grained pelagic sediment that has a low organic content. Of special interest in our consideration are the biota that inhabit the bathypelagic zone (1,000 to 4,000 m), the abyssopelagic zone (4,000 m to 6,000 m), and the sea floor in these areas.

2.2.2 Deep-Sea Biota

The faunas in the abyssal plains include organisms that consume organic material arising from the overlying waters or prey on resident organisms. Organic material arriving on the sea floor ranges in size from large fish, marine mammals, and invertebrates to small particles. Results from sediment trap studies have shown that the particles include foraminiferan tests, radiolarian skeletons, pteropod shells, diatom frustules, zooplankton fecal pellets, and amorphous aggregates of mucus material collectively known as marine snow (Sibuet and Juniper 1986).

The numbers of the bathypelagic faunas decrease exponentially with depth, the exponent differing from one type of organism to another and from one area to another. The density of organisms below 3,000 m is very low, the biomass not exceeding 1 mg wet weight per m³ regardless of the total depth of water, until a

richer zone in close proximity to the bottom is reached (Smith et al. 1986). This benthopelagic zone or benthic boundary layer is arbitrarily defined as a layer of about 100 m above the sea floor.

The organisms of primary concern with respect to radioactive waste disposal are those inhabiting the benthos. The density and biomass of the benthic community are considered to decrease exponentially with increasing water depth. On a global scale, one estimate puts the total biomass of the world's oceans at 9×10^{12} kg, with approximately 6×10^{12} kg occurring in the benthos, of which 5×10^{10} kg occupies depths $>3,000$ m (Menzies et al. 1973).

Benthic organisms include the epifauna, which live predominantly on the sediment surface, and the infauna, which live within the sediment. The faunas may be considered in four separate divisions based on their size. Unfortunately, the screens used to separate them into size classes have not been standardized, and as a result comparisons of numbers or projected biomass compiled by different investigators are not always possible. The microfaunas include bacteria, fungi, and soft-bodied protozoa and are not retained by the screens (0.40 and $0.63 \mu\text{m}$) used to separate out the meiofauna (Grassle and Grassle 1986). The meiofaunas are best defined by taxonomic group; nematodes, harpacticoid copepods, foraminifera, and podocypid ostracods are the most abundant groups. The dominant macrofaunas include polychaetes, bivalves, and peracarid crustacea, all of which are retained by $250 \mu\text{m}$ (or $300 \mu\text{m}$) sieves. The megafaunas are defined as the animals visible by eye and include echinoderms, corals, sponges, crustaceans, fishes, and cephalopods.

Attempts to characterize the organisms in benthic zones have included photographic surveys, enumeration of organisms collected in epibenthic sledges and other devices (Rice et al. 1982), as well as identification and quantification of organisms in cores. However, the number of completely processed quantitative samples (sieve size $>0.5 \mu\text{m}$ and separation of faunas to species) are less than 100 worldwide (Grassle and Grassle 1986). The polychaetes constitute the greatest numbers of species and individuals in benthic samples from both the continental shelf and the deep sea, and many of these species have not been identified (Grassle and Grassle 1986).

2.2.3 Species Diversity and Distribution

High-species diversity in the deep sea has been the subject of much discussion (Hessler and Sanders 1966; Sanders and Hessler 1969; Grassle and Sanders 1973; Wolff 1977). According to Grassle and Grassle (1986), deep-sea species diversity is extraordinarily high in comparison to the continental-shelf species diversity, and the number of undescribed species may rival that of tropical rain forests. The diversity of macrofaunal and megafaunal organisms has been shown to increase with depth below the continental shelf, reaching a peak near depths of between $2,000$ and $3,000$ m (Sanders 1968; Sanders 1969). Below $2,000$ and $3,000$ m, species diversities appear to decrease markedly with increased depth down to the abyssal plain (Sibuet 1977; Haedrich et al. 1980). The benthic faunas of the North Pacific have a higher diversity than those of the North Atlantic ocean. This may be attributed to their greater antiquity and their proximity to shallow-water faunas

that had not suffered faunal extinction as a result of glaciation (Gage 1979). In any case, there are many indications that diversity in the deep ocean is greater than would be expected from the fact that it is a relatively homogeneous environment.

The horizontal distribution of deep-sea benthic and pelagic organisms on a global scale is not well established. From analysis of faunistic affinities, there is some indication that about 85 percent of the major benthic groups are restricted to 1 of the 3 ocean regions each, and only 4 percent are truly cosmopolitan species (IAEA 1988).

Detailed studies of long-term temporal changes in deep-sea ecosystems have not been undertaken. However, data obtained during the last 12 years at deep-sea sites in the North Atlantic indicate that abyssobenthic meiofaunal, macrofaunal, and megafaunal abundances have a constant relationship with each other and with food input between sampling sites (Sibuet and Juniper 1986). This is not unexpected because the principal source of organic matter to the deep sea is from the overlying waters, and it can be expected that any long-term changes in these waters would be reflected in deep-sea food webs that are dependent on surface-water sources.

Dispersal ability of deep-sea species remains poorly known, and as many as half the species in major taxa with poor dispersal, such as the peracarids, may be endemic to ocean basins (Grassle and Grassle 1986). Some indication of low dispersal ability can be obtained from colonization studies. Rates of colonization of trays of azoic sediment indicate lower rates of colonization of most species in the deep sea (Grassle 1980). Even after 2 years, the number of individuals is an order of magnitude lower than in surrounding sediments, and most of the individuals are juveniles.

2.2.4 Adaptations to the Deep Sea

2.2.4.1 Introduction. The traditional concept is that the deep-sea environment is constant and nonseasonal and that life in the deep sea is at a slow pace. This slow pace is supposedly reflected in the biota by slow growth rates, long times to maturity, and long life spans. Although this concept is probably true to a large extent, results from more recent studies indicate that there may be considerable variability with season and with environmental perturbations, such as deep-sea storms (Dickson et al. 1982; Elliott and Thorpe 1983; Gardner and Sullivan 1981; Guennegan and Rannou 1979; Billett et al. 1983; Lampitt 1985; Deuser and Ross 1980; Deuser et al. 1981).

The low temperature, high pressure, and limited food supply of the deep sea appears to have resulted in physiological, biochemical, genetic, and behavioral adaptations of the indigenous species. Low temperatures appear to have a greater impact on metabolic processes than do high pressures (IAEA 1988). There are many indications that the adaptations that have occurred have resulted in significant differences between homologous species of shallow-water and deep-sea organisms in their metabolic and reproductive strategies and may have resulted in differences in their genomic potential.

2.2.4.2 Metabolic Adaptations to the Deep Sea. Metabolic rates measured on some deep-sea biota indicate that they have lower rates of respiration and enzymatic activities (Childress and Nygaard 1973; Childress 1975; Torres et al. 1979; Somero et al. 1983; Childress and Somero 1979; Smith and Baldwin 1982). Benthopelagic amphipods were found to have extremely low resting metabolism plus the capability of switching to increased rates to support rapid movements (Smith and Baldwin 1982). In contrast to this pattern, the eurybathic amphipod Eurythenes gryllus exhibited high beating frequencies of its swimming legs, indicative of high respiration (Yayanos 1978).

Metabolic strategies of deep-sea biota may reflect differences in their feeding behavior (Sibuet and Juniper 1986). One type of behavior is exemplified by the "sit and wait" predator and another by the strong-swimmer predator. In the case of the first type, organisms may be able to switch from an extremely low basal rate to a higher one when food is available or when a higher rate is required for survival. Because the food supply is highly intermittent, the changes in metabolic rate may be intermittent and be reflected in changes in growth rate and in reproduction. In the case of the second type, a comparatively high rate is maintained to allow for relatively continuous foraging for food.

Metabolic rates of some deep-sea organisms appear to have adapted to the high hydrostatic pressure. Bacteria, whose growth and metabolic rates are greater at high hydrostatic pressures than at atmospheric pressure, have been isolated both from inside benthic invertebrates and from sediments (Yayanos et al. 1981; Deming et al. 1981; Deming and Colwell 1985; Deming 1985). Although it has been demonstrated conclusively that barophylic bacteria are present in the deep sea, their overall importance within the microbial community has not been established.

2.2.4.3 Reproductive Adaptations to the Deep Sea. Reproductive strategies of deep-sea biota may affect critically their response to radiation. Unfortunately, very little information is available on the life history of most deep-sea biota. Important parameters include time to maturity (age at first reproduction), brood size, duration of gametogenesis, time from gametogenesis to spawning, and adult survival.

The few data available on reproductive strategy indicate that most deep-sea species show a trend toward "K-selected" type of species. K-selected species devote more of their scarce food supply to growth and less to reproduction than do the "r-selected" type, and they tend to be long-lived, reach sexual maturity later, and produce few numbers of large larvae throughout the year.

Evidence is available for both periodic and nonperiodic reproductive patterns in deep-sea biota, which makes generalizations about patterns difficult. A study of reproductive patterns in abyssal organisms was conducted by Rokop (1974) and revealed that 9 of 11 species reproduced continuously and recruit larvae throughout the year. However, there is a growing body of evidence for reproductive periodicity in deep-sea isopod crustaceans (George and Menzies 1967), echinoderms (Schoener 1968; Tyler et al. 1982), and a macrourid fish (Rannou 1975).

Reproductive periodicity may be related to the seasonal differences in food input into the deep sea (Grassle and Grassle 1986). For those species where periodic changes in food availability are not regular enough to influence the reproductive cycle, they may adopt other strategies. The production of long-lived lecithrophic larvae may allow survival until another episode of food enrichment is encountered, at which time the larvae may acquire sufficient food for its critical late-larval and post-larval growth phase. Such a strategy has been proposed for an abyssal ophiuroid population (Gage and Tyler 1982).

Information on reproductive strategy is available on both fishes (Mead et al. 1964; Hureau et al. 1979; Merrett 1978; Stein 1980; 1985; Childress et al. 1980; Pearcy et al. 1982; Rannou 1975) and invertebrates (Tyler et al. 1984; Lampitt and Billett 1985; Thurston 1979; Tyler 1980; Gage and Tyler 1981; 1982; Tyler and Gage 1980; Charmasson and Calmet 1987).

In fish living at deep bathyl and abyssal depths, varied life history strategies may be exemplified. One group of fish typically produces few but large eggs that hatch into advanced young. These develop either in bathy- or abysso-pelagical zones, and, thus, are subject to lower predation pressure than fish living near the sea surface (Mead et al. 1964). Another group provides parental care by means of viviparity or even mouth breeding. Careproctus kermadecensis, which has been caught at depths exceeding 6,000 m, had as few as 850 eggs of very different sizes, ranging from 0.5 to 9.0 mm. Only 34 eggs were larger than 7.5 mm in diameter, which may point to sporadic breeding. Numerous other species are also monoecious. Actually, little is known of the reproductive biology of most deep-sea fishes. The exceptions are the macrourids (rat-tail fishes) for which considerable data exist.

Rat-tail fishes are the most abundant fishes in numbers of individuals in all abyssal seas (Hureau et al. 1979). The macrourid Coryphaenoides armatus is the most common fish at depths from 4,000 to 5,000 m, at least in the northeast Pacific and Atlantic Oceans. The fecundity estimate, based on a single ripe female, is 6.2×10^6 eggs (Stein 1985). It is assumed that the development of eggs and hatching of larvae occur in the productive near-surface waters and that settlement of juveniles and the adoption of a benthic life occurs later (Stein 1980). No information exists on growth rate or on the lifespan of C. armatus, but the life span was estimated to be about 5 to 8 yr (Childress et al. 1980). In C. acrolepis, fecundity was found to vary widely; egg masses ranged in size from about 22,600 to 118,000 eggs. This species has been found from 1,430 to 9,900 m (Pearcy et al. 1982).

Deep-sea bivalves may produce hundreds of eggs, but some of the protobranchs produce one or two eggs per brood (Sanders and Allen 1973). Comparison of data available on species of Nucula that live at different depths indicates that the egg number and reproductive effort are lower in the deeper dwelling species (Grassle and Grassle 1986). Similar findings have been reported for echinoderms (Hendler 1975; Tyler and Gage 1984), and peracarid crustacea (Bishop 1982).

The data available on the life-history of deep-sea invertebrates are for a limited number of species, and often the information on their reproductive biology and growth strategies is incomplete (Table 1). Most invertebrates

Table 1. Data on reproduction in some benthic invertebrate species indigenous to different depths.

Species	Depth (m)	Spawning	Fecundity (egg number)	Egg size (µm)	References
Echinoderms					
<u>Ophiura ljungmani</u> (ophiuroid)	309 to 4070	Seasonal	57,000	90	Tyler and Gage 1982 Gage and Tyler 1980; 1981; Lightfoot et al. 1979
<u>Ophiomusium lymani</u> (ophiuroid)	700 to 4000	irregular	200 to 1400	420	Gage 1982
<u>Zoroaster fulgens</u>	367 to 3660	irregular	11,000	900	Tyler et al. 1984
<u>Freyella spinosa</u>	1880 to 4800	irregular	2,500	?	Tyler et al. 1984
<u>Peniagone azorica</u>	1400 to 8200	irregular	1500 to 10,000	300	Tyler et al. 1985a Hansen 1975
<u>Peniagone diaphana</u>	2000 to 5600	irregular	>100 to 10,000	300	Tyler et al. 1985a Hansen 1975 Tyler et al. 1985b
<u>Benthagone rosea</u>	1100 to 4100	irregular	>100 to 10,000	750	Hansen 1975 Tyler et al. 1985b
<u>Oneirophanta</u> sp.	2900 to 6000	irregular	>100 to 10,000	950	Hansen 1975 Tyler et al. 1985b
<u>Deima validum mutabilis</u>	720 to 4700	irregular	>100 to 10,000	700	Hansen 1975 Tyler et al. 1985b
<u>Amphiophiura bullata</u> (ophiuroid)	?	?	300	?	Hendler 1975
<u>Amphilepis ingolfiana</u> (ophiuroid)	?	?	500	?	Hendler 1975
<u>Homalophiura tessellata</u> Brachiopod	?	?	2,000	?	Hendler 1975
<u>Pelagodiscus atlanticus</u>	530 to 5500	?	?	?	Zezina 1975
Bivalves					
<u>Microgloma</u> sp.	Deep sea	?	1 to 2	?	Sanders and Allen 1973

Table 1. Data on reproduction in some benthic invertebrate species indigenous to different depths. (cont.)

Species	Depth (m)	Spawning	Fecundity (egg number)	Egg size (μ m)	References
<u>Nucula proxima</u>	shelf	?	4,120	?	Grassle and Grassle 1986
<u>Nucula annulata</u>	shelf	?	1,230	?	"
<u>Nucula granulosa</u>	slope	?	217	?	"
<u>Nucula cancellata</u>	530 to 4800	irregular	194	?	"
<u>Nucula verrilli</u>	abyss	?	260	?	"
Crustaceans					
<u>Orchomene gerulicorbis</u>	4860	twice	70	?	Thurston 1979
<u>Paralicella caperesca</u>	>3500 to 4800	once	90	?	"
<u>Leucon jonesi</u>	deep sea	?	6 to 12	?	Grassle and Grassle 1986
<u>Leucon medius</u>	deep sea	?	6 to 12	?	"
<u>Leucon nasica</u>	shallow water	?	30 to 60	?	"

studied have a low fecundity and large eggs and gametogenesis is typically asynchronous and nonseasonal. However, little information exists on the amount of time needed for gametogenesis. Ripe gametes may be either spawned during periods of adequate food supply or undergo resorption during periods of low food supply.

2.2.4.4 Genetic Adaptations to the Deep Sea. Genetic variability in deep-sea species may be an important factor in successful adaptation to periodic stress in their ecosystems. Some information is available that some species of deep-sea magabenthic invertebrates show a greater degree of genetic variation in the deep sea than do related species in shallow water (Ayala et al. 1975; Ayala and Valentine 1974; Murphy et al. 1976). Because larvae of these species are widely dispersed, the potential for adaptation to different biotic environments by selection for different alleles at specific loci on chromosomes may be available. Information is not available for species that have limited dispersal ability. If these species have low genetic variability, then they would be potentially more susceptible to extinction from adverse changes in radiation levels in their environment.

2.2.5 Conclusions

The information currently available on the deep sea indicates that this environment may be more variable than was previously believed, and the responses to environmental perturbations, such as radiation, may differ greatly with species. Differences in life-history characteristics among species appear to reflect the metabolic, reproductive, and genetic adaptations for survival that have occurred in the deep-sea environment.

The metabolic adaptations to low temperature and high pressure appear to affect enzymatic reactions. If these reactions determine the rates at which damage to radiation is incurred and at which it is repaired, then the responses to radiation of deep-sea biota may have a high degree of uncertainty.

Varying reproductive adaptations may influence the reproductive success of species exposed to ionizing radiation. Of greatest concern are low-fecundity species because, in these species, mortality of early life stages may be more likely to affect recruitment. In addition, reproductive strategies may affect the time period over which the radiation dose may be integrated and, thus, the magnitude of the damage that may be accumulated in potentially sensitive stages of gametogenesis. If in the reproductive cycle there is an extended time between the formation of the primary oocytes and spawning, damage could be accumulated and be manifested in the embryos that develop. Also, if reproduction is arrested at any time because the food supply is not adequate for completion of the cycle, there may be integration of the radiation dose with subsequent expression of the damage. Low fecundity and reproductive arrest appear to be common in deep-sea species, and these life-history characteristics may put deep-sea species at greater risk.

Genetic adaptations may also affect responses to radiation. The susceptibility of deep-sea species may be partially dependant on degree of genetic variability, a factor about which little information is available. Because genetic adaptations may alter

the resilience of species to radiation damage, more information is required on such adaptations before conclusions can be drawn about the radiation responses of deep-sea organisms compared to those of their shallow-water counterparts.

2.3 REVIEW OF RADIATION EFFECTS AND OBSERVABLE EFFECT LEVELS

2.3.1 Introduction

Several comprehensive reviews of published data on the effects of radiation on aquatic organisms have been published (Polikarpov 1966; Templeton et al. 1971; Chipman 1972; Ophel 1976; Blaylock and Trabalka 1978; Egami and Ijiri 1979; Woodhead 1984; Anderson and Harrison 1986). Consequently, it is not necessary to give a detailed account of the entire literature. In this section, we will summarize information primarily from the review of Anderson and Harrison (1986).

Radiation effects have been produced from both acute and chronic exposure to ionizing radiation. Detrimental effects may be lethal or sublethal. Effects of high and low doses may be markedly different and may be realized on different time scales. Effects of high doses of ionizing radiation are realized in the time frame of days to months when a threshold quantity of cells are destroyed in a key tissue or organ. Effects of low doses of ionizing radiation may be latent in development and result from reproductive impairment, developmental abnormalities in subsequent generations, and cancer. Because the mechanisms of effects of ionizing radiation are well understood, a mechanistic approach to hazard assessment is appropriate.

Categories of responses that have been defined are (1) lethal effects from acute exposure, (2) lethal effects from chronic exposure, (3) sublethal effects from acute exposure, and (4) sublethal effects from chronic exposure. Acute exposures are defined as those delivered in seconds or minutes and chronic exposures are defined as those occurring on a time scale of days to months. These operational definitions are needed because we will not use "chronic effects" to indicate, as has been done in the past, those effects only resulting from a full life-cycle exposure. In the discussion that follows, we will give more emphasis to sublethal effects from chronic exposure because at low-level radioactive waste disposal sites, it is expected that the biota will be exposed chronically to low levels of radiation and these exposure conditions generally result in sublethal effects. Because the mechanisms of effect of ionizing radiation are well understood, a mechanistic approach to hazard assessment is appropriate. Emphasis must be placed on the direct assessment of low-dose effects. For this reason, lethal effects of ionizing radiation are discussed only briefly.

Acute effects of ionizing radiation exposure are defined differently than are acute effects of other contaminants studied in aquatic toxicology. Exposure times for acute radiation studies are not typical 96-h bioassays. In most radiation studies, animals are either delivered a single dose in seconds or minutes or they are exposed continuously for a relatively long period of time (Anderson and Harrison 1986). When a short exposure is used, the time to 50 percent mortality is determined. Great variability exists in the minimum time of observation after exposure, and this may range between 30 days to a year (Anderson and Harrison 1986).

For the purposes of this report, exposure and effects must be clearly distinguished. For radiation research, it is most logical to refer to acute and chronic exposures as distinguished from lethal and sublethal effects. In addition, we refer to both total doses and dose rates.

2.3.2 Lethal Effects from Acute Exposures

Several factors modify the levels at which lethal effects are observed after acute exposures. These include the life stage irradiated, the time period of observation after exposure, the species studied, the intensity of ionization of the radiation source, and other factors such as dose rate, temperature, and salinity. Lethal responses have been documented in adult fish after radiation doses ranging between 3.75 and 100 Gy (Anderson and Harrison 1986). Exposure of adult invertebrates has resulted in lethal responses between 2.1 and 566 Gy (Anderson and Harrison 1986). In contrast, data available on embryos of three fish species demonstrate effects between 0.16 and 0.9 Gy. Embryo mortality in additional species of fish and invertebrates has been observed below 10 Gy, although the levels vary greatly, depending on the time period of observation, the species, and the exact embryonic stage. Although many factors of the experimental design influence effect levels, embryonic stages of fish exhibit mortality at lower doses than any other life stage studied. However, few developmental studies have been conducted using invertebrates.

2.3.3 Lethal Effects from Chronic Exposures

Few data exist to evaluate lethal effects of ionizing radiation following chronic exposures of marine fishes and invertebrates. Of the four studies that exist, the lowest effect level observed was an increase in mortality of blue crab adults exposed to 6.9 Gy/d for 70 d (Engel et al. 1974).

2.3.4 Sublethal Effects from Acute Exposures

Several types of sublethal effects have been evaluated after acute exposures of aquatic organisms to radiation. These include pathophysiological measurements as well as more commonly studied reproductive and developmental effects. In addition, molecular indicators of cellular effects have been investigated in selected animals, and multigeneration experiments designed to evaluate mutation frequencies have been performed on several species.

Of the pathophysiological indicators studied, decreased lymphocyte counts in fish are the most sensitive indicators of stress. Decreased counts have been documented as low as 1.0 Gy (Schechmeister et al. 1962) in adult goldfish and tench. Other pathophysiological measurements evaluated include damage to intestinal epithelial cells, impaired osmoregulation, and abnormal tissue development in various organs. However, these effects occurred at doses of 10 Gy or more.

Detrimental reproductive effects have been observed in fishes and invertebrates at doses as low as 2.2 Gy. Welander et al (1948) observed a 90 percent decrease in the number of primordial germ cells in chinook salmon (Oncorhynchus tshawytscha) after x-ray doses of 2.5 Gy. Hopperheit (1973) observed reduced egg-production rate in amphipods (Gammarus duebeni) at 2.2 Gy, and Harrison and Anderson (1983a) observed a decrease in fecundity of the polychaete Neanthes arenaceodentata at doses greater than 1 but less than 5 Gy. Evaluations of chromosomal aberration induction in 2 species of fish, Umbra limi (Kligerman et al. 1975) and Ameca splendens (Woodhead 1976), and one invertebrate, N. arenaceodentata (Harrison et al. 1985; Anderson et al. 1990), have demonstrated that effects are observed between 0.25 and 3.25 Gy. The range in levels of significant effects may be due to differences in experimental design or cell kinetics of the tissue studied or in actual differences in sensitivity between species.

Several experiments designed to evaluate mutagenic effects in fish have demonstrated induction of effects such as major eye malformations and vertebral column abnormalities at doses between 0.25 and 10 Gy. These experiments are performed typically by irradiating gametes externally after spawning and then raising progeny of exposed and unexposed gametes; the malformations are observed in the progeny.

2.3.5 Sublethal Effects from Chronic Exposures

Studies of reproductive and developmental effects of chronic exposure to radiation have provided the data of greatest relevance in evaluation of hazards of low-level radioactive waste disposal. For fishes and invertebrates, detrimental reproductive and developmental effects are observed at dose rates between 0.005 and 0.10 Gy/d. These data are summarized in Table 2 as are the other low-dose and low-dose-rate effects discussed below. Although these effects are well documented, their significance at the population level is unknown. Donaldson and Bonham (1964) exposed salmon embryos to ^{60}Co radiation continuously for 80 d beginning immediately after fertilization. At dose rates of 0.5 R/d (about 0.005 Gy/d), they observed an increased incidence of opercular defects in smolt. Interestingly, they also observed a significantly greater average weight in this same dose group as compared to controls, and subsequent studies revealed an increase in the rate of return of migrating chinook salmon at this same dose rate (Bonham and Donaldson 1966; Donaldson and Bonham 1970).

Harrison and Anderson (1988b) delivered doses of ^{60}Co to polychaete worms (Neanthes arenaceodentata) beginning within a day of fertilization through an entire life cycle ending within 1 d after spawning of the F_1 generation. Developmental success of F_2 progeny was quantified. At the lowest dose rate tested, 0.005 Gy/d, the percent normally developing embryos was significantly reduced. Evaluation of population-level impacts using these data is currently in progress.

Populations of both fish and snails introduced into radionuclide-contaminated White Oak Lake have been the subjects of extensive evaluations (Table 2). While valuable data have been obtained on reproduction and development of these

Table 2. Summary of biological effects observed at low dose rates and low total doses in aquatic organisms (from Anderson and Harrison 1986 and Harrison and Anderson 1988a,b).

Dose rate or total dose (Source)	Observed or projected effect	Reference
<u>Aquatic organisms</u>		
0.5 R/d (^{60}Co)	Increased incidence of opercular defects in salmon after 80 d of irradiation beginning immediately after fertilization; significantly greater average weight in the irradiated group was also observed.	Donaldson and Bonham 1964
0.005 Gy/d (^{60}Co) 0.5 Gy (^{137}Cs)	Significant reduction in the percentage of live embryos in the broods from mated pairs given life time exposure.	Harrison and Anderson 1988a,b
0.59 R/d (mixed source, White Oak Lake sediment)	Increased embryo mortality in the mosquitofish <u>Gambusia affinis</u> relative to those from an unirradiated field control site; no concomitant decrease in fecundity was observed.	Trabalka and Allen 1977
0.65 R/d (mixed source, White Oak Lake sediment)	Frequency of egg-capsule production was reduced in an irradiated population of the aquatic snail <u>Physa heterostroph</u> a; however, an increased number of eggs per capsule also occurred.	Cooley 1973
4.08 rad/d (^{137}Cs)	Reduced total fecundity at lowest dose tested over the entire life cycle of the guppy <u>Poecilia reticulata</u>	Woodhead 1977
6.8 rad/d (^{137}Cs)	Increased percentage of unfertilized eggs from ricefish <u>Oryzias latipes</u> adults irradiated for 120 d; no statistically significant effects were observed at lower doses	Hyodo-Taguchi 1980
10 R/d (^{60}Co)	Gonadal development retarded in Chinook salmon irradiated for smolts 80 d after fertilization; lower smolts exposures showed no such effects; the percent return of migrating salmon was slightly higher in the return of migrating salmon was group irradiated at 0.5 R/d.	Bonham and Donaldson 1972; Donaldson and Bonham 1970
16 R (x ray)	LD ₅₀ of silver salmon (<u>Oncorhynchus kisutch</u>) embryos irradiated in the one-cell stage and observed 150 d after irradiation.	Bonham and Welander 1963
25 rad (^{60}Co)	Increased frequency of major eye malformations in trout embryos from irradiation of trout sperm.	McGregor and Newcombe 1972a
25 and 50 rad (^{60}Co)	Trout sperm at 25 and 50 rad produced decreased embryo mortality; increased embryo mortality from 200-400 rad to sperm.	McGregor and Newcombe 1972b

^a Units given are those provided by the investigators.

organisms under conditions of constant field exposure, it is not known whether the detrimental effects observed are entirely due to radiation exposure, because other contaminants have also been disposed of in the lake.

Two separate studies using fish have determined effect levels for long term ^{137}Cs exposure between about 0.04 and 0.06 Gy/d. Woodhead (1977) evaluated fecundity of guppies (Poecilia reticulata) irradiated over an entire life cycle. He noted a reduction in fecundity at the lowest dose tested, 0.04 Gy/d. Hyodo-Taguchi (1980) observed an increase in the percent of unfertilized eggs from ricefish (Oryzias latipes) adults irradiated for 120 d at 0.06 Gy/d.

While low-dose-rate effects are of greater interest in risk assessment, it is important to evaluate some cases in which higher effect levels have been observed to discuss potential mechanisms of realized damage. Cooley and Miller (1971) delivered doses of ^{60}Co gamma rays to pond snail (Physa heterostrophica) adults and embryos. They observed a significant decrease in percent hatching of embryos only at 2.4 Gy/d. Conversely, growth of adults was significantly increased following doses of 6.0 and 2.4 Gy/d. Marshall (1962) performed classic studies on the population-level consequences of radiation exposure using Daphnia pulex, a water flea, commonly used in toxicity testing. He noted a decrease in birth rate only at 12 Gy/d. The intrinsic rate of natural increase in the population was decreased to zero at 16.8 Gy/d. These dose rates are higher than those observed to be lethal to blue crab (6.9 Gy/d). Therefore, in two classic studies using different invertebrate species, effect levels were observed to be at least two orders of magnitude higher than those discussed above. This may reflect differences in the innate sensitivity of the organisms, but given the common cellular mechanism of radiation damage, it is more likely due to differences in the way that molecular damage is realized at the organismal level because of differences in reproductive strategy.

The reasons for the observed differences in effect levels for similar sublethal effects are not known, but a few hypotheses exist. Anderson and Harrison (1986) noted that low effect levels observed in radiation studies on mammals and terrestrial vertebrates coincide with the lowest effect levels observed in studies on aquatic organisms (Table 2 and Table 3). It is apparent that low-dose effects are obtained in four types of experiments: (1) cautious documentation of mutagenesis in multigeneration experiments, (2) observations of decreased fecundity of organisms in full life-cycle studies or in specific gametogenic stages, (3) observations of developmental problems in organisms irradiated beginning immediately after fertilization, and (4) observations of induction of chromosomal aberrations and sister chromatid exchanges in cells of numerous organisms.

The coincidence of these four types of effects producing similar results in mammals and aquatic organisms is probably due to the common molecular mechanism of radiation damage. Radiation causes DNA damage that may either be translated to subsequent generations in the case of mutagenic effects or cause cell death. It is not surprising that mutagenic effects occur at similar levels across phylogenetic boundaries.

Table 3. Summary of biological effects observed at low dose rates and low total doses in some terrestrial organisms (from Anderson and Harrison 1986).

Dose rate or total dose (Source)	Observed or projected effect <u>Terrestrial organisms^{a,b}</u>	Reference
0.11-0.31 R (γ rays) plus 0.001-1.6 R (α rays)	Increased frequency of chromosomal aberrations in human lymphocytes	Pohl-Ruhling and Fischer 1979
0.043-0.43 R/d (γ rays)	Increased percent sterility in male dogs exposed to 0.043 or 0.086 R/d compared to control; whereas exposure to 0.43 R/d made male dogs sterile in one year	Luckey 1980
0.3 rad/d (HTO)	Decreased brain weight in F ₂ generation of rats irradiated from conception to term	NRC 1980
1 R/d (γ rays)	Decrease in percent survival in first 6 months of life in desert rodent <u>Perognathus formosus</u> ; thereafter, percent survival was increased relative to controls	Whicker and Schultz 1982
1 R/d (¹³⁷ Cs)	Significant increase in mutation rates in mouse spermatogonia versus controls	UNSCEAR 1977
1.1-1.37 rad/d (γ rays)	Sterility induced in leopard lizard and whiptailed lizard from lifetime irradiation	Whicker and Schultz 1982
2 rad/d (γ rays)	No significant differences in sex ratios or egg distributions observed in populations of the lizard <u>Uta stansburiana</u>	Whicker and Schultz 1982
2.5 R/d (⁶⁰ Co)	Prenatal and postnatal mortality observed in rats irradiated from conception to term	NRC 1980
3 rad/d (HTO)	Reduced brain, testis, and ovary weights in rats irradiated from conception to term	NRC 1980
3.3 rad/d (HTO)	No effect on lifespan of rats irradiated from conception to term	NRC 1980
5 rad (HTO) and 8 R (x ray)	LD ₅₀ of mouse oocytes	NRC 1980
8 rad (HTO)	LD ₅₀ of monkey oocytes	NRC 1980
8.4 R/d (¹³⁷ Cs)	Complete sterilization of female mice irradiated 20-40 d postconception	NRC 1980

^a Examples selected to represent the range of organisms, effects, and effect levels examined, as well as to illustrate the presence of positive and negative effects at low dose rates. Other examples for terrestrial organisms are given in Luckey (1980); NRC (1980); UNSCEAR (1977); UNSCEAR (1982); and Whicker and Schultz (1982).

^b Many examples selected for the section on terrestrial organisms are cited from secondary references.

It is also not surprising that fecundity is reduced in many species at similar levels. Gametes are cells of critical importance because their number is limited in many organisms. Of course, human females have no ability to replace damaged oocytes, but many aquatic species do have this capability. It follows that irradiation of an organism throughout its life cycle would impair its ability to replace damaged gametes. It has been hypothesized that one of the reasons for the very low effect levels observed for Neanthes arenaceodentata is that the species is a terminal spawner, that is, females spawn once and then die. Therefore, under normal circumstances they are less likely to exhibit asynchronous oocyte development. Similarly, the whiptailed lizard and leopard lizard are believed to be sensitive to irradiation as are human primates because both exhibit low fecundity (Table 3). It is possible that some deep-sea species may be equally or more sensitive because of the high incidence of low fecundity species in the deep sea.

The question then arises as to why fish fecundity can be severely reduced at doses two orders of magnitude below those affecting similar processes in the daphnid and pond snail. In the guppy experiments performed by Woodhead (1977), irradiation was initiated on 0- to 3-d-old embryos and exposures lasted for at least 1 yr. In the case of the pond snail, irradiation was begun on 45-d-old snails, and it lasted 25 weeks. And in the case of the daphnids, irradiation was conducted over the entire life cycle, but the life cycle is relatively brief (20 to 30 d) with rapid cell turnover. It is hypothesized that the total dose absorbed by the guppy gametes was much greater than the dose absorbed by the gametes of the other species. As such, not only does low fecundity make a species susceptible to greater damage but slow generation times and gamete synchrony are also factors that could result in high cumulative doses to the gametes. Arrested oocyte development and slow embryo development may be much more prevalent in deep-sea species than in coastal species, rendering the deep-sea species more susceptible to radiation damage.

Data on developmental end points also indicate that impacts on selected cells can result in significant realized damage. Interestingly, the lowest developmental effects demonstrated in studies on both mammals and aquatic organisms were observed in experiments beginning immediately after fertilization. Depending on the developmental pattern of the organism, an impact to one cell of a 2-celled embryo is more likely to have a detrimental effect on development than would an impact on one cell of a multithousand-celled embryo. In addition, irradiation immediately after fertilization may encompass the sensitive period of second meiosis.

Because death of critical cells appears to determine the lowest effect levels, there is great interest in determining whether indicators of molecular damage and cell death are also adequate indicators of effects at the cellular and organismal levels, such as reduced fecundity. Anderson et al. (1990) determined that chromosomal aberrations were induced in juvenile polychaetes (Neanthes arenaceodentata) at the same doses at which reduced fecundity was observed in adults. In addition, levels at which chromosomal aberrations have been induced in fish tissues are similar to the levels at which embryo mortality has been observed (Table 1).

2.3.6 Conclusions

The data discussed indicate that the goal of radiation protection in aquatic ecosystems is similar to the goals of human-health protection. The goal should be to focus on characteristic low-dose effects. These include (1) mutagenesis, (2) reproductive effects resulting from long-term or life-cycle exposures, (3) developmental effects resulting from exposures beginning at fertilization, and (4) chromosomal aberrations induced in selected cells as an indicator of other realized effects at the cellular level.

Clearly, the state of the art of these hazard assessments is very sophisticated relative to assessments for other aquatic contaminants. Because common mechanisms of effect exist and because similar low effect levels are noted for both mammals and aquatic organisms, scientists can be reasonably certain that they have obtained state-of-the-art assessments of the low observable effect levels. The stochastic nature of mutagenic effects precludes determination of a no observable effect level or NOEL.

Aquatic hazard assessment for radiation, therefore, has arrived at the same frontiers as has 3 to 4 decades of biomedical research on mammals. Other mutagenic contaminants discharged into the aquatic environment have not been evaluated this extensively, and a mechanistic approach has not been applied. As such, development of numerical or bioassay criteria based on these data potentially affords greater certainty than do data bases used for other contaminants.

In summary, very little uncertainty exists regarding what constitutes low effect levels. Not only are effect levels similar for a wide range of species and types of effects (Table 3), but effects also appear to be within or close to the range in which hormesis effects ("positive stimulation by subharmful quantities of any agent to any system," Luckey 1980) are observed. Of course, all of these findings (Table 3) are not strictly comparable, but convergence is evident in the findings.

Uncertainty does exist, however, in terms of relative effect levels for specific radionuclides and in predicting the outcomes of ingested particles. Uncertainty also exists in extrapolation of effects on coastal species to effects on deep-sea species. Of particular interest is the effect of cold temperature on DNA repair in cold-adapted species and differences in life-history characteristics between coastal and deep-sea species. The latter exhibit a greater frequency of low-fecundity species and of species in which gamete development may be arrested during periods of stress.

2.4 REVIEW OF POTENTIAL EXPOSURE SCENARIOS

According to existing and future regulations, all low-level radioactive waste destined for ocean disposal will be packaged (IAEA 1986). Extensive source characterizations and exposure assessments have been reported elsewhere (see p. 1).

2.4.1 Exposure of Organisms on the Surface of the Packages

Exposure levels on the surface of the packages will be limited to no greater than 0.048 Gy/d. Exposures will be due exclusively to gamma emitters because the radiation from alpha and beta emitters will be attenuated by the packaging. It also follows that, if packaging remains intact, dose to organisms will be external only and no ingestion of radionuclides will occur.

2.4.2 Exposure of Organisms in Sediment

Exposure of organisms to radionuclides in sediment and pore waters would occur if packaging failed for any reason. Assessment of exposures in this case is far more complex. In this case, the spatial and temporal scales of exposure can only be determined by modeling the leaching rates of specific radionuclides from the solid matrix coupled with specific predictions of radionuclide fate. The water-sediment distribution coefficients for specific radionuclides and specific sediments vary greatly, and dispersal from the site will be highly site specific. Half lives and distribution coefficients for selected radionuclides likely to be present in low-level waste are provided in another publication (IAEA 1988).

Estimation of exposure rates for organisms in sediment is also complicated by the fact that the types of radionuclides available are more numerous and the route of entry is more complex. Unlike the case for the packaged wastes, alpha- and beta-emitting radionuclides are now potentially available to the organism, and ingestion is a possible route of entry.

Despite the complexity of modeling exposures in sediment, two general scenarios are most reasonable. In the first scenario, it could be assumed that the outer packaging has failed but the block of solidified waste remains intact. In this scenario, exposures to marine organisms will occur through colonization of the waste block. Therefore, the exposures will be similar to those for intact packaging except that doses will be greater because of the availability of alpha and beta emitters and the potential for ingestion. In addition, localized increases in radionuclides will occur in the sediment surrounding the packages. Due to slow leaching rates and dispersal, these will only be local effects, provided the total number of packages in a unit area covers only a fraction of the area available.

In the second scenario for sediment exposures, it is assumed that both the packaging and the solidification matrix fail. In this case, all the radionuclides are potentially biologically available and may be subject to dispersal. As such, the temporal scale of the exposure is more variable than in the other two scenarios. Provided that the total activity disposed of in a given area is low, then packaging failure would produce a localized higher exposure at first, with possible dispersal or burial.

Two biological phenomena could result in otherwise local exposures having more widespread consequences. Occurrence of significant attraction of organisms to the disposal area due to the introduction of artificial habitat could produce more widespread effects. In addition, any potential for bioaccumulation of radionuclides from sediment exposure could result in more widespread ecological consequences.

Smith et al. (1987) have reviewed the potential for significant bioaccumulation and reef effects to occur as a result of low-level radioactive waste disposal activities. They conclude that significant bioaccumulation could occur, but that it would be realized only on a timescale of decades. They cite four specific examples in which significant accumulation of radionuclides have been attributed to ocean-disposal activities. Additionally, Smith et al. (1987) provide detailed assessments of techniques that could be used to conduct ecological monitoring of the dumpsite at the community level. They recommend that monitoring be conducted to evaluate potential reef effects.

2.5 COMPARISON OF EXPOSURE AND EFFECT LEVELS

Comparison of the potential for overlap between the levels at which detrimental biological effects occur and anticipated exposures reveals that highly localized effects may occur in the immediate vicinity of the packages. However, these effects would only be realized in organisms receiving constant exposures over the course of several months to years (Figure 2).

The minimum dose rate at which detrimental sublethal effects are known to occur for marine organisms is 0.005 Gy/d over at least 3 months of constant exposure (Harrison and Anderson 1988b). Lethal effects of radiation exposure will only occur at doses of approximately 5 Gy/d (Figure 2).

Exposure levels were assessed in three scenarios. It was determined that dose rates at the surface of intact packages would be no greater than 0.048 Gy/d. Dose rates in two types of sediment-based exposures will be higher on a local basis than those occurring on the surface of intact packages because of the greater biological availability of alpha- and beta-emitting radionuclides after packaging failure. However, burial, leaching, and dispersing of these radionuclides could occur over time. These processes would cause the potential for effects to be diminished.

Comparison of exposure and effect levels indicates that detrimental sublethal effects to biota could occur as a result of low-level radioactive waste disposal. However, the area of potential impact is only the immediate vicinity of or the actual surface of the packages themselves. The effects realized would not be lethal effects, and exposures would have to be constant for several months to realize effects. Unless the dumping rate is very high per unit area or extraordinary reef effects occur, it is predicted that there will be little significant biological impact even in sediment exposure (Figure 2).

Uncertainties do exist, however, that should be discussed in summarizing this risk assessment. First, sediment exposures are far more difficult to predict than are exposures on the packages. Second, very little is known about the basic life history and physiology of deep-sea organisms; as such, assessment of hazard is based on extrapolations between the sensitivity of coastal species and the sensitivity of abyssal organisms. Third, the ramifications of potential reef effects are not well understood. Fourth, multigeneration effects of ionizing radiation on marine populations are not well documented, especially for low fecundity species (Anderson and Harrison 1990).

HAZARD ASSESSMENT

Lethal effects of chronic exposure:

6.9 Gy/d

Sublethal effects of chronic exposure:

0.005 Gy/d

EXPOSURE ASSESSMENT

Packaging intact:
Instantaneous release:

0.048 Gy/d

- Site specific modeling required

Solidification intact:

- Site specific modeling required

RISK ASSESSMENT

For all exposure scenarios:

- no lethal effects
- sublethal effects in constant exposures over several months to several years
- area of effect limited to surface of packaging or within immediate vicinity of breached package

SOURCES OF UNCERTAINTY

- sediment exposures
- physiology of deep-sea species
- life history of deep-sea species
- potential for reef effects
- multigeneration effects

Figure 2. Summary of risk assessment findings. Lowest observed effect levels and most conservative exposure assessments are presented as is the predicted risk to aquatic life.

Chapter 3

TOOLS FOR RISK MANAGEMENT

3.1 GENERAL APPLICATIONS OF CONTAMINANT-SPECIFIC AND TOXICITY-BASED CONTROLS

The risk assessment in the previous chapter documented the potential for localized detrimental effects to aquatic life as a result of low-level radioactive waste disposal. However, the risk is not only local in scale but subtle in nature, with any effects occurring only after exposures on the order of months to years. Consequently, a need for water-quality based controls exists, but they must be developed in a reasonable yet conservative manner. It must also be acknowledged that an extraordinary level of scrutiny has been applied to this question relative to other types of waste discharges for which significantly greater risks to both human health and aquatic life exist.

Two types of water-quality based criteria exist. They are numerical criteria for specific constituents and toxicity-based criteria. Toxicity-based criteria are most frequently recommended for use when a mixture of contaminants is to be assessed for toxicity or when the biological availability of a single contaminant cannot be predicted easily. Toxicity-based criteria are being widely used to control toxic effects of complex effluent discharges (EPA 1985). However, the application of toxicity standards to contaminated sediment is still developmental, with several approaches being used in different areas. Toxicity-based standards are usually based on determination of No Observable Effect Levels (NOELS) using standard chronic toxicity tests. As such, these standards have operational definitions that may vary based on the techniques used to detect chronic effects and the duration of the tests. Commonly, short-term chronic tests lasting 7 d are recommended. If toxicity-based standards are applied to disposal of low-level radioactive waste, the most commonly used approaches will be inadequate because longer exposures of more selected lifestages would be required to detect Lowest Observable Effect Levels.

Numerical criteria for specific contaminants are usually recommended when the contaminant of concern has been identified and when specific responses, which are not reliably assessed in routine testing, are to be controlled. The latter includes phenomena such as bioaccumulation, teratogenesis, and mutagenesis. The advantages of constituent-specific limits over bioassay limits are that contaminant fate is more easily assessed in the constituent-specific approach, source reduction is more easily assessed and controlled, and costs of analytical testing are frequently cheaper than costs of chronic bioassay procedures (EPA 1985). Numerical criteria are calculated using toxicity data generated in rigorous laboratory testing on a range of species.

Both constituent-specific and bioassay criteria are applied in conjunction with exposure analyses and policies regarding acceptable mixing zones. In most waste-disposal activities, a small zone in which detrimental effects could occur is

allowable. However, to our knowledge, no such policies have been developed for contained radioactive wastes. If such policies are to be explored, distinctions must be made between dispersal of radionuclides and attenuation of radiation dose through water.

3.2 APPROPRIATE APPLICATION OF CONTAMINANT-SPECIFIC CONTROLS

The appropriate application of constituent-specific limits depends on the exposure scenario to be considered and the information available to develop limits. First, we consider the scenario in which packages remain intact. In this case, only organisms colonizing the surface of the packages will receive exposures of any significance. The only radiation will be from gamma emitters, and there are no complications associated with the fate of the radionuclides or their biological availability. As such, application of a numerical limit on activity levels at the surface of the packages is a highly recommended approach. Sufficient information exists in the aquatic literature to develop criteria for doses from gamma emitters, if only external doses are to be considered. In fact, the majority of reliable data on radiation effects has been obtained using sealed gamma-emitting sources (Anderson and Harrison 1986).

Development of numerical criteria or numerical guidelines for a specific activity would require efforts specifically focused on that goal; however, some general guidance is described below. For protecting against potential detrimental reproductive effects on aquatic life on the surface of the packages, dose rates at the surface should be less than 0.005 Gy/d. Dose rates one to two orders of magnitude higher would protect against lethal effects of chronic exposures several weeks in duration.

In contrast to the rather clear cut scenario for exposures on the container surface, development of numerical limits for specific radionuclides in sediment is considerably more complex. First, insufficient information is available to predict the distribution and residence time of ingested radionuclides. Second, few data are available on alpha emitters that could be used to predict accurately sediment toxicity; and third, the development of numerical sediment quality criteria, in general, is still experimental. With these limitations in mind, the development of radionuclide-specific criteria for sediment is not recommended for near future application. However, determination of safe sediment exposure levels for classes of radionuclides should be a high priority subject for future research.

3.3 APPROPRIATE APPLICATION OF TOXICITY-BASED CONTROLS

Application of toxicity-based controls should be focused on consideration of sediment exposures. For exposures in which the packages remain intact, there is no technical rationale for implementation of a bioassay testing program. This is because neither the biological availability of the radiation penetrating the container is in question nor is the level at which detrimental effects are observable. In this exposure scenario, there is only one general class of constituents of concern, gamma-emitting radionuclides. Detrimental effects of these radionuclides should be controlled using numerical guidelines.

Toxicity-based controls are highly recommended for sediment-exposure scenarios. This is because the biological availability of the different sediment-bound radionuclides is not adequately documented, because a number of radionuclides may occur in combination with toxic constituents either in the waste or already present in the sediment, because ingestion will be an important exposure route, and because potentially applicable bioassay protocols exist.

A toxicity-based approach should only be considered if, from a policy standpoint, there is a need to protect against occurrence of sublethal radiation effects in sediment. To date, very little work has been conducted nationally or internationally to assess sublethal effects on aquatic animals from exposure to contaminated sediment. Sediment testing is, however, a rapidly developing area, and it is advisable that the radiation community apply the most sophisticated tools available.

The narrative criterion to be met should be to maintain sediment quality such that detrimental reproductive and developmental effects of radiation exposure are not detectable. Detectable should be defined using reasonable adaptations of bioassay tests (specified herein) that are amenable to standardization.

3.4 IDENTIFICATION OF BIOASSAY PROCEDURES

Adequate bioassay tools must be identified for toxicity-based standards or control measures to be applied. Toxicity testing would be applicable either to predisposal testing or to testing of sediment toxicity from sediment or pore-water sample collected at dumpsites. Many of the assays described also have application for future development of numerical standards for specific radionuclides.

Two types of sediment exposure will be considered. For the first type, we will assume that both the packaging and the solidification would fail. For the second type, we will assume that the packaging failed but the solidification is partially intact.

Our first step in identifying promising bioassay techniques was to develop selection criteria. These were (1) potential for detecting sublethal effects related to reproduction, development, or genotoxicity, using relatively long exposure periods; (2) potential for use with sediment exposures either by direct exposure to sediment or through use of pore water, sediment extracts, or elutriates; (3) use of marine species; and (4) use of standardized protocols or protocols amenable to standardization. It was assumed that most assays would have to be modified to some extent to accommodate special considerations associated with unusual waste configurations and the need for relatively long exposure periods.

Application of the preliminary criteria listed above resulted in the exclusion of some commonly used bioassay tests. Water column tests were considered only if they might be adaptable to testing with blocks of solidified waste or if they required only very small water volumes, making them amenable to use with pore waters or sediment extracts. All tests that did not have relevant sublethal end points and long exposure times were also excluded. Bioassays excluded for this reason are (1) the Rhepoxynius abronius infaunal amphipod sediment bioassay (mortality and behavioral end points only), (2) the mollusc 48-h embryo test (short exposures), (3) the sea urchin fertilization and

development tests (1- to 48-h exposures), (4) all acute testing protocols (no sublethal end point measured), and (5) short-term growth tests using the inland silverside Menidia beryllina and the sheepshead minnow Cyprinodon variegatus (growth end point not sufficiently sensitive in radiation research). In addition to the exclusions mentioned above, no macroalgae tests were considered because macroalgae do not occur in the deep sea.

Bioassay protocols to be considered fall into two categories. First are protocols that have been applied to sediment elutriate or pore-water exposures. Second are protocols for water-column tests that could conceivably be modified for testing with introduced blocks of solidified wastes. This approach is considered valuable because animals could be exposed directly to the solid waste.

3.4.1. Tests Recommended for Sediment Exposures

Four potential tests were identified for use with direct sediment or pore-water exposures (Table 4). They are (1) the Ampelisca abdita reproduction test, (2) the Neanthes arenaceodentata dominant lethal and reproductive tests, (3) the Capitella capitata full-life-cycle reproductive test, and (4) the Dinophilus gyrociliatus pore-water test. The latter three species are polychaete or archiannelid worms. We do not recommend that they all be applied, but their relative merits should be discussed before tests are selected.

A reproductive test for the amphipod Ampelisca abdita may be very useful for either predisposal testing of wastes that have not been solidified or for postdisposal monitoring of disposal site sediments. A variety of applicable protocols have been identified for use (e.g., Gentile et al. 1985). Most frequently, females with embryos in the brood sac are used to initiate the experiment and, following 28-d exposures, fecundity of F₁ females is evaluated (Table 4). Exposures may be increased to approximately 40 d to evaluate survivorship of F₂ progeny or to 56 d to evaluate fecundity of F₂ females. A. abdita inhabits relatively fine-grained sediment throughout its life cycle, except for a brief period in which spawning is believed to occur. This species will not tolerate sand or similar substrates.

While detailed protocols for the A. abdita tests have not been subject to intensive review as of yet, the tests have already been used in a variety of field situations (e.g., Gentile et al. 1988 and Long et al. in press). This test can be recommended for immediate application with no modifications required. However, the fact that the radiosensitivity of this species is unknown is a significant disadvantage that should be rectified with further study. While this particular species does not exist in the deep sea, it is fortuitous that standard culture procedures and protocols for long-term reproductive tests exist for any infaunal amphipod. Animals are exposed directly in sediment, and they are excellent biological integrators of sediment exposures. The only drawback to this assay is that it has never been applied in radiation biology.

Table 4. Summary of sediment toxicity tests evaluated.

Species	Effects measured	Medium of exposure	Exposure	Length of life stages	References
<u>Ampelisca abdita</u> amphipod	Reproduction	Sediment	28 to 56 d	Full life cycle	Gentile et al. 1985
<u>Neanthes arenaceodentata</u> polychaete	Reproduction and survival	Sediment	3 to 4 mo	Full life cycle	Harrison and Anderson 1988a;b
<u>Capitella capitata</u> polychaete	Reproduction, growth, and survival	Sediment	35 to 50 d	Full life cycle	Chapman and Fink 1984
<u>Dinophilus gyrociiliatus</u> archiannelid	Reproduction and survival	Pore water	7 to 10 d	Full life cycle	Carr et al. 1986

Reproductive and multigeneration dominant lethal and recessive lethal mutation tests have been developed for the polychaete worm Neanthes arenaceodentata (Reish 1980). These tests have been modified by Harrison and Anderson (1988a) and used in a number of studies on radiation effects (Table 4). A method using Trypan Blue staining (Harrison and Anderson 1988a;b) was developed to distinguish live and dead embryos. This end point is sensitive and less time consuming than methods described by Reish (1980). The species is known to exhibit sublethal effects of radiation exposure at the lowest doses documented for any aquatic organism. Assays with N. arenaceodentata are normally conducted in water-column tests. However, protocols are adaptable to some types of sediment exposure.

Chapman and Fink (1984) have described a life-cycle test for sediment and sediment-elutriate exposures using the polychaete worm Capitella capitata (Table 4). In this test, a number of sublethal end points are evaluated, among them are reproductive and developmental effects. Exposures were initiated with trochophore larvae and were terminated 50 d later. The number of females bearing eggs and mortality during developmental stages were quantified.

This assay cannot be recommended for immediate use, but it may be of value with further development. This assay is a specific adaptation for sediment exposure of the water-column methods of Reish (1980). Thus far, the reproductive end point is not sufficiently refined, but future detailed work could rectify this concern. To our knowledge, no data exist on use of this species in radiological studies.

The fourth species of interest for sediment testing is the archiannelid Dinophilus gyrociiliatus. Carr et al. (1986) recently proposed a short-term life-cycle test for this species (Table 4). Unpublished modifications of this test have been developed for use with pore-water samples, and field validation studies have been conducted (e.g., Long et al. in press). In this test, hatching success and the number of juveniles released from egg cases are quantified.

This species has a very short life cycle of only 10 d. This characteristic is an advantage in many types of testing but is not a benefit for studies of radiation effects where long-term cumulative doses are desirable. However, it is possible that modification of this assay for multigeneration exposures could be accomplished with relatively little effort. Successive multigeneration exposures could be sensitive in detecting dominant and recessive lethal mutations. However, based on experience described in Chapter 2 with Daphnia pulex, species with rapid life cycles are potentially insensitive to radiation effects.

This test cannot be recommended for immediate application because the short life cycle may imply that radiation effects that could potentially be detected using other assays will not be detected. However, this concern could be resolved by conducting a chronic exposure experiment. In addition, because the test does not employ sediment but pore-water exposures, it has few advantages over the more well-developed N. arenaceodentata tests.

3.4.2 Tests Evaluated for Exposures Using Solidified Waste

Just as sediment toxicity tests are the best means of evaluating sediment exposures, toxicity of contaminants in solidified or partially solidified wastes is best evaluated by simulating a close association with those solidified wastes. To our knowledge, this approach has not yet been utilized, but it could be a very practical one. To use this approach, we must select species for which some sensitive life stage can be brought into close contact with the surface of the waste material. By providing a close association with the waste during gametogenesis or the early stages of development, sensitive lifestages are directly exposed to the waste and dosimetry is controlled far more effectively than if free-swimming life stages were used. There are a number of fish and invertebrates for which a portion of the life cycle could be evaluated in this manner. Modifications of existing tests are described, as are some feasible experimental approaches.

The first recommended test approach is use of either the inland silverside Menidia beryllina or the sheepshead minnow Cyprinodon variegatus in a modification of the standard 28-d embryo-larval tests (Table 5). In the standard tests, embryos are raised in brood cups made of plastic with nylon-mesh bottoms. Tests are initiated shortly after fertilization, and the embryos rest on the bottom until hatching occurs (approximately 7 d). The test is terminated after 28 d, and growth and survival are measured. Tests may be considered invalid if less than 60 percent survival is achieved in the controls. Separate tests may be run in parallel to determine hatching rate and embryological success. These tests may be considered invalid if less than 80 percent of the embryos survive in the controls.

Modification of this test for association with solidified wastes would not require major new developments. For the first 7 d, brood cups could be placed a few millimeters above the waste surface. Ideally, small samples of the waste could actually be solidified in the bottom of a convenient test container, such as a glass petri dish. After hatching, the height of water in the brood cup should be

minimized to promote the closest possible association between free-swimming fry and the solid waste. The free-swimming fry could be removed from the brood cups and cultured in direct contact with the waste material.

The principal advantage of this test approach is that it is practical and could be conducted by most of the better-qualified contract laboratories. The principal disadvantage is that only 7 d of clear-cut association with the waste block are provided and that development is not as sensitive an end point as is reproduction for the evaluation of radiation effects. With some further development, species could be identified that could be used for improved exposure scenarios.

The second recommended test approach is exposure of epibenthic species to the waste blocks during sensitive periods of gametogenesis. Animals would be exposed for varying periods of time and reproductive success of the progeny evaluated. Tests on four species that might be considered are described below. The species are the crustaceans Palaemonetes pugio and Mysidopsis bahia and the polychaete worm Neanthes arenaceodentata (Table 5).

The Palaemonetes pugio life-cycle test developed by Tyler-Schroeder (1979) begins with 12- to 19-mm juvenile shrimp and is terminated when the F₁ progeny reach juvenile stage. This requires approximately 3 to 4 mo. Shrimp are exposed in aquaria until the females are ready to spawn. At that time, the females are transferred to spawning chambers. Waste exposures could be conducted in the aquaria with water volume minimized to discourage movement from the bottom. It is also possible that exposures could be continued into the post-spawning period if modified spawning chambers could be constructed. While this test is no longer experimental, it could only be conducted by a select group of the most qualified bioassay consultants.

A protocol for full-life-cycle tests with the mysids has been described by Nimmo et al. (1977). Mysidopsis bahia could be cultured for approximately 27 d with exposures to the waste block. Reproductive success is evaluated. The P. pugio test is recommended over the mysid test because a longer exposure period could be utilized.

The Neanthes arenaceodentata full-life-cycle test has been described above. No substantial modifications for exposures to solidified wastes would be required. EPA could require that subsamples of waste be prepared in a convenient configuration, such as covering the bottom of plastic petri dishes, and the assay could be performed in that dish. It may be necessary to adjust the external texture of the solidified waste because N. arenaceodentata will not survive on highly abrasive surfaces. This test would be more reliably performed by a variety of laboratories than the P. pugio test, but highly trained bioassay specialists are required. The Trypan Blue modification described by Harrison and Anderson (1988a) simplifies the test greatly and should be considered for standard use.

Table 5. Summary of test evaluated for exposures using solidified waste.

Species / Tests	Effects measured	Length of exposure	Life stages	References
<u>Menidia beryllina</u> Inland silverside	Embryo development	28 d	0 to 28 d	ASTM in prep
<u>Cyprinodon variegatus</u> Sheepshead minnow	Embryo development	28 d	0 to 28 d	ASTM in prep
<u>Palaemonetes pugio</u> grass shrimp	Reproduction	3 to 4 mo	Juvenile to F1 juvenile	Tyler-Schroeder 1979
<u>Mysidopsis bahia</u> opossum shrimp	Reproduction	127 d	24h-one brood release	Nimmo et al. 1977
<u>Neanthes arenaceodentata</u> polychaete worm	Reproduction	3 to 4 mo	Gravid adult to F2 progeny	Harrison and Anderson 1988b

The test approaches that emphasize reproduction as an end point will be most sensitive to radiation effects in long-term exposures (Anderson and Harrison 1986). Therefore, the test approaches utilizing development of fish embryos (Menidia beryllina and Cyprinodon variegatus) are not preferred, but it is strongly recommended that a test utilizing a fish species be developed.

Reproductive effects could also be evaluated in a variety of yet-to-be-developed experimental approaches. For example, species could be selected with relatively low fecundity or with brooding development and cultured in association with solidified wastes. The frequency of live versus dead embryos could be evaluated at varying time points after spawning. Additionally, tests utilizing fish species that are epibenthic feeders could be developed.

3.4.3 Costs of the Bioassay Tests and Suggested Numbers and Types of Tests

Assessing reasonable cost of bioassay requirements is an important goal in environmental management. The costs of individual tests must be weighed together with the number of types of assays that must be run and the number of times the tests must be repeated to assess variability in waste composition.

EPA has made several determinations on what might be considered reasonable costs over the past several years. For protection of aquatic life, EPA (1985) has recommended that short-term chronic bioassay tests at a specific discharge or disposal site be conducted on a minimum of three species (to assess phylogenetic diversity in response to contaminant exposure). Assessment of variability is determined on a site-specific basis, but a typical sewage-treatment plant servicing an urbanized area may conduct a battery of 3 species tests approximately 18 times over

1 yr to assess variability. Short-term chronic tests, such as the Ceriodaphnia sp. reproduction tests, cost approximately \$1,000 per test. Therefore, typical site-specific studies cost \$50,000 to \$100,000.

The strategy for assessing toxicity of low-level radioactive waste should emphasize fewer tests, because the waste is less variable in composition, but the tests will be more expensive. It is assumed that all applicants for ocean disposal of radioactive waste have financial resources at least equal to those of a typical sewage-treatment plant, and that a prepermit program costing approximately \$50,000 to \$100,000 would be considered reasonable. The cost of a typical full-life-cycle test will range between \$25,000 and \$40,000 each if appropriate quality assurance is included.

It is recommended that a minimum of two long-term tests be conducted in sediment exposures and that one be conducted using solidified waste for each waste type under evaluation.

3.4.4 Summary of Tests Suggested for Immediate Use

Tests most highly recommended for use are shown in Figure 3. Although these tests are not considered experimental, their practical application using radioactive waste has not been validated. It is recommended that specific adaptations of these tests to selected waste configurations be developed. Development of test with a fish species should be considered a high priority. In addition, a genotoxicity assay should be developed using cells of a marine fish or invertebrate that may be capable of integrating chronic doses of ionizing radiation.

SEDIMENT EXPOSURES

Ampelisca abdita (amphipod)
Neanthes arenaceodentata
(polychaete worm)

- reproductive test by Gentile et al. (1985)
- reproductive test by Harrison and Anderson (1988a;b)

SOLID WASTE EXPOSURES

Palaemonetes pugio (shrimp)
Neanthes arenaceodentata
(polychaete worm)

- reproductive test by Tyler-Schroeder (1979)
- reproductive test by Harrison and Anderson (1988a;b)

RESEARCH AND DEVELOPMENT PRIORITIES

- development of bioassay using an epibenthic fish species
- development of a genotoxicity assay for long-lived cells of a marine fish or invertebrate

Figure 3. Summary of bioassays recommended for use and priorities for development of improved bioassay methodologies.

Chapter 4

PROPOSED RISK MANAGEMENT APPROACH—SUMMARY OF PREDISPOSAL TESTING RECOMMENDATIONS

The recommended approaches to predisposal testing differ depending on whether the assumption is made that packaging will remain intact. If packaging remains intact, there is no rationale for predisposal toxicity testing because activity measurements on the surface of the drum are sufficient to predict biological effects. However, if packaging fails, then there is a rationale for a predisposal toxicity testing program in selected cases. These two scenarios are summarized in Figures 4 and 5.

The proposed management approach for the scenario in which packaging remains intact (Figure 4) is to develop a gamma-activity limit for the surface of the packages. Deep-sea biota would receive no internal dose and no exposure to complex mixtures. Consequently, there is no rationale for a prepermit bioassay testing program based on this exposure scenario. Nevertheless, it is strongly recommended that enforcement activities include (1) a carefully developed activity limit for gamma exposures, (2) a program to enforce compliance with activity limits, and (3) reporting and data-management requirements for tracking compliance with reported activities at the drum surface and for tracking dumping rates in given geographical areas.

The principal policy decision to be made is a determination of whether chronic effects are allowable at the surface of the packages. Evaluation of effect levels and packaging requirements indicates that it is feasible that acute effects on the container surface be prevented. Therefore, this latter issue should not be debated. Our assessment of possible chronic effects indicates that there may be subtle chronic effects on organisms living on the surface of the packages. It is advisable that surface-activity limits be reduced as much as possible. However, if no reduction is attainable, subtle chronic effects on the surface of the packages may be considered analogous to mixing zones of coastal discharges in which chronic effects are allowed.

The proposed management approach for the exposure scenario in which packaging fails (Figure 5) is to develop a program with four basic provisions. The program would first emphasize compliance with a specified gamma-activity limit, as is recommended for the exposure scenario in which the packaging remains intact. Secondly, it is recommended that reporting of waste characteristics be carefully developed and detailed, emphasizing waste minimization and waste segregation. The third would be the development of a prepermit-bioassay-test program. While such a program was not recommended for the first exposure assessment, it is recommended for this scenario because deep-sea biota would be exposed to alpha and beta emitters and complex mixtures of wastes, and matrix effects may affect the response. Such exposures make predictions of bioavailability from numerical criteria very difficult.

EXPOSURE SCENARIO

- no internal dose
- no matrix effects
- no complex mixtures
- γ -radiation only
- space and time can be defined

POTENTIAL IMPACT

- only those organisms constantly inhabiting the surface of packages for months to years may be impacted with detrimental reproductive effects
- no acute toxic effects will be observable

PROPOSED MANAGEMENT APPROACH

- set an objective for safe gamma activity on the surface of packages
- require reporting of activities measured and compliance with safe limit
- do not impose bioassay program

Figure 4. Risk-management approach with the assumption that packaging remains intact; animals are exposed only on the surface of the packages. Characteristics of predicted exposures and impacts are given as are proposed management approaches to minimizing impacts.

EXPOSURE SCENARIO

- external dose from γ emitters and internal dose from α , β , and γ emitters
- matrix effects
- complex mixtures
- sediment and pore-water exposures
- space and time of exposure difficult to predict
- variable dispersal anticipated

POTENTIAL IMPACT

- effects, observed (if any), would be extremely subtle
- effects realized after months or years of constant exposure
- effects, if at all, would be observed only in organisms residing in the sediments immediately adjacent to breached packages

PROPOSED MANAGEMENT APPROACH

- require reporting for compliance with a specified activity limit on the surface of packages
- require reporting of waste characteristics
- evaluate need for waste-segregation program
- require predisposal toxicity testing if waste characteristics are complex
- formulate sediment toxicity objective and consider development of sediment quality criteria for individual radionuclides
- develop sediment radionuclide and ambient biotoxicity monitoring requirements at disposal site over extended timescale

PROPOSED TOXICITY TESTS

- 2 sediment tests
- 1 test with solidified waste
- develop an epibenthic fish biotoxicity test and an improved genotoxicity test

Figure 5. Risk-management approach with the assumption that packaging fails; animals are exposed by rapid or slow releases of radionuclides to water and sediments. Characteristics of predicted exposures and impacts are summarized as are proposed risk-management approaches to minimize impacts.

The recommended bioassay program (described in Section 3.4) was developed for specific application to low-level radioactive waste disposal. The following were emphasized (1) need for long-term exposures to appropriate life stages, (2) potential for evaluating effects related to reproduction, development, or genotoxicity, (3) potential for use in sediment exposures, and (4) preference for use on marine species and protocols amenable to standardization.

Three species and bioassay test protocols were identified after adaptation for use with the waste types in question. These are reproductive assays utilizing the polychaete worm Neanthes arenaceodentata, the amphipod Ampelisca abdita, and the shrimp Palaemonetes pugio. A fish embryo test is also described but not recommended for immediate application. Development of an improved genotoxicity assay to detect cumulative effects in long-lived cells, such as gametes, is also recommended.

The fourth provision of the program is that sites be monitored for sediment contamination. Initially, monitoring could be limited to determination of radionuclide concentrations in sediment of disposal areas. However, ambient biomonitoring could be incorporated over the next decade, because progress in evaluating effects in sediment is developing rapidly.

The imperative technical and policy actions associated with this fourth provision are the formulation of a toxicity objective for sediment exposures and the development of sediment-quality criteria for specific radionuclides or classes of radionuclides. It is recommended that the long-term goal of the program be to prohibit chronic effects due to sediment exposures. However, this goal is not yet enforceable. This is because state-of-the-art sediment toxicity evaluations are undeveloped and the available tests are not always good predictors of effect. The same is true with respect to methods used in the development of sediment-quality criteria. As such, it is recommended that goals be adopted with timelines for re-evaluation of the attainability of those goals. This type of approach is common in drinking-water regulations, where goals or objectives for specific chemicals may not be technically enforceable because of high-detection limits. Nevertheless, unattainable goals are set as statements of policy.

Protocols to specifically assess genotoxic effects were also considered. There are two tests that have been used to evaluate effects in contaminated sediment. They are a sister-chromatid-exchange assay using the polychaete Neanthes arenaceodentata (Pesch et al. 1985a;b) and an anaphase aberration assay using fish embryos (Tetra Tech 1986). Genotoxic effects of ionizing radiation exposure in N. arenaceodentata have also been evaluated (Anderson et al. 1990). These assays were not recommended for immediate implementation because they cannot be applied to long-term exposures without extensive modification. For these assays to be as sensitive as the long-term reproductive assays, a genotoxicity assay must be designed for use on cells that are long lived and can accumulate the dose delivered (Harrison and Anderson 1988b).

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