Environmental Management Science Program

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Determining Significant Endpoints for Ecological Risk Analyses

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Research Objective

Our goal is to establish a protocol for assessing risks to non-human populations exposed to environmental stresses typically found on many DOE sites. We think that we can achieve this by using novel biological dosimeters in controlled, manipulative dose/effects experiments, and by coupling changes in metabolic rates and energy allocation patterns to meaningful population response variables (such as age-specific survivorship, reproductive output, age at maturity and longevity). This research is needed to determine the relevancy of sublethal cellular damage to the performance of individuals and populations exposed to chronic, low-level radiation, and radiation with concomitant exposure to chemicals. We believe that a scientifically defensible endpoint for measuring ecological risks can only be determined once we understand the extent to which molecular damage from contaminant exposure is detrimental at the individual and population levels of biological organization. Our experimental facility will allow us to develop a credible assessment tool for appraising ecological risks, and to evaluate the effects of radionuclide/chemical synergisms on non-human species.

Research Progress and Implications

This report summarizes work completed midway of a 3-year project that began in November 1996. Emphasis to date has centered on three areas: 1) developing a molecular probe to measure stable chromosomal aberrations known as reciprocal translocations, 2) constructing an irradiation facility where the statistical power inherent in replicated mesocosms can be used to address the response of non-human organisms to exposures from low levels of radiation and metal contaminants, and 3) quantifying responses of organisms living in contaminated mesocosms and field sites.

We have had wonderful success in adapting a molecular technique used to measure stable chromosomal aberrations in human atomic-bomb survivors to a biological dosimeter for determining ecological risks. Stable aberrations, known as reciprocal translocations, unlike others, remain detectable for periods long after the initial exposure. The methodology was a spin-off of the human genome project of the DOE. Until recently, however, technique development and application of the approach for ecological risk analyses would have required a monumental effort for each organism studied. It is now possible to achieve the same goal with much less effort in isolating the necessary probes by using chromosome microdissection techniques followed by polymerase chain reaction (PCR) amplification.

We are excited about our development of the first chromosome-specific probe for a non-mammalian species, the yellow-bellied slider turtle (Trachemys scripta). The technique requires microdissection of specific chromosomes followed by polymerase chain reaction amplification and fluorescent in-situ hybridization. The probe, when fluorescently labeled, ‘paints’ that chromosome in any cell of that species treated with the probe. The labeled DNA is then hybridized back to cells sampled from individuals of the same species that have been exposed to contaminants. Reciprocal translocations that have occurred over the life of the exposed organism can then be quantified by

observing the fluorescent marker on translocated chromosomes. Thus, a biological dosimeter that measures cumulative damage in a long-lived vertebrate is now possible. The yellow-bellied slider turtle was chosen as a model organism for this phase of the work because it can accumulate appreciable doses over a lifetime of 30 years, it has a chromosomal constitution amenable to cytogenetic analysis of aberration induction, it has a wide geographical range and is thus found at numerous DOE sites, and it is mobile enough in both terrestrial and aquatic systems to sample a reasonable range of its contaminated environment. Our development of this tool is fundamental to our determining what constitutes a 'significant risk' in ecological risk analyses. We have conducted in vitro experiments using irradiated turtle fibroblasts to document the efficacy of the molecular probe in detecting reciprocal translocations, and we have also developed dose response curves for total aberrations and reciprocal translocations following acute irradiation. Our second area of advancement is in the design and construction of a mesocosm irradiation facility. This facility will allow us to determine: 1) chromosome damage at various radiation exposures and metal contaminant concentrations, 2) relationships between cellular damage and metabolic rate, 3) treatment effects on an individual's energy allocation pattern, growth and survival. The greatest advantage of using mesocosms (outdoor, above-ground tanks in which artificial pond communities are established) is the ability to replicate treatments such that powerful statistical methods can be used. A location on the Savannah River Site was chosen for the array of mesocosms due to its proximity to the PAR Pond Radioecology Laboratory, its isolation from the general public, and the availability of water and electrical power. Land has been cleared of trees, graded, and storm drains installed. Because we anticipate using turtles, fish and amphibians as model organisms, considerable effort was spent designing a mesocosm suitable for a variety of species. Each individual mesocosm will have a sealed ^{137}Cs source suspended above it, thus designs had to consider the need to homogenize the dose distribution within a mesocosm, and to minimize the dose a human would receive working in the field of 50 mesocosms. A subcontractor recently completed construction of the 50 mesocosms. We have installed a prototype irradiator over one of the mesocosms and our initial dose distributions indicate an unexpected heterogeneity that the manufacturer of the sealed ^{137}Cs source is now trying to remedy.

Our first field experiment was designed to test the effects of contaminants on mosquitofish (Gambusia) and snails (Campeloma) reared in a radioactive seepage basin, metal contaminated site (coal ash basins), and a control pond. The design provided animals that were exposed to each type of stress for the entire study (four months), or for pairwise combinations of stress types during different portions of the study. Surprisingly, all animals transplanted to the metal-polluted site died during the first half of the study, whereas survival in the radiation site and reference site was high. Unfortunately, prior to the scheduled end of the second phase of the study, a prolonged drought resulted in drying of the radiation basin and subsequent loss of animals in that site as well. Although the mortality was disappointing, we acquired valuable dosimetry data from the radiation basins and the lessons learned will be applied in future experiments.

Even though our mesocosm facility is not finished, we have designed it such that organisms can be irradiated from the prototype source we are testing. We are now nearing completion of an experiment that examined the effects of low-level radiation exposure, alone and in conjunction with heavy metal contaminants, on larval salamanders. This is our first experiment conducted in the mesocosm facility and we hope to present results at the Society of Environmental Toxicology and Chemistry annual meeting in November.

Planned Activities

Determine if the probe developed for Trachemys scripta works on other species of turtles (July 1998)

Submit a manuscript entitled Turtle Chromosome Specific DNA Libraries for Ecological Risk Assessment of Irradiated Populations to the Journal of Radiation Biology. The paper describes the development of the probe and the acute irradiation dose-response curve. (August 1998)

Conduct an experiment, similar to what was described above for larval salamanders, using freshwater shrimp (September 1998)

Complete mesocosm facility (May 1999)

Compare dose response of turtle lymphocytes (as opposed to fibroblasts) irradiated in vitro vs. in vivo; conduct dose-rate experiments using turtle lymphocytes (June 1999)

Other Access To Information

Dr. Muhlman-Diaz presented a poster publication detailing the development of the chromosomal probe at the Third International Clinical FISH symposium in Steamboat Springs, CO. Brant Ulsh presented a poster detailing the response of turtle fibroblasts to acute irradiation at the National meeting of the Radiation Research Society in Louisville, KY (for which we won a student travel award).