
Determining Significant Endpoints for Ecological Risk Analyses

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Research Objective

Our interest is in obtaining a scientifically defensible endpoint for measuring ecological risks to populations exposed to chronic, low-level radiation, and radiation with concomitant exposure to chemicals. To do so, we believe that we must understand the extent to which molecular damage is detrimental at the individual and population levels of biological organization. Ecological risk analyses based on molecular damage, without an understanding of the impacts to higher levels of biological organization, could cause cleanup strategies on DOE sites to be overly conservative and unnecessarily expensive. Our goal is to determine the relevancy of sublethal cellular damage to the performance of individuals and populations. 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).

Research Progress and Implications

This report summarizes our third year's progress as of June 1999. Please refer to earlier annual reports for work that began in November 1996. Research has concentrated on three areas: 1) developing a molecular probe to measure stable chromosomal aberrations known as reciprocal translocations, 2) construction of an irradiation facility where the statistical power inherent in replicated mesocosms can be used to address the response of non-humans organisms to exposures from low levels of radiation and metal contaminants, and 3) quantifying responses of organisms living in contaminated mesocosm and field sites.

We have successfully developed a biological dosimeter that measures cumulative damage in a long-lived vertebrate, the yellow-bellied slider turtle. The technique requires microdissection of specific chromosomes followed by polymerase chain reaction amplification and fluorescent in situ hybridization. An acute dose-response curve has been generated, and currently we are investigating the dose-rate effect under chronic exposures. The probe has been developed for chromosomes 1, 2, and 3, which represents 49% of the scorable genome (41% of the total genome). Coverage of this fraction of the genome is a major achievement and will allow us to examine the effects of lower doses than would otherwise be feasible – maximum detection efficiency means fewer cells have to be scored and statistical precision will be maximized. We are also developing a pancentromeric probe that will allow unequivocal identification of symmetrical vs. asymmetrical translocations. We have found the molecular probe to work on all species of turtles tested to date, thus making it a generic tool applicability across numerous species and useful in a wide range of DOE sites.

Our successful research to date has been with turtle fibroblasts; our work with turtle lymphocytes is proving more challenging. While procedures for obtaining mammalian lymphocytes and stimulating them to mitosis are well established, this is not the case for nonmammalian species, including reptiles. For turtles, which have nucleated erythrocytes (unlike humans), the commercially available density gradient solution (ficoll) has not been effective at separating lymphocytes. Another challenge has been stimulating the lymphocytes to mitosis. The available mitogens are proving to be ineffective

with turtle cells. We are experimenting with a few of the more uncommon mitogens, and we are also trying various methods of premature chromosome condensation to address this problem. We are also seeing high levels of apoptosis in our cultured turtle blood. We are experimenting with some apoptosis-inhibiting compounds and using flow cytometry to determine which are effective and which are not.

Our second area of advancement is in the design and construction of a mesocosm irradiation facility. The mesocosms are outdoor, above-ground tanks in which our test organisms (turtles, fish and amphibians) are maintained. Each individual mesocosm is chronically irradiated from a sealed ^{137}Cs source suspended above it. Source activities constitute a treatment and vary from 0.2 to 1.2 Ci. This design allows us to determine: 1) chromosome damage at various radiation exposures and metal contaminant concentrations, 2) relationships between cellular damage and metabolic rate, and 3) treatment effects on an individual's energy allocation pattern, growth and survival. The greatest advantage of using mesocosms is the ability to replicate treatments such that powerful statistical methods can be used. We have had major delays in our experiments because the sealed source vendor was unable to produce a source to meet our design criteria. Dose distributions within the mesocosms have been too heterogeneous and scatter from the source has caused excessive doses to humans working in the area of the irradiator. Only this month was a successful prototype developed that met our specifications for research and safety. Because of this delay we have asked for a one-year no-cost extension to our grant. We will now be able to finish the construction of the irradiation facility, and anticipate numerous controlled dose-effects experiments to be forthcoming.

Our third area of research examines responses of organisms living in contaminated environments. The potential effects of coal ash sediments alone were examined in experimental mesocosms using larval marbled salamanders (*Ambystoma opacum*). Eight tanks were established as artificial ponds: four tanks contained ash basin sediments and four controls contained clean sediments. Tanks were filled with water in early February, leaves were added as a nutrient source, and zooplankton were added as a food source. Twenty hatchling *A. opacum* were placed in each tank in mid-February. A homogenized mixture of zooplankton was added to each tank as supplemental food approximately weekly throughout the course of the experiment. Larvae began to metamorphose in late April; recently metamorphosed salamanders were trapped from tanks and returned to the lab for measurements of body size and standard metabolic rate. Animals were also collected from a natural wetland for comparison. Individuals reared in the ash basin treatment metamorphosed approximately seven days earlier than clean tank animals, and at a smaller body size (0.96 ± 0.08 g wet mass vs. 1.22 ± 0.04 g). Survivorship did not differ between treatments, nor did size-adjusted metabolic rate ($\text{mL O}_2/\text{g} \cdot \text{hr}$). Numerous limb deformities were noted in the ash-basin treatment, however. The frequency of malformation that we observed (~50%) in animals from the ash basin treatment was far greater than the ~3% found in natural populations or the level (0%) observed in the control tank animals. A second salamander species, *A. talpoideum* (mole salamander) is currently being reared in the same set of tanks. These individuals will also be examined for limb deformities, as well as for differences in standard metabolic rates.

In another experiment, Dr. Betsy Sutherland examined DNA taken from tadpoles living in a clean pond and compared them to DNA taken from tadpoles living in a pond contaminated with coal fly ash. DNA was isolated in agarose plugs as double-stranded molecules, and the size of the single strands quantified on alkaline agarose gels. Results of five replicate gels indicated that the DNA from the ash basin animals (14.6 ± 1.0 kb) was clearly smaller than that from animals living in the clean pond (21.7 ± 2.1 kb). Using Unidirectional Pulsed Field electrophoresis the frequency of oxidized purines was also determined, based on the frequency of Fpg sites. The level of Fpg sites in DNA from the ash basin pond (29.1 ± 2.5 per Mb) was apparently lower than those in the clean pond (34.8 ± 2.4). These results suggest that the frequency of single strand breaks in the DNAs from animals from ash basin ponds is higher than that from animals from clean ponds. This has often been taken as a measure of DNA damage, and this may be the case. However, the similar –or even lower– levels of oxidized purines in DNA in the ash basin animals suggest that repair of such damages is increased in animals exposed to ash basin components. Thus the excess of single strand breaks in the ash basin animal DNAs could reflect that increased repair, as incision into the phosphodiester backbone is a normal step in repair of DNA damage. This possibility will be tested in future experiments.

Planned Activities

- acquire a one-year no-cost extension so that we can complete the irradiation facility consisting of 50 mesocosms.
- initiate controlled dose-response experiments using the irradiation facility.
- compare *in vivo* vs. *in vitro* turtle lymphocyte response to acute irradiation and determine the stability of symmetrical exchanges through time
- conduct a dose rate effect study on lymphocytes irradiated *in vivo*.
- determine the frequency of reciprocal translocations in free ranging turtles maintained within a highly contaminated radioactive basin on the SRS. The turtles will have radio transmitters and TLDs attached to them so that repetitive blood samples can be taken and acquired doses estimated.

Information Access

We have submitted our data on the successful development of the chromosome probe, determination of the acute dose-response curve, and hybridization of the probe to several species of turtles in a manuscript entitled *Turtle Chromosome Specific DNA Libraries for Ecological Risk Assessment of Irradiated Populations*.

The principal investigator has taken knowledge gained from this research and used it in his work with the Department of Energy's Biota Dose Assessment Group (BDAG). BDAG is currently reviewing ecological risk concepts and establishing guidelines for conducting ecological risks on DOE facilities.