NUCLEAR FUSION

- **Sublethal Effects of Tritium on Aquatic Systems**
- **Effects of Low-Level Chronic Irradiation on Embryonic Development**
- **Effects of Beryllium and Lithium on Aquatic Systems**
- o **Teratogenic Effects of Low-Level Magnetic Fields**

It is conceivable, and possible, that with increasing applications of nuclear energy, greater quantities of potentially harmful radionuclides will be released to the environment. This may be particularly true for advanced reactor designs such as the fusion reactor where radionuclides, principally tritium, may be expected to escape from the plant both in gaseous and liquid effluents in quantities significantly greater than for present PWR or BWR designs. Foreseeing such contingencies, the research programs described herein are in response to the need to measure the potential radiation effects of tritium releases on individuals, and ultimately populations and biotic communities. As a first approach, our efforts are directed to determine effects of low-level chronic exposures on developing embryo and larval stages, clearly the most radiosensitive.

The anticipated increase in the release of beryllium and lithium from mining, refining, and fabrication of materials used during construction of fusion reactors has also caused concern as to potential adverse effects on the environment. Accordingly, FY-78 fusion related research will include efforts to study the metabolism of each metal in the living organism, and to determine at what levels toxicity may be expected. Again, the effects of beryllium and lithium will be assessed in embryo and larval stages of selected aquatic organisms, with ultimate interest in the response of populations and communities.

Our fusion related research in FY-78 will also include preliminary experiments on the effects of low-level magnetic fields. It is conceivable that magnetic fields of 70-450 gauss will be encountered by attendant personnel working in the transport and hot cell areas of fusion reactors. Also, those personnel assigned to the areas immediately surrounding the reactor may be exposed for substantial durations to

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o Work order agreement

field strengths of 1-70 gauss. The current PNL program is aimed at developing different measurement end-points for the early detection of developmental and adult chronic effects. Work involving mammalian tissue and cell systems is reported in PNL-2500, Part 1, Biomedical Sciences; work involving nonmammalian animal systems is reported here. Dr. Dennis Mahlum is coordinator for the comprehensive program. The approaches described here will utilize the embryo and larval stages of aquatic organisms which should provide meaningful data applicable to ultimate assessment of effects in life processes of higher vertebrates.

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Continuing studies of the sublethal effects of tritium on freshwater species emphasize the potential for genetic transmission of suppressed immune competence in offspring of parental rainbow trout, Salmo gairdneri, exposed to tritium (0, 0.04, 0.4, 4.0, 40.0 rads) over embryogenesis. It is planned that the offspring of recently conducted test crosses will be assayed for agglutinating antibodies in response to the bacterial antigen, Flexibacter columnaris, and decreased levels of circulating antibody indicating the potential for transmission of altered genetic material mediating immune competence.

Continuing studies with marine species, Pinnixa occidentalis, Hemigrapsus nudus, and Cragnon sp., examine uptake and release of tritium over varying life stages and evaluate radiation effects on development and survival.

In another study to begin in FY-78, interest is focused on the potential effects of beryllium and lithium on aquatic systems. Increased levels of each metal are likely to be encountered in surface waters due to mining and refining subsequent to their use in the construction of fusion reactors. Planned studies include an evaluation of potential toxicity of beryllium and lithium on embryological life stages of rainbow trout, Salmo gairdneri, and an assessment of fate and effects in artificial stream habitats.

Studies to be initiated in FY-78 include an evaluation of the effects of low-level magnetic fields on embryologic development of rainbow trout, Salmo gairdneri. It is the objective of these studies to provide data which will have application to assessment of potentially harmful

effects of low-level magnetic fields encountered by attendant personnel working in the transport and hot cell areas of fusion reactors. This approach is less costly, provides large numbers of experimental organisms for meaningful statistical analysis, and permits examination of potential late effects in a representative vertebrate.

Sublethal Effects of Tritium on Aquatic Systems

Present studies are directed to determine the potential for genetic transmission of suppressed immune competence in offspring of parental rainbow trout, Salmo gairdneri, sublethally exposed to tritium as tritium oxide (HTO) (0, 0.04, 0.4, 4.0, and 40.0 rads) during embryogenesis. Previous studies demonstrated that the primary immune response of parental stocks at 5 and 17 months of age was significantly suppressed and permanently altered at doses as low as 4.0 rads.

As the race of experimental fish presently reared in the Hanford Hatchery did not mature until winter of their 3rd yr of growth, offspring of test crosses conducted in February-March 1977 will not be of sufficient size to assay for diminished immune competence until November-December 1977.

However, it is planned that the offspring of each test cross will be compared as parental stocks were in previous studies; that is, for agglutinating antibodies in response to the bacterial antigen, Flexibacter columnaris, as detected by agglutinin assay. Decreased levels of circulating antibody in the serum of offspring from tritium irradiated stocks will serve to indicate the potential for transmission of altered genetic material controlling or mediating immune competence.

Challenge techniques employing virulent strains of the pathogen, F. columnaris, will also be applied in this context. The procedure as adopted for such subsequent experiments will involve the injection of 0.1 cc of a standard suspension and tenfold dilutions of the pathogen into separate lots of 10 or 20 fish each from the respective test crosses. Mortality will be recorded for 5 consecutive days and the LD_{50}, or the dilution at which 50% mortality occurs will be calculated. If permanent genetic alteration has occurred, it may be expected that significantly lower LD_{50} endpoints will result when the offspring of tritium irradiated parental stocks are tested.

Effects of Low-Level Chronic Irradiation on Embryonic Development of Marine Fish and Invertebrates

During FY-77, efforts were centered on the determination of HTO turnover rates in young postlarval stages or eggs of three marine crustaceans. Additionally, larvae of two species of commercial shrimp were reared in low levels of tritiated seawater.

Measurements of HTO turnover rates were determined in the megalopa and crab stages I to V of Pinnixa occidentalis, a brachyuran crab. Mean values for rate constants and half-times were 3.27 ± 0.40 ($\bar{x}$ ± S.E.) hr and 0.28 ± 0.04 hr, respectively. Differences in rates of HTO turnover for different stages were not statistically significant. Therefore, body size or life stage apparently did not influence HTO turnover rates in this crab.

Rate constants and half-times for HTO turnover in the eggs of the crab Hemigrapsus nudus were 0.32 ± 0.02 hr and 2.20 ± 0.10 hr, respectively; values for the eggs of shrimp Crangon sp. were 7.58 ± 2.99 and 0.20 ± 0.06 for rate constants and half-times, respectively. These data indicate that HTO turnover was very rapid in Crangon sp. compared to H. nudus and were reflective of the habitats of the two species. H. nudus exists in the intertidal environment and is frequently exposed to the atmosphere. Crangon sp., on the other hand, is a subtidal species which inhabits a relatively stable marine environment.

Larvae of the shrimps Pandalus platyceros and P. danae were reared in seawater containing 3×10^{-4} and 3×10^{-3} μCi/ul tritium as HTO. Survival, molting frequency, and duration of larval development were similar in control and tritium-exposed individuals of both species.

Effects of Beryllium and Lithium on Aquatic Systems

The anticipated increase in the release of beryllium and lithium from mining, refining, and fabrication of materials used

during construction of fusion reactors has caused concern as to potential effects on the environment. In order to assess and predict with assurance the effects of beryllium and lithium released to the environment, it is necessary to know how readily each metal is metabolized by living organisms, and at what levels toxicity may be expected. Entry of these metals, particularly beryllium, into the food chain with biomagnification may present a serious hazard to man. However, few studies relevant to these needs are providing the results necessary to make an environmental assessment.

Beginning in FY-78, we plan to identify and measure the acute and chronic effects of lithium and beryllium compounds on specific aquatic organisms and associated freshwater communities. Since this study is designed to reflect actual perturbations caused by the accidental release of these metals into freshwater ecosystems, it differs from a standard toxic bioassay in that the observed and measured effects will be viewed under ecologically dynamic conditions. Thus, the expressions of response will be more relevant to the assessment of environmental impact associated with mining, refining, and fusion plant operation.

Summary of Study Plan

Task I. Toxicity. In initial experiments to establish acute (96 hr) and chronic (30 days) effects, eggs and embryos of rainbow trout will be exposed to varying concentrations of beryllium and lithium. Injury will be assessed by the proportion of embryos failing to reach larval stage, and by the proportions of morphologically abnormal embryos (relative to total reaching larval stage). For the latter, anomalies scored will include major malformations of head and body. Changes in rate of embryonic development will also be quantitated for definite, recognizable developmental stages. Metal accumulation will be studied by periodic removal and analysis of embryos and larvae. Turnover rates will be estimated by transfer of embryos and larvae to single-pass incubation systems where similar measurements will be conducted.

As time provides over the 1st yr, additional exposures will be conducted over different stages of development to determine relative sensitivities. As well, exposures of varying duration will be tested. The metals beryllium and lithium will be added to the exposure medium in their respective ore forms, or as oxides, hydroxides, fluorides, or other salts as appropriate.

Task II. Community Response. During the initial period of this study, artificial streams will be developed in hatchery troughs to resemble a low velocity habitat of the Columbia River. Flow rates of unfiltered Columbia River water passing through the troughs will be regulated to permit optimal growth for periphyton, benthic invertebrates, and certain fishes. These experiments will be performed outside, under natural thermal and solar regimes, during spring, summer, and fall seasons. These streams will be arranged in a parallel fashion to permit nearly identical conditions in experimental and control systems. Artificial streams will undergo natural colonization, however, fishes will be added to enhance the trophic structure of the community. By late spring, sufficient colonization will be attained to begin experimental additions of lithium and beryllium compounds. The effects of these compounds in the microcosms will be detected and measured in terms of significant changes in:

- community diversity,
- biomass,
- primary production of periphyton communities,
- photosynthesis (respiration ratios),
- rates of recolonization on bare substrates,
- survival and growth of specific organisms,
- seasonal periodicity of species occurrences as reflected by the control streams.

The compounds of greatest interest in this work will be the fluorides, oxides, and hydroxides of lithium and beryllium. Concentrations of these compounds will be measured and maintained in experimental systems by direct sampling and continuous delivery procedures. Ranges of concentrations for testing will be specifically determined by preliminary toxicity bioassays and toxicological information gathered from the literature.

Teratogenic Effects of Low-Level Magnetic Fields

Development of magnetic fusion reactors will result in occupational exposure of personnel to varying strengths and geometries. It is conceivable that magnetic fields of 70-450 gauss will be encountered by attendant personnel working in the transport and hot cell areas of the reactor. Also, those personnel assigned to the areas immediately

surrounding the reactor may be exposed for substantial durations to field strengths of 1-70 gauss.

Working in cooperation with the Biology Department, our study objective is to determine the potential effects of low-level magnetic fields on sensitive life stages of lower vertebrates. Our initial approach is to study the teratogenic effects of magnetic fields on the embryonic life stages of rainbow trout, Salmo gairdneri. Several advantages may be gained by examining magnetic effects on a lower vertebrate. This approach is less costly and will provide large numbers of experimental organisms for meaningful statistical analysis. It also permits study of potential latent effects resulting from exposure during embryogenesis. In addition, the data obtained will be applicable to processes occurring in higher vertebrates.

A literature review began in August 1977 to initiate this program. Although a significant number of investigations of the biological effects of magnetic fields on both plants and animals have been conducted, most studies have employed high field strengths over short periods of exposure, with little attention given to the effects of low-level or chronic exposures. Many of these studies were deficient in using inadequate numbers of test organisms for statistical evaluation, and the data obtained were of high variability and were often not reproducible. Since August 1977, we have obtained both permanent magnets and a large Varian electromagnet. Field strengths of 4 kilogauss may be obtained with available permanent magnetic sources; while field strengths of 10 kilogauss are achievable with the electromagnet. Incubation chambers for specimen exposure to magnetic fields were designed and are being tested for their ability to sustain normal development of trout embryos. Experimental protocals for both types of magnets were also completed.

Initial tests are scheduled for November 1977 and will determine effects of magnetic fields on rainbow trout eggs during and after incubation. Trout embryos will be exposed to both homogeneous and nonhomogeneous sources. Mortality, abnormality, delayed development and hatching will be critically monitored. If preliminary tests demonstrate significant differences between control and treatment groups, subsequent tests will be designed to quantify critical maximum field strengths, as well as the relative sensitivity of different developmental life stages.