# Synergistic Bio-effects of Oil and Irradiation in an Aquatic Organism, *Taricha granulosa*

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Radiation sensitivity, radiation response, and radionuclide metabolism of aquatic and terrestrial organisms, including man, are influenced by the presence of pollutants. In order to develop knowledge on which to base recommendations for new radiation exposure standards and other measures to control radiation emissions and exposure, it is imperative to investigate possible interactions which may exist between radiation and other environmental stresses, especially if each pollutant affects the same vital organ system. Furthermore, if acute concentrations of each pollutant results in a synergistic bioeffect, further investigation is warranted using multiples of the maximum permissible level of each pollutant in the environs.

The biological damage to many species from oil pollution needs further study, and the combined effects from oil and radiation (co-insults) are virtually unknown. Previous work by Brunst (1) and Lappenbusch and Willis (2) strongly suggests that Urodeles (tailed amphibians) subjected to x-ray exposures up to 12 kR die predominately from hematopoietic failure and skin disorders, causing anemia and respiratory failure. Furthermore, it is hypothesized that oil may also affect the surface, skin, or membranes of aquatic life. As a result, this study was designed to determine the lethal toxicity of oil and to report the possible synergistic effects of oil on the radiosensitivity of the rough-skinned newt (Taricha granulosa).

## MATERIALS AND METHODS

### Collection and maintenance of animals

Adult female newts, weighing 11-13 g each, were collected in a small lake in Western Oregon (Willamette Valley) during June. Animals were immediately shipped by air to Rockville, Maryland, acclimatized at 10$^{o}$C and maintained unfed, simulating winter conditions, both before and after irradiation, and before and during oil treatment. Newts were placed in aquaria and maintained under normal alternating light and dark periods, since many Urodeles have been shown to be governed by a strong biological rhythm, characterized by a bi-phasic circadian cycle (3). After newts were subjected to irradiation, they were returned to their initial aquaria containing a specified concentration of oil or water.

### Treatment before exposure

An initial oil toxicity study was conducted in which 20 animals were placed in 4,000 ml of water containing 0, 0.5, 1.0, 2.5, 5, 10, 20 or 50% by volume regular non-detergent, motor oil. The water and oil was gently mixed via upwelling using a pump. A definite oil slick resulted on the water's surface, simulating realistic environmental conditions.



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After oil toxicity levels were determined, additional newts were randomly classified in one of the following four groups ten days prior to irradiation:

(I) Controls: Newts receiving neither oil or irradiation;
(II) Irradiated: Newts not exposed to oil, but irradiated;
(III) Oil-treated: Newts continuously exposed to 0.5% oil, but not irradiated;
(IV) Oil-treated, irradiated: Newts continuously exposed to 0.5% oil and irradiated.

Radiation exposure

Animals absorbed (whole-body) 0, 1,000, 2,500, 5,000, or 10,000 rads of x rays between 1-4 pm E.S.T. under the following conditions: 250 kVp, 15 mA, HVL of 0.55 mm Cu, TSD of 75.0 cm with a dose rate of 109.2 rads/minute. Twenty animals were irradiated per radiation dose and oil treatment. Newts were taken out of their water or oil environment, held at 10°C, irradiated in air at 22.5°C, and returned to the water environs.

Data post-exposure

After the newts were subjected to irradiation and/or oil treatment, their behavior, physical appearance and activity, and mortality were observed daily. At the time of death, several animals of each group were necropsied and tissues were processed for histopathological examination.

RESULTS

Toxicity of oil

The toxicity of oil was found to be acute, not chronic, since newts immersed in 2.5-20% oil died primarily between Days 2 and 5 after the initial exposure (Figure 1). Furthermore, higher oil concentrations resulted in earlier lethality. A definite shoulder to each survival curve existed. Newts subjected to continuous exposure to 0 or 0.5% did not die during the first 130 days, and only one newt submerged in 1.0% oil died during the same period. Mortality occurred immediately thereafter; however, untreated newts also died due to starvation, as described by Lappenbusch and Willis (2). Animals immersed in oil concentrations of 10, 20 or 50% appeared irritated and immediately made continuous efforts to climb out of the solution. Newts subjected to lower oil concentrations made no special effort to escape from their containers.

Co-insult of oil and radiation

Animals subjected to 0, 1,000, 2,500, 5,000, 10,000 or 12,000 rads had mean survival times (MST) of approximately 143, 141, 117, 85, 54 and 30 days, respectively; newts subjected to both 0.5% oil and radiation (co-insults) had significantly lower ($P < 0.05$) MST of 148, 98, 75, 53, 29 and 21 for the same respective radiation doses (Figure 2).

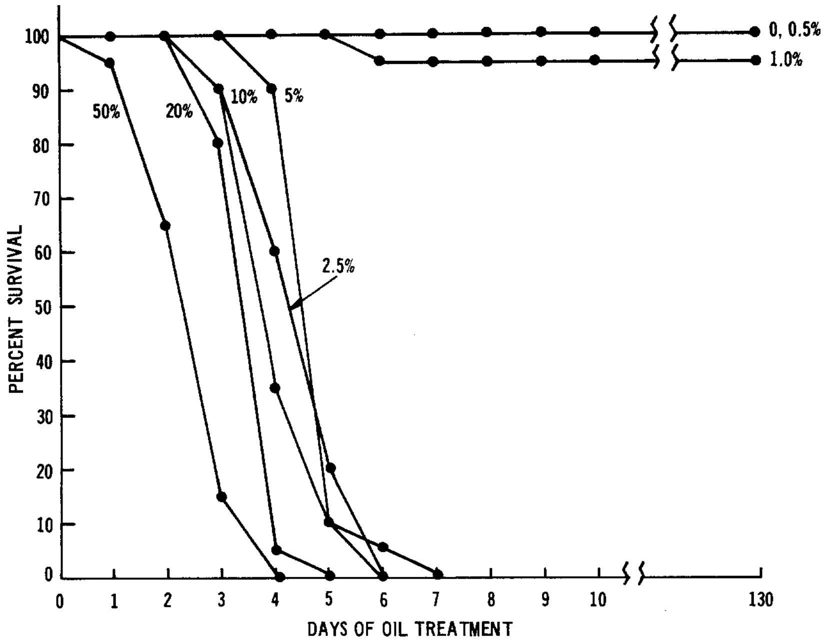


Figure 1. Effect of continuous oil treatments on the survival of newts. Twenty female newts per treatment. Animals maintained at 10°C.

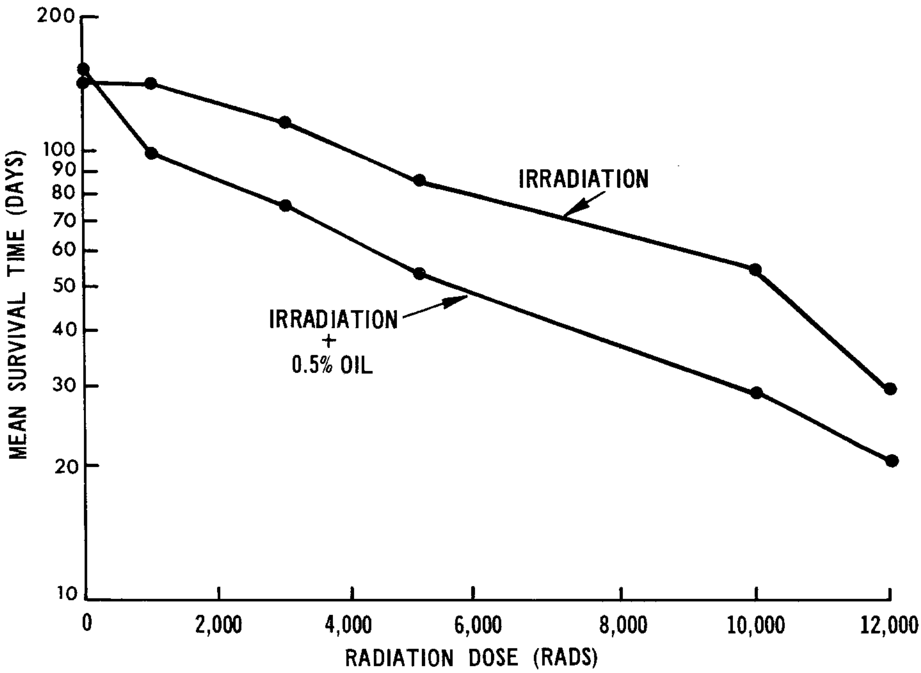


Figure 2. Effect of continuous oil (0.5%) treatment on radiosensitivity of newts. Twenty female newts per treatment. Animals maintained at 10°C.

Pathology of newts exposed to x-irradiation and/or oil

Lesions in rough-skinned newts (*Taricha granulosa*) exposed to 10,000 or 5,000 rads only or to 10,000, 5,000 or 2,500 rads and maintained in 0.5% oil were similar. Variable degrees of the same changes were noted, with those dying earliest (23 days post-irradiation) revealing the most severe lesions. Most lesions probably resulted from the effects of irradiation. The most consistent finding was depletion of hematopoietic cells in the liver and spleen. Other lesions included focal and diffuse necrosis of cells in the oviduct, kidney, lung, pancreas, and gastro-intestinal tract. Nephrosis was prominent in most newts dying early. Portal fibrosis was observed in livers of a few newts dying later. Vacuolization and necrosis of epithelial cells in the skin were noted in several newts. Changes in the poison glands were difficult to interpret because normal glands in control newts varied greatly in morphology from small islands of cells enclosing a small lumen to glands with large lumina. Glands appeared to be greatly dilated in many newts exposed to irradiation only, in newts exposed to irradiation and 0.5% oil, and in newts exposed to 50% and 2.5% oil only. Edema of the dermis was seen in several newts. The only lesions observed in newts exposed to 50% and 2.5% oil were those glandular changes described above. No other lesions were seen which would be expected to contribute to death in these newts. No lesions were seen in the skin of newts exposed to 0.5% oil only.

## DISCUSSION

The cause of death of irradiated newts appears to be due to anemia and lesions in various tissues including the skin. In this experiment, complete depletion of hematopoietic cells in the liver and spleen is strong evidence of hematopoietic failure and anemia, especially since newts lack bone marrow. Irradiation also caused degeneration and necrosis of the epithelium of the skin and dilation of poison glands, possibly interfering with respiration, one of the vital skin functions of newts. This is supported by previous findings by Brunst (4) who reported that hematopoietic failure and skin ulcerations were primarily responsible for the lethality of adult axolotls exposed up to 12 kR of x rays. Lethality from skin damage is also supported by Jakowska et al. (5) who found that *Notophthalmus viridescens* died from ionizing radiation because of osmo-regulatory dysfunction of the skin and by Czopek (6) who reported that the skin, itself, is vital since it accounts for 74% of the respiration in *Triton vulgaris*.

High concentrations of oil (2.5-50%) without irradiation resulted in dilatation of poison glands and skin denudation, probably also interfering with respiration. Although low oil concentrations (0.5%) did not result in skin lesions, these levels may have interfered with normal skin function and hence the skin may be more susceptible to irradiation.

Oil and radiation are two environmental pollutants that affect the same vital organ system (skin) in the newt and, thus, accounts, in part, for their increased susceptability to radiation. This does not discount the hypothesis that animals subjected to the co-insult did not die partially from hematopoietic failure and loss of function of other tissues as well. Nevertheless, the co-insult increases radiosensitivity of *Taricha granulosa* synergistically, especially since 0.5% oil, by itself is not lethal.

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