Toxicity to Fish of Two Organic Reactor Coolants

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INTRODUCTION

One aspect of current research at Whiteshell Nuclear Research Establishment (WNRE) is the use of certain terphenyl compounds as primary coolants for nuclear reactors. The application of these "organic coolants" in the Canadian reactor programme, and their development, has been described by Campbell et al. (3).

Very few studies of the toxicity of terphenyl compounds to aquatic animals have been reported. However, Truelle (4) Holland et al. (5) and Mallet (6) have studied the effects of terphenyl-like compounds in aquatic environments.

This paper summarizes the results of bioassays carried out to determine the acute (48 hr.) toxicity of Santowax OM* + 30% high boilers and HB-40* to rainbow trout (Salmo gairdneri). Santowax OM is a mixture of ortho-, meta-, and para-terphenyls. HB-40 is a mixture of hydroterphenyls and terphenyls. "High boilers" are the tar-like decomposition products produced as a result of the exposure of terphenyl compounds to a high radiation field. The composition and physical properties of Santowax OM and HB-40 have been described by Hatcher and Tomlinson (7). An Atomic Energy of Canada Limited report describing in detail the methods used and results obtained in this study is available (8).

*Monsanto Chemical Company proprietary name.

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MATERIALS AND METHODS

Coolant Suspensions

Two coolants were investigated: Santowax OM + 30% high boilers and HB-40. Santowax OM + 30% high boilers was prepared by mixing Santowax OM (lot No. WS/P3) with a high boiler fraction produced in the United States OMRE reactor. Since organic coolants are almost insoluble in water, suspensions for toxicity testing were prepared by discharging the heated coolant into water with nitrogen at 17.2 bars, from a pressure vessel apparatus. This apparatus and the physical characteristics of the coolant-water suspension have been described (9). Precautions were taken to make reproducible suspensions. Nevertheless, the settling rates of the suspensions differed sufficiently to make bioassay results from tests run longer than two days unreliable. The concentration of Santowax OM + 30% high boilers, or HB-40, in a coolant-water suspension was measured by extracting replicate samples with carbon tetrachloride, then measuring the amount of coolant in the extract by infra-red spectrometry (10).

Test Fish

Rainbow trout (*Salmo gairdneri*), 6 - 8 cm in length were obtained from a Manitoba Government Provincial Fish Hatchery. Groups of 80 fish, the "test-stock", were kept in 110 litre aquaria maintained at 12 $\pm$ 1 C in a constant temperature tank. All fish were acclimated for three weeks before testing.

Test Method

The bioassay method used to measure the toxicity of various concentrations of the two coolants was essentially that described by Doudoroff *et al*. (11), except more frequent observations of mortality were made. Food was withheld for 24 hours before a test. All aquaria water and water used to dilute coolant suspensions, was filtered through an activated charcoal column prior to use. No chlorine was detected in this water, the total alkalinity of which was 20 to 22 mg/l as calcium carbonate. Except where otherwise noted, the dissolved oxygen level of the coolant water suspension was maintained at, or greater than, 8 mg/l by bubbling oxygen through a fine porosity filter-stick

placed in each 4 l beaker, the test vessel, and manually controlling the aeration rate via a recording dissolved oxygen meter. This aeration technique did not affect the settling rate of the suspensions during a test (9).

The same experimental criteria as those adopted by Sprague (12) were used in the study. The measure of a fish's resistance to a coolant concentration was taken as the time to 50% mortality (ET50). The criterion of death was absence of respiratory movement and lack of response to being touched by a glass stirring rod. Survival for at least 48 hours was considered to indicate absence of direct or acute toxicity (11).

Analysis of Results

Results were analysed by the methods of Litchfield (13). The application of these methods to fish toxicity studies has been described by Sprague (12). All the results from bioassays conducted in the same concentration of coolant were pooled and treated as a single statistical unit.

RESULTS

Behaviour

In concentrations exceeding 25 mg/l of Santowax OM + 30% high boilers the following behaviour pattern was generally observed. Within one half hour of being placed in the coolant suspension, the fish appear disturbed. The controls usually remained motionless near the bottom of the test vessel, but the test fish swam continuously. This may have been an avoidance reaction. Santowax suspensions containing more than 25 mg/l were cloudy, making observation of the fish difficult. Pronounced reddening of the gill covers occurred within one to five hours, depending on the coolant concentration. Swimming movements slowed; balance and avoidance reactions were also affected. Many fish continued swimming on their sides at or near the surface, an indication of respiratory difficulty. The fish would continue to move about in this unnatural position, in some instances for several hours, before dying. Less than five percent of the fish exhibiting evident signs of distress recovered before the end of the test period. In one series of experiments reported

elsewhere (8), 90% of the moribund fish recovered when transferred to clean water.

Coolant Toxicity

The ET50's of trout fry exposed to concentrations up to 100 mg/l of Santowax OM + 30% high boilers are shown by the solid line in Figure 1. The dissolved oxygen level in these tests was at least 8 mg/l. To estimate ET50's by Litchfield's methods (13) at least 50% of the test population must die within the test period. Less than 25% of the fish died within 48 hours in HB-40 suspensions containing 100 mg/l or less, hence no results from the toxicity tests with this coolant appear in Figure 1. The difference between the toxicity of Santowax OM + 30% high boilers and HB-40 at comparable concentrations is significant at the 95% confidence level. Detailed results have been reported (8).

Pooled ET50's for Santowax OM + 30% high boilers bioassays are plotted in Figure 1. The ET50 for the 10 mg/l concentration differs significantly from those of the 50 and 100 mg/l concentrations at the 95% confidence level. ET50 confidence limits for concentrations less than 10 mg/l could not be calculated, because less than 50% of the fish died during the test period. Sprague and Ramsey (14) reported a sharp differentiation between lethal and non-lethal concentrations of copper and zinc. Their curves relating toxicant concentration to survival time broke sharply and ran parallel to the ET50 axis at this point. Sprague (personal communication) calls this critical concentration the "lethal threshold concentration". The results plotted in the upper curve of Figure 1 suggested that the lethal threshold concentration for Santowax OM + 30% high boilers is about 5 mg/l. Extrapolated ET50 values for coolant concentrations less than 5 mg/l were about 200 hours. They are marked ⌒ in Figure 1.

Effects of Dissolved Oxygen on Toxicity

The dissolved oxygen content of a lake or river may fluctuate appreciably. Lloyd (15) exposed fish to graded concentrations of several pollutants at different dissolved oxygen concentrations, none of which was low enough to be lethal. His study indicated a relationship between the increase in toxicity of each poison and the reduction in dissolved oxygen concentra-

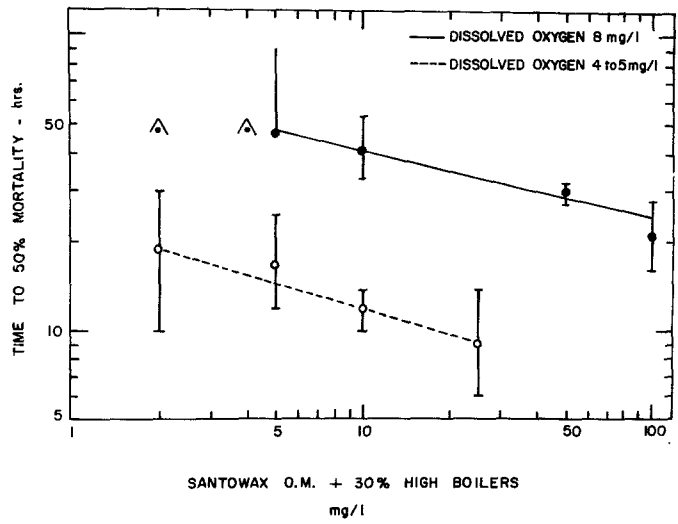

Figure 1. Mortality times of rainbow trout exposed to various concentrations of Santowax OM + 30% high boilers. Verticle bars indicate 95% confidence limits. ⩓ is an extrapolated value (see text).

tion. Thus oxygen tension of the water is an important environmental factor in a pollution study and its effect, therefore, on terphenyl toxicity was investigated.

The bioassays were repeated at the same concentrations of Santowax OM + 30% high boilers in water as before but the dissolved oxygen level was maintained between 4 and 5 mg/l. The results of this series of tests are shown by the broken-line curve in Figure 1. As would be expected, the ET50 of a coolant concentration was below optimum. The slope of the lower curve in Figure 1, tested by Litchfield's methods (13), does not differ significantly from that of the solid-line curve. However, the respective ET50's are different at the 95% confidence level.

The solubility, density, and melting points of the two coolants tested makes the preparation of comparable suspensions in water difficult. The variability in settling rate of different suspension preparations undoubtedly accounts for much of the variation in the bioassay results (Figure 1). It probably also explains recovery of the moribund fish. The apparatus used to make the coolant suspensions, however, simulated the conditions which might be expected to accompany a puncture of an organic cooled reactor heat exchanger tube (9), with subsequent release of the coolant into a river.

The 48 hour toxicity of HB-40 is significantly less than that of Santowax OM + 30% high boilers. This finding is important in view of recent chemical engineering evidence, obtained at WNRE, which suggests that HB-40 is the superior organic reactor coolant (S. R. Hatcher, WNRE, private communication). The dissolved oxygen concentration is seldom a limiting factor in a quickly flowing river. However, the lower curve in Figure 1 clearly shows the consequences of accidentally releasing Santowax OM + 30% high boilers into water in which the dissolved oxygen concentration is a limiting factor. Evidence of pathological damage to fish gills, kidney and liver possibly resulting from the test exposures, is still being examined.

SUMMARY

The use of terphenyl compounds to cool nuclear reactors prompted an enquiry into the effect of these substances in the aquatic environment, should they be accidentally released.

Santowax OM + 30% high boilers and HB-40 are two terphenyl compounds under study as nuclear reactor coolants at Whiteshell Nuclear Research Establishment. The acute toxicity of these two coolants to rainbow trout fry has been investigated, and it was found that Santowax OM was the more toxic. The behaviour of fish exposed to suspensions of Santowax OM in water is briefly described.

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