BEHAVIORAL AVOIDANCE/ATTRACTANCE RESPONSE OF RAINBOW TROUT TO FIRE-RETARDANT CHEMICALS

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EXECUTIVE SUMMARY

Behavioral Avoidance/Attractance Response of Rainbow Trout to Fire-Retardant Chemicals

The avoidance/attractance response is the primary response of organisms to unfavorable or hazardous environmental conditions. Avoidance of potentially harmful conditions is an adaptive behavior that often reduces exposure to contaminants through behavior that may limit contact with, or residence in unfavorable or contaminated habitats. In contrast, failure to avoid contaminated areas or attraction to contaminated areas may result in increased exposure of organisms to hazardous substances leading to injury or death. Laboratory avoidance studies were conducted with the fire-retardant chemicals Fire-Trol® GTS-R and Phos-Chek® D75-R to determine if rainbow trout (*Oncorhynchus mykiss*) avoided low concentrations of these substances. The fish were given a choice between untreated well water and water treated with GTS-R in concentrations ranging from 0.65 to 26 milligrams (mg) dry concentrate GTS-R per liter of water (mg /liter), or with D75-R in concentrations ranging from 0.27 to 27 mg dry concentrate D75-R per liter of water. In an effort to understand the role that different components of GTS-R play in eliciting the avoidance/attractance response, tests were also performed with (1) GTS-R without ferrous oxide colorant, (2) GTS-R without ferrous oxide or YPS (a corrosion inhibitor), and (3) YPS alone. Avoidance of fire-retardant substances was determined in a counter-current avoidance chamber. Avoidance was apparent when fish spent significantly less total time in the side of the chamber containing water treated with fire-retardant substances compared with the side of the chamber containing uncontaminated well water (control). (NOTE: All concentrations, dilutions and mixtures are based on the dry concentrate weight unless otherwise noted.) The following results were determined:

- Rainbow trout consistently avoided water treated with GTS-R at concentrations as low as 0.65 mg/liter (Figure 1), which is approximately 10 percent of the concentration lethal to 50 percent of rainbow trout test in 96 hours (LC50-6.6 mg/liter).
- The threshold concentration of D75-R ranged between 0.27 to 1.3 mg/liter (Figure 2). This concentration is less than 1 percent of the LC50 (168 mg/liter). The magnitude of the avoidance response increased with increasing D75-R concentrations.
- Ferrous oxide and YPS did not appear to contribute to the repellant qualities of GTS-R, since fish avoidance responses were not significantly diminished during tests of GTS-R formulations without ferrous oxide and/or YPS (Figure 3). In addition, fish did not avoid YPS when this was tested alone.
- Given the similarity of response induced by the various formulations of GTS-R and D75-R, the salinity (measured by specific conductance) of the test water may be the sensory stimulus that induces the avoidance response. Conductance of the test water increased as the concentration of fire retardant increased.

These results suggest that, if given the opportunity, rainbow trout will escape chemical gradients of GTS-R in the field. Other species of fish and different life stages of the same species may not avoid the same chemical substances, and competing biological drives may override the response. Environmental variables such as ash plumes and heightened water temperatures could inhibit avoidance responses

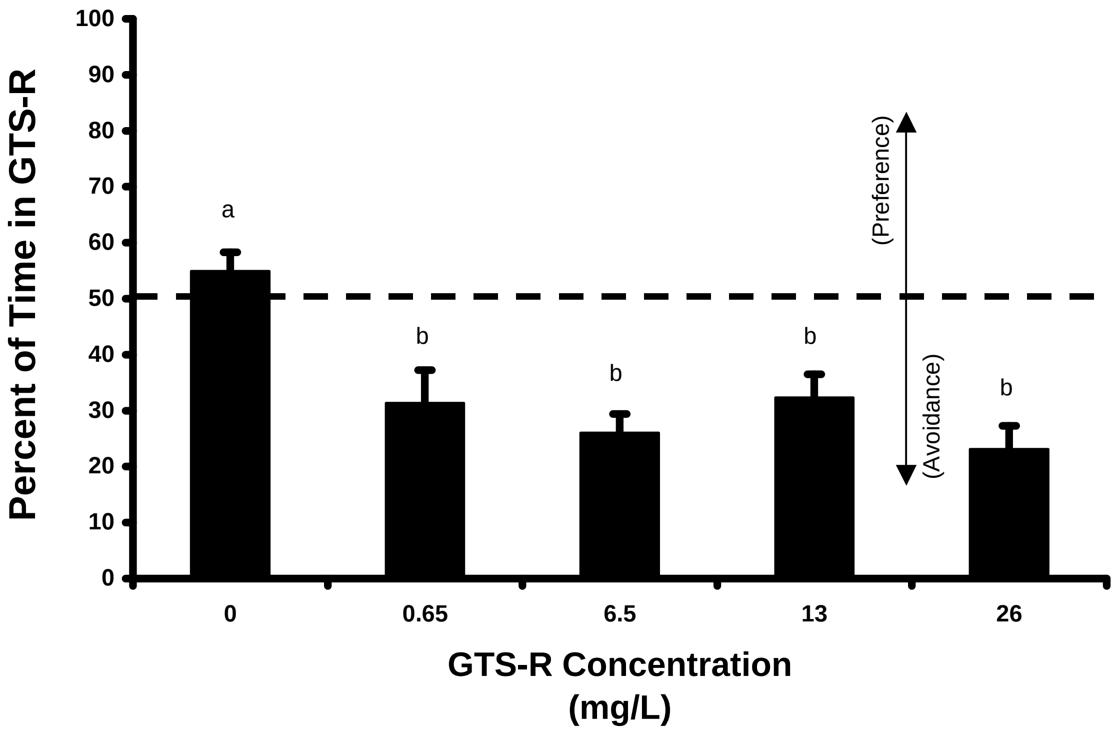

Figure 1. Percent time fish spent in the side of the counter-current avoidance chamber containing Fire-Trol® GTS-R. Bars above each histogram indicate standard error of the mean. Histograms accompanied by a different letter are significantly different. Statistically, the response to different GTS-R concentrations is compared with the response when uncontaminated water is present in both sides of the chamber (0 mg/liter GTS-R). Note that in the counter-current chamber, fish are either in the contaminated side or in the uncontaminated side. Thus, when fish are in the side containing GTS-R for 25 percent of the time, they are in the uncontaminated side of the chamber for the remaining time, or in this example, 75 percent of the time.

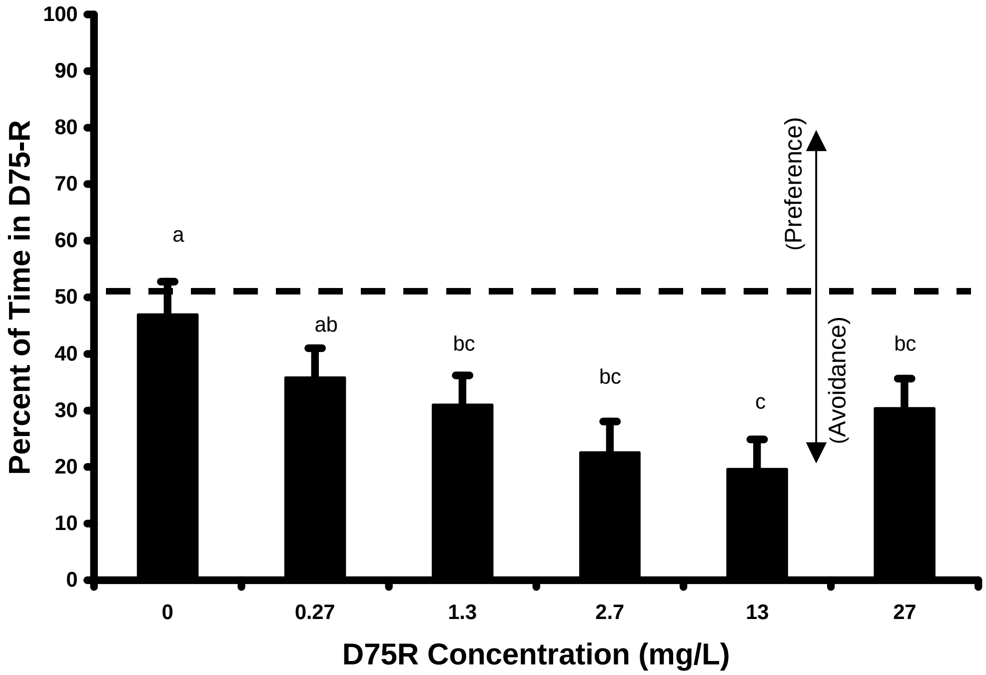

Figure 2. Percent time fish spent in the side of the counter-current avoidance chamber containing Phos-Chek® D75-R. Bars above each histogram indicate standard error of the mean. Histograms accompanied by a different letter are significantly different. Note that when fish are not in the side of the counter-current apparatus containing D75-R, they are in side containing uncontaminated well water.

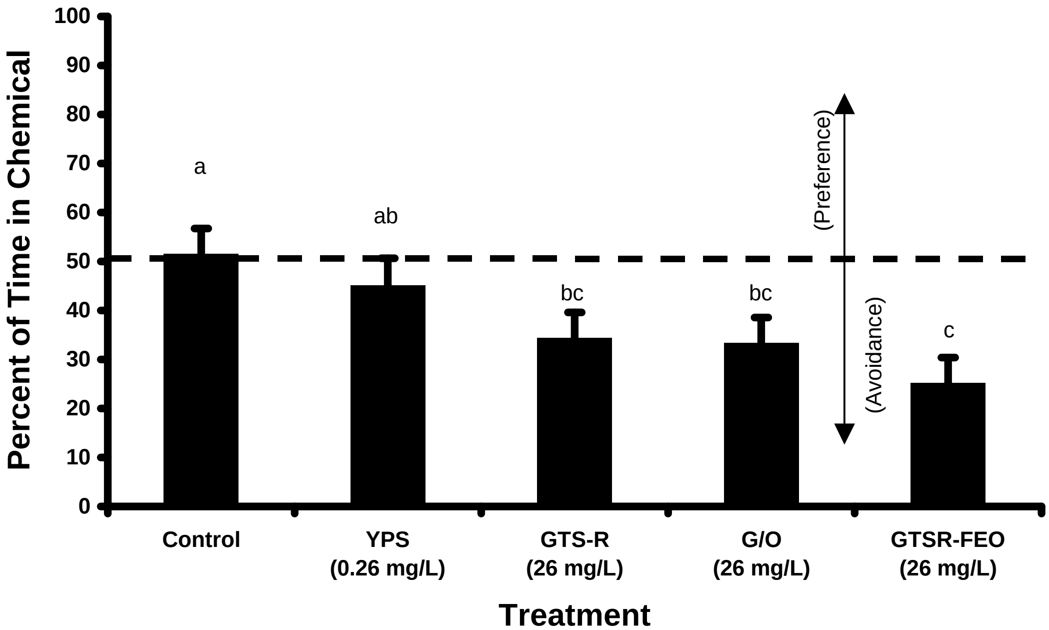

Figure 3. Percent time fish spent in the side of the counter-current avoidance chamber containing (1) uncontaminated water (Control), (2) YPS (yellow prussiate of soda or sodium ferrocyanide), (3) Fire-Trol® GTS-R, (4) GTS-R without iron oxide and YPS (G/O), and (5) GTS-R without iron oxide (GTSR-FEO). Bars above each histogram indicate standard error of the mean. Histograms accompanied by a different letter are significantly different. Note that when fish are not in the side of the counter-current apparatus containing fire retardant materials, they are in side containing uncontaminated well water.

INTRODUCTION

Millions of liters of fire-retardant chemicals are applied in the United States annually to control wildland fires. These chemicals are often applied in relatively pristine and environmentally sensitive wildland areas that are potentially inhabited by endangered, threatened, or sensitive aquatic species (USDOI/DA, 2000). Effective fire management and use of these substances requires an understanding of the potential ecological hazards they may pose. This requires information about the toxicity of fire-retardant chemicals, the persistence of these substances in the environment, natural variables that influence persistence and toxicity of these substances, and exposure of flora and fauna to expected environmental concentrations. Exposure involves not only the doses received through various pathways, but also the behavioral reactions of the organism to the exposure. Avoidance/attractance responses to chemical plumes or gradients are of major toxicological significance because exposure and injury can be minimized when the organism avoids a substance (DeLonay et al., 1996; Little et al., 1993; 1985). In contrast, exposure and likelihood of injury greatly increase if the substance is attractive. For fish, there are numerous examples of chemical substances that are aversive or attractive (Beitinger, 1990). Fish may avoid concentrations of chemical substances well below levels that may cause mortality or reductions in growth (Little et al., 1985), but avoidance of lethal concentrations is protective against injury since duration of exposure is limited.

To our knowledge, no avoidance/attractance data are available for fire-retardant chemicals. However, casual observation suggests that fertilizer-based fire-retardants might be avoided. During recent experimental stream tests with Fire-Trol® GTS-R (GTS-R), we observed that bluegill readily moved downstream, and crayfish moved out of the water in response to addition of this substance to the stream (Little and Calfee, 2002).

The objective of the experiments described in this report was to determine if juvenile rainbow trout (*Oncorhynchus mykiss*) avoid or are attracted to low concentrations of GTS-R and Phos-Chek® D75-R in a laboratory setting. These formulations are widely used as fire retardants in the United States. They may enter aquatic systems through stormwater runoff resulting in high concentrations relative to concentrations that are lethal to fish. Other avenues of contamination such as direct, accidental application may also occur. Tests were also conducted to determine if the colorant (ferrous oxide) and corrosion inhibitor (YPS, yellow prussiate of soda, an archaic term for sodium ferrocyanide) components of the GTS-R formulation play a role in inducing the avoidance response.

MATERIALS AND METHODS

Test Organisms

Rainbow trout were obtained as eyed eggs from a federal fish hatchery and cultured at USGS Columbia Environmental Research Center (CERC) in incubator trays that received flowing deep well water (pH 7.8, hardness 283 mg/liter as calcium carbonate [$CaCO_3$], temperature 18 °C). The fish were transferred to 400-liter tanks at the onset of exogenous feeding. The fish were used in avoidance tests when they were approximately 90 days post-hatch.

Chemical Receipt and Handling

Chemicals selected for testing include GTS-R, GTS-R without YPS and without ferrous oxide colorant (G/O), GTS-R without ferrous oxide colorant (GTSR–FEO), YPS, and D75-R. These chemicals were provided by the U.S. Forest Service Wildland Fire

Chemical Systems Program (WFCS) of the Missoula Technology Development Center, Missoula, Montana via over night courier in sealed 19-liter plastic containers. GTS-R, D-75-R, and YPS were received on April 28, 2001. GTS-R formulations without ferrous oxide colorant, with YPS, and without YPS were received on May 18, 1998. Upon receipt, the shipping containers were inspected for damage and the security seals were inspected for evidence of tampering. The chemicals were held in the CERC chemical storage facility at room temperature in the absence of light.

All concentrations are based on the weight of dry concentrate of GTS-R or D75-R per liter of water unless otherwise specified. Stock solutions of YPS were mixed based on the weight of these components estimated in the dry concentrate of the fire-retardant chemicals. Stock solutions of GTS-R (10 grams/liter) and D75-R (10.3 grams/liter) of comparable salinity were prepared daily by mixing the dry concentrates of either of these fire-retardants with well water using a magnetic stirring device and stir bar at a velocity just below the vortex point in a clear glass container. During this mixing period the stock solutions were irradiated for 20 hours with UV light (280 to 400 nanometers) at 4 microWatts UVB per square centimeter ($\mu W/cm^2$) to simulate a 4-day exposure to the sunlight that may potentially photoactivate substances in the fire-retardant formulation to increase toxicity to fish (Little and Calfee, 2000; Burdick and Lipschuetz, 1950). Stock solutions were stored at room temperature in a glass amber bottle.

Experimental Design and Test Conditions

Test apparatus: A counter-current avoidance chamber was used to test the avoidance/attractance behavior of juvenile rainbow trout (DeLonay et al., 1996). The chamber produces a steep, central gradient between a control situation and a test treatment (Figure 4). The chambers were constructed of clear Plexiglas cylinder (11 cm diameter x 92 cm length) with six centrally located drain holes. Screens to prevent the fish from escaping were placed 11 cm from each end of the chamber, creating a 70-cm observation area. Fish were added to and removed from the chamber through access openings on the top of the chamber. Well water was pumped into the chamber at opposite ends at a flow rate of 1 liter per minute, exiting through the adjustable drains at the center of the chamber.

The irradiated stock solutions of (1) D75-R, (2) GTS-R, (3) GTS-R without ferrous oxide and YPS (G/O), (4) GTS-R without ferrous oxide (GTSR-FEO), or (5) YPS alone were introduced into one end of the test chamber (randomly selected) using a Masterflex® digital metering peristaltic pump at flow rates of 0.07 to 2.6 milliliters (ml) per minute. The three identical chambers were placed side by side and enclosed in a black plastic shroud to shield from external motion.

Avoidance test procedure: Prior to any experimentation, the operation of all components of the apparatus was verified and the establishment of a steep gradient was documented (Figure 5) using water-tracing dye (Bright Dyes®). Calibration of each experimental chamber and chemical delivery tubing was checked and adjusted daily before each trial. Maximum tolerances were ±2% for chamber flow rate and ±10% for the Masterflex® peristaltic pump. Preliminary trials were conducted to determine the appropriate acclimation period and a 20 minute acclimation period was found to be sufficient for acclimation and free movement about the chamber.

The behavioral trials consisted of a 20-minute acclimation period followed by the introduction and equilibration of the test treatment solution (Table 1). Ten minutes were required for the steep gradient to be established. Ten minutes following the introduction of the test treatment solution, the fish were observed and behavioral responses were recorded on videotape for later analysis. Behavioral responses were recorded using the first 5 minutes of the observational period as the proportion of time spent in the test solution versus the time spent in the uncontaminated well water. The presence of the fire-retardant gradient on one side of the chamber and uncontaminated water on the other side resulted in the fish either being in the gradient or in the uncontaminated water. Thus, the reciprocal of the proportion of time spent in the contaminant gradient was the amount of time spent out of the gradient. The number of times the fish crossed into the chemical gradient (trips) and the duration of visit per gradient crossing into the treatment condition (trip time) were also recorded. Tests were rejected when there was inconsistent water chemistry, water temperature, water quality; or when diseased animals, or abnormal behavior were detected. All fish tested within each series of tests were of similar length. Each fish was only tested once.

GTS-R test series: During the GTS-R concentration threshold avoidance-attractance study, a series of trials was conducted using all three chambers simultaneously. During a given trial, the same GTS-R concentration was delivered to each of the chambers. The responses observed from the 3 chambers were averaged to yield the mean value for the replicate. All tests provided uncontaminated well water on one side of the chamber and a fire-retardant chemical treatment on the opposite side. The experimental control treatment was the 0 mg/liter or uncontaminated well water treatment. Thus, during control tests uncontaminated well water was introduced into both sides of the counter-current apparatus. All treatment combinations within a replicate were tested on the same day. The order in which each GTS-R concentration was tested and the side of the chamber that received the treatment were randomized. The series of treatment combinations (0, 6.5, 13, and 26 mg/liter GTS-R) in the first block of tests was repeated 6 times (6 replicates of individual fish tested in 3 separate chambers) for each test concentration. A second block of tests with 0, 0.65, and 6.5 mg/liter GTS-R was repeated 3 times (3 replicates of individual fish tested in 3 separate chambers) for each test concentration.   Limited availability of fish necessitated a smaller sample size for the latter block of tests.

D75R test series: During the concentration threshold avoidance/attractance study of D75-R, a series of trials was conducted using a balanced incomplete block design (Cochran and Cox 1957). Each of the three chambers was used simultaneously, but independently of each other. This allowed testing more than three different treatments (concentrations) within incomplete blocks of time. The treatment scheme for this study was as for above. The treatment levels of D75R used in this assay spanned 2 orders of magnitude (or one hundred fold) and included 0 (Control), 0.26, 1.3, 2.7, 13, and 27 mg/liter D75-R. The D75-R concentrations were selected to match the conductivities observed during GTS-R tests. Tests of each concentration were repeated 10 times (10 replicates).

GTS-R components tests series: During the avoidance/attractance study of GTS-R and its formulation components, a series of trials was conducted using a balanced incomplete block design. Each of the three chambers was used simultaneously, but independently of each other. This allowed us to test different treatments using incomplete blocks of time.

The treatment scheme for this study was as for above. Tests of each treatment included a Control (0 mg/L), GTS-R (26 mg/L), GTS-R without YPS and without color (26 mg/L), GTS-R without color (26 mg/L), or YPS alone (0.26 mg/liter) were repeated 10 times (10 replicates).

Chemical Analysis

Water quality characteristics (temperature, pH, dissolved oxygen, and specific conductance) were sampled before and after each treatment within each trial. We measured pH with an Orion® model 290A pH meter and universal combination pH electrode. Dissolved oxygen was determined using YSI® model 58 dissolved oxygen meter. Specific conductance and temperature were measured using an Orion® model 142 conductivity meter and four-electrode conductivity cell with integrated temperature sensor. Specific conductance of the test water was measured during each test as an indirect means of monitoring fire-retardant concentrations. All instruments were calibrated daily before use.

Aqueous samples for the determination of cyanide and ammonia concentrations were taken from one of the three chambers for each concentration, within each replicate trial. Samples for the determination of weak acid dissociable (WAD) cyanide (500 mL) were preserved with 10 Normal NaOH and refrigerated until shipment to Severn Trent Laboratories (Arvada, CO) for analysis by method 4100 CN-I from Standard Methods for the Examination of Water and Waste Water (APHA 1989). Shipments were packed in ice inside insulated ice chests and sealed with security tape. Samples taken for the determination of ammonia (20 mL) were preserved with H_2SO_4 and refrigerated until analysis by automated flow-injection analysis (Technicon Auto Analyzer II) using method 4500 NH3-H from Standard Methods for the Examination of Water and Waste Water (APHA 1989).

Statistical Analysis

The avoidance data were determined to meet the assumptions for the analysis of variance (ANOVA) technique. A general linear model (GLM) ANOVA was conducted followed by least squares means comparisons to make inferences regarding treatment differences on the spatial selection of fish in the avoidance apparatus (SAS 2000). Statistical significance was assigned at $P \leq 0.05$.

RESULTS

GTS-R test series

Results of avoidance/attractance trials from our experiments indicate that rainbow trout are capable of detecting and avoiding low concentrations of dissolved GTS-R in full formulation in a laboratory setting (Table 2). Rainbow trout spent significantly less time in the treatment condition versus the control condition at concentrations as low as 0.65 mg/liter (10 percent of the LC50 concentration) as measured by total cumulative time ($p = 0.0016$). The magnitude of the avoidance response did not appear to follow a dose response relationship, but rather was an all-or-none response. Although the number of trips fish made into the treated area was similar among controls and GTS-R treatments ($p = 0.1993$), the average trip time was significantly shorter for each GTS-R concentration than were visits into the control treatment ($p = 0.0092$).

Water temperature in the counter-current chambers ranged from 18.5 to 18.7 °C.

Dissolved oxygen and pH did not vary significantly between GTS-R treatment and control conditions for any trial (Table 3). Specific conductance of the test water was linearly correlated to GTS-R concentrations (Conductivity = 1.4247[GTS-R] + 643.0909, $p<0.0001$, $R^2=0.9990$), and provided the most effective means of monitoring these fire-retardant concentrations applied during the avoidance tests (Figure 6). Total ammonia concentrations ranged from 5.2 mg/liter at the nominal 26 mg/liter GTS-R treatment to 0.1 mg/liter at the 0.65 mg/liter. WAD cyanide ranged from 16.3 µg/liter for the nominal 26-mg/liter GTS-R concentrations to below detection limits (10µg/liter) at the 13-mg/liter treatments and lower.

D75-R test series

Rainbow trout significantly ($p = 0.004$) avoided D75-R at concentrations as low as 1.3 mg/liter as measured by percent of total time spent in the treatment condition versus the control condition (Table 4). This is less than one percent of the LC50 for D75-R. The magnitude of the avoidance response tended to increase with increasing D75-R concentration. The frequency of trips into D75-R and uncontaminated water did not differ significantly, but the average trip time was significantly greater for fish entering uncontaminated water. Dissolved oxygen, pH, and temperature did not vary significantly among D75-R treatments and control conditions for any trial (Table 5). Differences in temperature between ends of the same chamber at a given time did not vary by more than 0.2 °C.

Specific conductance of the test water was linearly correlated to D75-R concentrations (Conductivity = 1.2284[D75-R] + 646.12, $p<0.0001$, $R^2=0.9943$), and provided the most effective means of monitoring concentrations of this fire-retardant during the avoidance tests (Figure 7). WAD cyanide was below detection limits for all D75-R and control tests.

GTS-R components test series

Tests with different formulations of GTS-R indicate that the absence of the ferrous oxide colorant, the YPS corrosion inhibitor, or both ferrous oxide and YPS did not diminish the avoidance response of rainbow trout (Table 6). Fish spent significantly less time in the apparatus side receiving the dilute concentrations of these materials. The number of trips into fire-retardant and control conditions and the average time of these visits did not differ significantly. Rainbow trout did not avoid YPS, when this substance was tested alone (Table 6).

Dissolved oxygen, pH, and temperature did not vary significantly among GTS-R or YPS treatments and control conditions for any trial (Table 7). Specific conductance of the test water was similar (Figure 8) among the full formulation GTS-R (y= 1.4989[GTS-R], $R^2 = 1.000$) GTS-R without YPS and ferrous oxide (Y= 1.5546[G/O], $R^2 = 0.999$), and GTS-R without ferrous oxide (Y=1.5415[GTSR–FEO], $R^2 = 0.9949$). The relative specific conductance of GTS-R was somewhat greater in deionized (less than 5 mg/L hardness as $CaCO_3$) water (Y=1.7395x + 1.3) than in well water (Y= 1.576x + 640) that had a water hardness of about 280 mg/L (Figure 9).

DISCUSSION

Fish kills have been reported following accidental releases of fire-retardant chemicals into aquatic systems from spills, overspray, and contamination from cleaning equipment. However, adherence to protocols for the application of fire-retardant products prevents most releases into aquatic systems. A likely route of entry of fire-retardant chemicals into aquatic systems from properly applied substances would occur by rainwater runoff from treated watersheds. The hazards posed by this pathway would depend upon the spatial extent of watershed treated, rate of application, binding or sequestration of material by the substrates, dilution by receiving waters, and environmental persistence. If the material reaches aquatic habitats, there may also be behavioral defenses as organisms avoid the chemical gradient and thereby limit exposure and injury.

Many compounds, including metals, pesticides, chlorinated compounds, industrial chemicals, and complex effluents, are known to induce avoidance responses in fish (Atchison et al., 1987; Beitinger, 1990). Avoidance responses observed in laboratory tests have been confirmed in the field. Sprague et al. (1965) and Saunders and Sprague (1967) reported that copper and zinc contamination from mining activity in the drainage of a New Brunswick stream reduced upstream migration of returning adult Atlantic salmon. Geckler et al. (1976) observed avoidance behavior in a stream that was intentionally dosed with copper where resident fish populations limited exposure by actively seeking out areas of low copper concentration. Hartwell et al. (1987) documented avoidance of a mixture of copper, chromium, arsenic, and selenium by fathead minnows (*Pimephales promelas*) in the laboratory and natural streams. Woodward et al. (1995) postulated that avoidance of low concentrations of metals associated with mining activities was in part responsible for the distribution and decline of salmonids in the Clark Fork River.

The data clearly indicate that when rainbow trout are presented with a choice between untreated control water and water treated with GTS-R or D75-R, the fish are able to detect and avoid concentrations of less than 10 percent of the LC50 concentrations determined by Little and Calfee (2000). From this perspective, avoidance of 0.65 mg/liter GTS-R or 1.3 mg/liter D75-R would protect rainbow trout from hazardous exposure.

There are several factors to consider in the interpretation of avoidance tests. Field variables such as water temperature and water quality, particularly pH, hardness, and dissolved carbon content, can influence the response by altering the sensory stimuli of the chemical substance. The tests presented in this report were conducted with a water quality corresponding to ASTM's classification of very hard water (ASTM 1998). Specific conductance was greater when fire-retardant was mixed in deionized water (less than 5 mg/liter as $CaCO_3$, less than 10MΩ Resistivity) than when prepared in well water of high water hardness (about 280 mg/liter as $CaCO_3$), thus fire retardants mixed in water of low hardness may induce a more robust avoidance response. Studies of other fish species in response to metals suggest that a heightened avoidance response may occur as water hardness and alkalinity decrease (Hartwell et al., 1987; Woodward et al., 1995).

Long-term fire-retardant chemicals such as GTS-R and D75-R are composed of ammonium salts such as ammonium sulfate and ammonium phosphate that significantly increase the salinity (measured by specific conductance) of water. Solutions of these fire-retardant chemicals were applied at similar specific conductance during the avoidance tests. Specific conductance may be linked to the sensory cue that induced the avoidance response. Fish chemosensory systems are highly sensitive to salts (Little 1983). The exclusion of the YPS corrosion inhibitor and the ferrous oxide colorant did not diminish the repellant qualities of GTS-R.

Environmental variables can compete with avoidance stimuli (Little et al. 1985; Little et al. 1993) such that responses to temperature, pH, or ash may override the avoidance reaction to the fire-retardant. The environmental circumstances caused by fire within the organism's watershed could cause a number of disruptions in aquatic habitats including inputs of ash that clog gill membranes, cause turbidity, and increase temperature, as well as obstructions to the flow of water by debris.

The chemical gradient, particularly in a lake may not provide sufficient directional cues to direct the organism's movement to uncontaminated areas. High concentrations of chemical substances may startle or confuse the organism, causing it to be disoriented. Biological factors can also influence the response. Different species or different life stages of the same species may not respond similarly to a chemical substance. The acclimation history of the test organisms and the influence of competing motivational variables may also influence the response to some degree. Organisms may become unresponsive to the chemical through habituation or sensory adaptation. Competing drives such as migration may override avoidance reactions.

Although the avoidance of fire-retardant chemicals is advantageous to aquatic organisms, long-term avoidance may result in displacement of fish from preferred habitats to areas that are less optimal for survival in terms of shelter, food, reproduction, or protection from predators (Atchison et al., 1987). In more extreme cases, avoidance of contaminants by aquatic organisms may result in the effective loss of habitable resources, the interruption of essential migratory behaviors, and the loss of viable populations in the field (Sprague et al., 1965; Saunders and Sprague, 1967). Localized declines in fish populations and the loss of habitable resources due to the behavioral avoidance of environmental contaminants may alter aquatic ecosystems and cause significant biological and economic injury to natural resources (Lipton et al., 1996). Avoidance responses, therefore, are an important measure of sublethal effect resulting from exposure to hazardous substances.

CONCLUSIONS

Rainbow trout consistently avoided water treated with GTS-R at concentrations as low as 0.65 mg/liter and D75-R at concentrations as low as 1.3 mg/liter, which are less than 10 percent of the concentration lethal to 50 percent of rainbow trout test in 96 hours (LC50). The increased salinity of water containing these fire-retardant chemicals may be a sensory cue used by fish to avoid plumes of fire-retardant in aqueous habitats. The YPS corrosion inhibitor and the iron oxide colorant did not appear to influence the avoidance response at the concentrations evaluated in this study.

ENVIRONMENTAL IMPLICATIONS

Results suggest that, if given the opportunity, rainbow trout will escape chemical gradients of GTS-R and D75-R in the field. As an example, consider a scenario of a 2.5 cm of rainfall over a 100 square foot area of watershed treated at the recommended minimum application rate of one gallon per 100 square feet (GPC). This is equivalent to 755 grams GTS-R or 554 grams D75-R resulting in a maximum runoff concentration of 3.2 grams GTS-R/liter or 2.3 grams D75-R/liter. These estimated concentrations are considerably greater than those inducing avoidance responses which are less than 10 percent of the 96-hour LC50 of 6.5 mg/liter GTS-R or 168 mg/L D75-R. Therefore, the avoidance response could provide the fish short-term protection from hazardous exposure. Avoidance responses may differ among species and lifestages of the same species, and competing biological drives may override the response.

Environmental variables such as ash plumes and heightened temperatures could inhibit avoidance responses. Steep concentration gradients likely to occur in streams would more likely induce avoidance than shallow gradients that may occur in ponds and lakes. Because avoidance responses force fish from preferred habitats, long-term avoidance may be deleterious as fish are subjected to less favorable habitats with greater predation pressure, less forage base, or greater competition from other species.

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Table 1. Sequence of Avoidance Test Procedure

Calibration: Flow rates and water temperature of supply lines to counter current apparatus are equilibrated. Metering pumps are calibrated for target delivery concentration.

Pre-test: Water quality assessed for conductivity, temperature, DO, and pH.

Acclimation: 20 minutes: Uncontaminated water introduced into both ends of the countercurrent avoidance apparatus. Fish observed for movement throughout apparatus.

Gradient Formation: 10 minutes. One end of apparatus randomly selected to receive fire-retardant treated water. Concentration of that treatment is randomly selected. Metering pumps are activated.

Behavioral Observation: 5 minutes. Overhead video tape is activated. The number of times fish cross into treatment area is counted. Cumulative time spent in the contaminated area is measured. Time spent during each trip is measured during play back of the video tape.

Test Conclusion: Water samples taken for conductivity, total ammonia, WAD cyanide Temperature, DO, and pH. Fish removed for length measurements, then discarded

Table 2. Avoidance/attractance responses of juvenile rainbow trout as measured by average (± standard error of the mean) percent time spent in the Fire-Trol® GTS-R gradient (Time %), number of trips into the gradient (Trips) and mean time per trip (seconds) in gradient. Common letters signify no significant statistical difference between treatments ($p \leq 0.05$). N indicates the number of replicate tests.

GTS-R *mg/L*	*n*	Time *%*	Trips	Mean Trip Time *(seconds)*	Mean Fish Length *(mm)*
0	9	54.9 ± 3.4 [a]	21.5 ± 1.3 [a]	17.5 ± 9.4 [a]	67 ± 2.4 [a]
0.65	3	31.3 ± 4.3 [b]	17.8 ± 2.2 [a]	5.2 ± 0.2 [b]	71 ± 2.6 [a]
6.50	9	26.0 ± 2.9 [b]	17.0 ± 2.0 [a]	4.3 ± 0.5 [b]	70 ± 1.4 [a]
13.00	6	32.2 ± 5.8 [b]	17.9 ± 1.3 [a]	5.1 ± 0.8 [b]	67 ± 1.0 [a]
26.00	6	23.1 ± 3.5 [b]	18.3 ± 2.2 [a]	4.0 ± 0.9 [b]	68 ± 1.8 [a]

Table 3. Mean (± standard error of the mean) pH, temperature, dissolved oxygen (DO), and specific conductance for Fire-Trol® GTS-R. Common letters signify no significant statistical difference between treatments. N indicates the number of replicate tests.

GTS-R (mg/L)	*n*	pH	Temp (°C)	DO *(mg/L)*	Test Specific Conductance (µS/cm)
0	9	7.3 ± 0.03 [a]	18.6 ± 0.03 [a]	8.6 ± 0.05 [a]	642 ± 0.2 [a]
0.65	3	7.3 ± 0.05 [a]	18.6 ± 0.01 [a]	8.6 ± 0.08 [a]	645 ± 0.2 [b]
6.50	9	7.3 ± 0.04 [a]	18.6 ± 0.05 [a]	8.6 ± 0.05 [a]	653 ± 0.2 [b]
13.00	6	7.3 ± 0.06 [a]	18.5 ± 0.08 [a]	8.6 ± 0.06 [a]	662 ± 0.2 [b]
26.00	6	7.3 ± 0.04 [a]	18.6 ± 0.04 [a]	8.5 ± 0.05 [a]	680 ± 0.5 [b]

Table 4. Avoidance/attractance responses of juvenile rainbow trout as measured by average percent time spent in the Phos-Chek® D75-R gradient (Time %), number of trips into the gradient (Trips) and mean trip time in gradient (± Standard Error of the Mean). Common letters signify no significant statistical difference between treatments ($p \leq 0.05$). N indicates the number of replicate tests.

D75-R *(mg/L)*	*n*	Time %	Trips	Mean Trip Time *(seconds)*	Mean Fish Length *(mm)*
0	8	47.0 ± 10.51 [a]	15.0 ± 2.9 [a]	43.5 ± 36.7 [a]	35 ± 0.8 [a]
0.27	10	35.8 ± 3.49 [ab]	17.4 ± 1.6 [a]	6.3 ± 0.5 [b]	33 ± 0.8 [a]
1.3	10	31.0 ± 4.43 [bc]	15.6 ± 1.8 [a]	6.1 ± 0.8 [b]	32 ± 1.1 [a]
2.7	9	22.6 ± 3.78 [bc]	14.3 ± 2.6 [a]	4.8 ± 0.3 [b]	33 ± 1.5 [a]
13	10	19.7 ± 3.82 [c]	11.9 ± 2.6 [a]	4.8 ± 0.7 [b]	32 ± 1.0 [a]
27	10	30.4 ± 5.26 [c]	17.2 ± 2.6 [a]	5.3 ± 0.7 [b]	33 ± 0.7 [a]

Table 5. Mean (± standard error of the mean) pH, temperature, dissolved oxygen (DO), and specific conductance for Phos-Chek® D75R. Common letters signify no significant statistical difference between treatments. N indicates the number of replicate tests.

D75R (mg/L)	*n*	pH	Temp (°C)	DO *(mg/L)*	Test Specific Conductance (µS/cm)
0	10	7.6 ± 0.02 [a]	18.1 ± 0.08 [a]	8.6 ± 0.06 [a]	646 ± 0.3 [a]
0.27	10	7.6 ± 0.02 [a]	18.1 ± 0.07 [a]	8.6 ± 0.05 [a]	646 ± 0.4 [a]
1.3	10	7.6 ± 0.02 [a]	18.1 ± 0.08 [a]	8.6 ± 0.05 [a]	648 ± 0.4 [b]
2.7	10	7.6 ± 0.02 [a]	18.1 ± 0.08 [a]	8.6 ± 0.06 [a]	650 ± 0.4 [b]
13	10	7.6 ± 0.02 [a]	18.1 ± 0.08 [a]	8.6 ± 0.05 [a]	652 ± 0.9 [b]
27	10	7.6 ± 0.02 [a]	18.1 ± 0.07 [a]	8.6 ± 0.05 [a]	680 ± 1.7 [b]

Table 6. Avoidance/attractance responses of juvenile rainbow trout as measured by average percent time spent (Time %) in uncontaminated water (Control), yellow prussiate of soda (YPS), Fire-Trol® GTS-R, GTS-R without YPS or ferrous oxide (G/O) and GTS-R without ferrous oxide (GTRS-FEO). Also shown are number of trips into the chemical gradient (Trips) and mean time per trip in the chemical gradient (± standard error of the mean). Common letters signify no significant statistical difference between treatments ($p \leq 0.05$). N indicates the number of replicate tests. Concentration tested appears in parentheses beneath each chemical treatment.

Chemical (*mg/L*)	*n*	Time %	Trips	Mean Trip Time *(seconds)*	Mean Fish Length *(mm)*
Control (0)	10	51.5 ± 6.91 [a]	18.0 ± 2.5 [a]	23.9 ± 17.3 [a]	37 ± 1.2 [a]
YPS (0.26)	9	45.1 ± 8.31 [a b]	19.8 ± 2.7 [a]	11.9 ± 7.3 [b]	37 ± 0.8 [a]
GTS-R (26.00)	10	34.4 ± 3.35 [bc]	19.1 ± 2.4 [a]	5.9 ± 0.7 [b]	38 ± 0.8 [a]
G/O (26.00)	10	33.3 ± 3.71 [bc]	20.1 ± 2.3 [a]	5.4 ± 0.8 [b]	39 ± 1.3 [a]
GTSR-FEO (26.00)	10	25.1 ± 4.05 [c]	17.8 ± 2.8 [a]	3.9 ± 0.6 [b]	38 ± 0.8 [a]

Table 7. Mean (± standard error of the mean) pH, temperature, dissolved oxygen, and specific conductance for Uncontaminated water (Control), yellow prussiate or soda (YPS), Fire-Trol® GTS-R, GTS-R without YPS or ferrous oxide (G/O) and GTS-R without ferrous oxide (GTSR-FEO). Common letters signify no significant statistical difference between treatments ($p \leq 0.05$). N indicates the number of replicate tests. Chemical concentrations tested appear in parentheses beneath each chemical.

Chemical (mg/L)	*n*	pH	Temp (°C)	DO *(mg/L)*	Test Specific Conductance (µS/cm)
Control (0)	10	7.3 ± 0.14 [a]	18.1 ± 0.19 [a]	8.7 ± 0.13 [a]	649 ± 0.2 [a]
YPS (0.26)	10	7.4 ± 0.07 [a]	18.1 ± 0.17 [a]	8.7 ± 0.09 [a]	649 ± 0.3 [a]
GTS-R (26)	10	7.3 ± 0.14 [a]	18.1 ± 0.16 [a]	8.8 ± 0.18 [a]	683 ± 0.9 [b]
G/O (26)	10	7.3 ± 0.13 [a]	18 ± 0.13 [a]	8.7 ± 0.12 [a]	688 ± 1.3 [b]
GTSR-FEO (26)	10	7.4 ± 0.08 [a]	18.1 ± 0.24 [a]	8.7 ± 0.11 [a]	688 ± 1.1 [b]

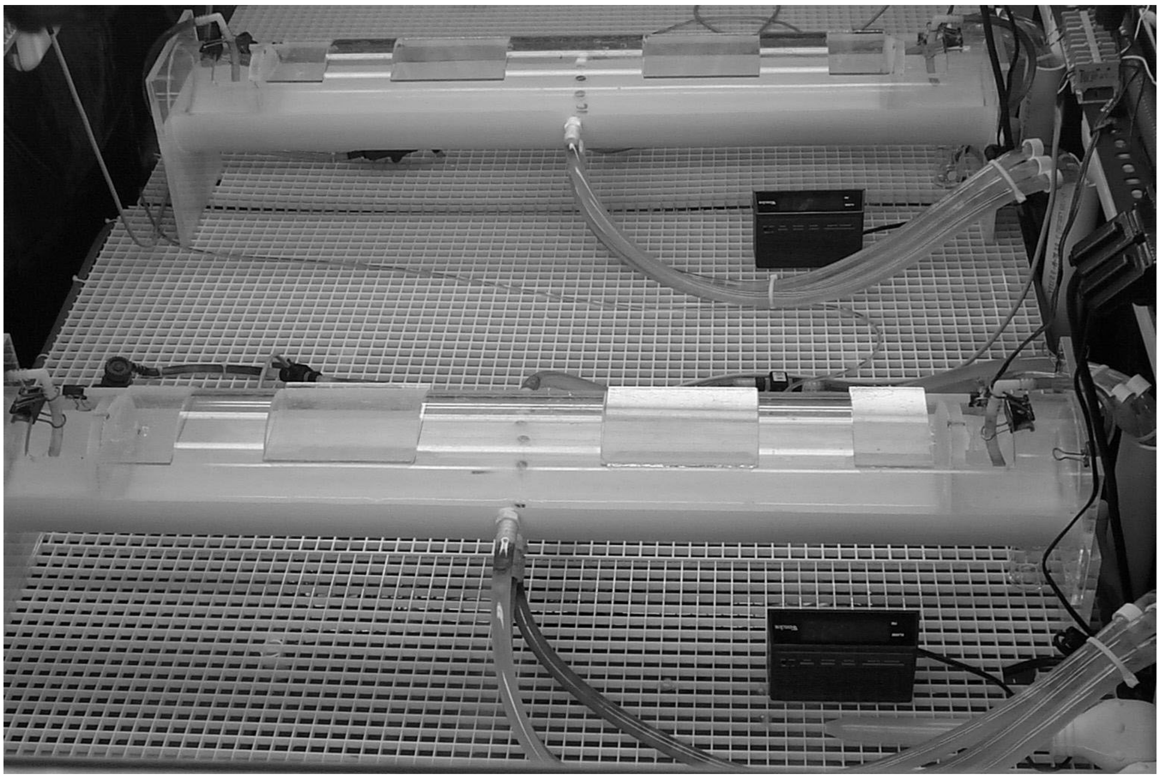

Figure 4. Counter-current avoidance chambers used to determine the avoidance/attractance by juvenile rainbow trout to water treated with fire-retardant chemicals.

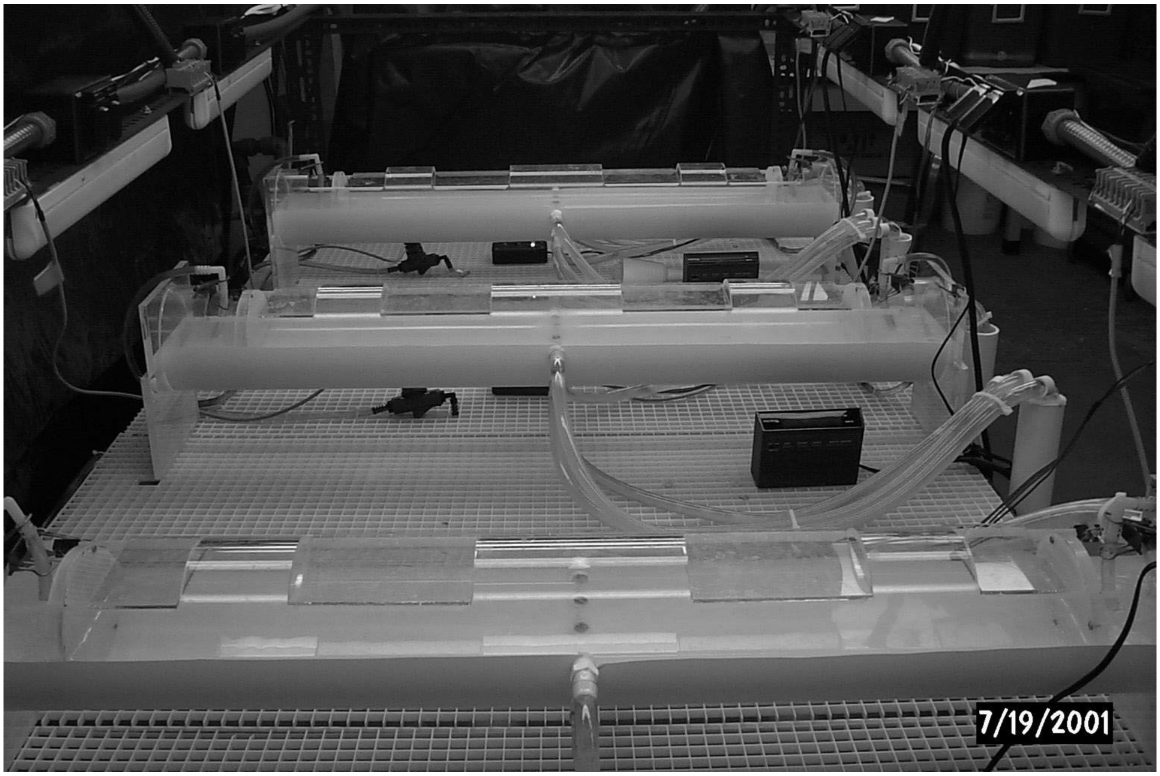

Figure 5. Counter-current avoidance/attractance chamber showing dye gradient on right side of apparatus. Note: Fire-retardant treated water introduced on the left end of the chambers while untreated well water is introduced into right end of each chamber. The apparatus drains at the center forming a steep chemical gradient.

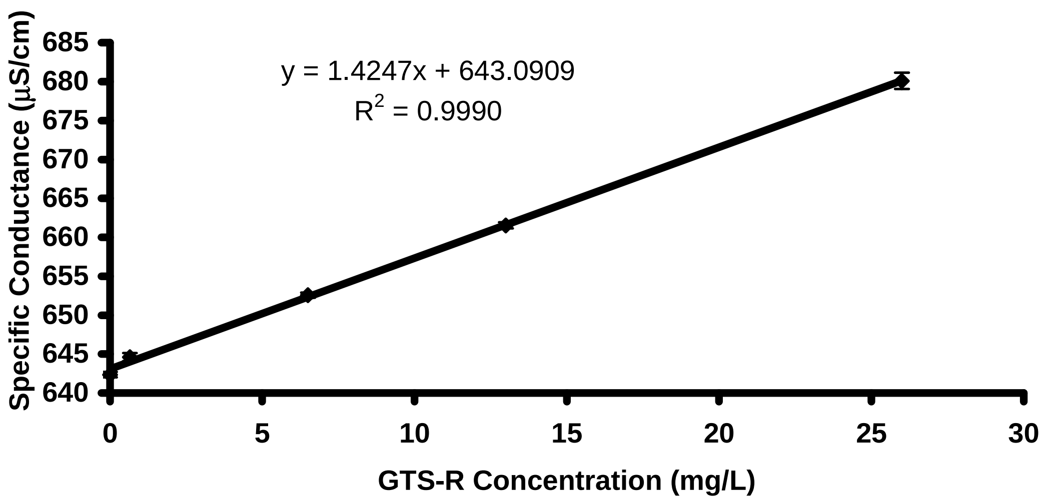

Figure 6. Specific conductance of Fire-Trol® GTS-R observed over a range of concentrations applied during avoidance/ attractance tests.

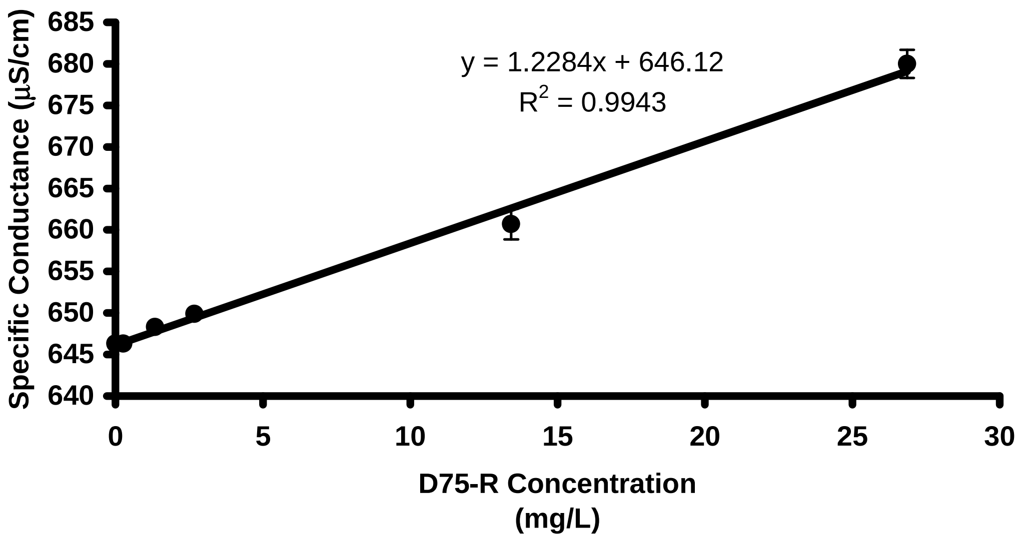

Figure 7. Specific conductance of Phos-Chek® D75-R observed over the range of concentrations applied during avoidance/attractance tests.

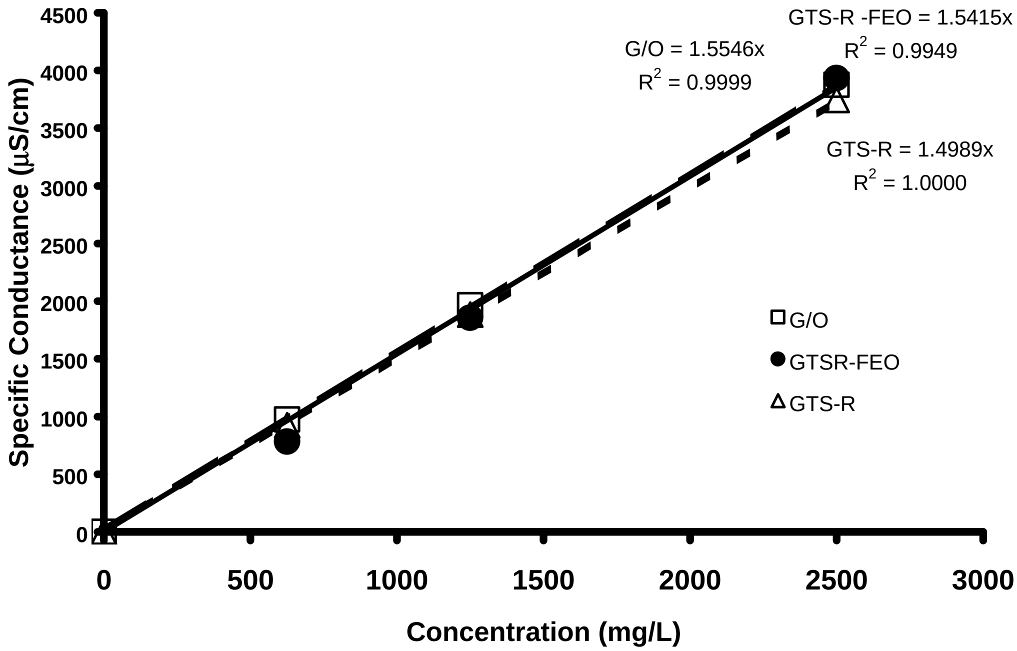

Figure 8. Relative specific conductance of Fire-Trol® GTS-R, GTS-R without YPS and ferrous oxide (G/O), and GTS-R without ferrous oxide (GTSR-FEO) observed during avoidance/attractance tests.

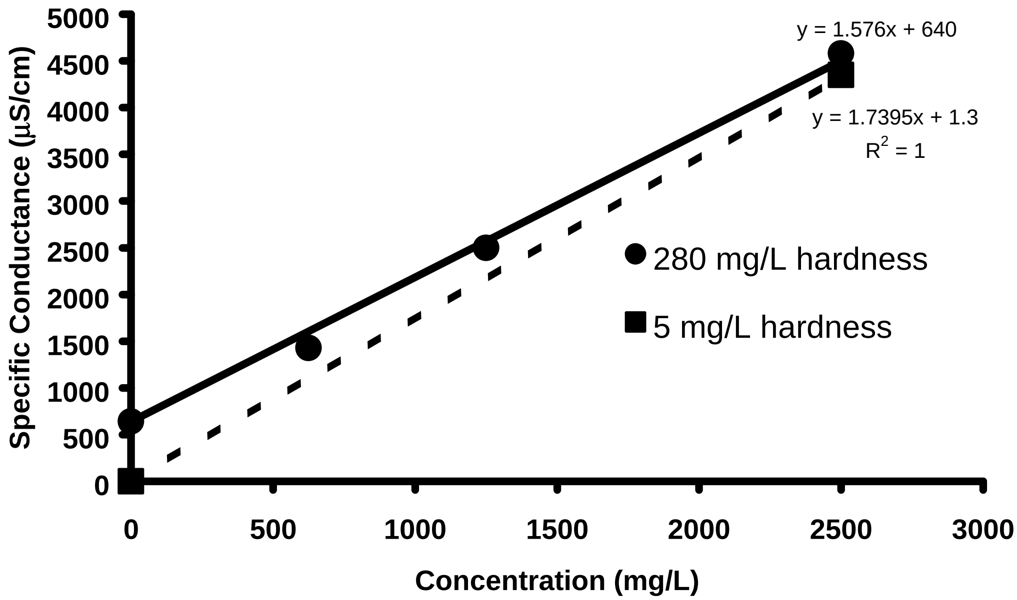

Figure 9. Influence of water hardness of the specific conductance of GTS-R without ferrous oxide.