THE EFFECTS OF UVB RADIATION ON THE TOXICITY OF FIRE-FIGHTING CHEMICALS

Final Report

Submitted by

Edward E. Little and Robin D. Calfee
U.S. Geological Survey, Columbia Environmental Research Center
4200 New Haven Road
Columbia, MO 65202

Submitted to

Cecilia Johnson
USDA Forest Service
Wildland Fire Chemical Systems
Bldg. 1, Fort Missoula
Missoula, MT 59804

March 23, 2000

TABLE OF CONTENTS

EXECUTIVE SUMMARY

The Effects of UVB Radiation on the Toxicity of Fire-Fighting Chemicals

Fire retardant chemicals are widely used in the United States and Canada to suppress and control wildland fires. These chemicals may be applied in environmentally sensitive areas potentially inhabited by endangered, threatened, or sensitive aquatic organisms. There is relatively little information on the toxicity of these chemicals to aquatic organisms and even less on the interactive effects of fire retardant chemicals and ultraviolet (UV) radiation.

The toxicity of some chemicals are known to be photoenhanced in the presence of natural solar UV (Oris and Giesy, 1985; Pelletier et al., 1997). During photoenhanced aquatic toxicity, a chemical transformation of the substance takes place in the presence of UV to create forms that are more toxic to aquatic organisms (Zaga et al., 1999; Calfee et al., 1999; Cleveland et al., In Press). One ingredient of some fire retardant chemicals, yellow prussiate of soda (YPS) or sodium ferrocyanide, is used as a corrosion inhibitor. In earlier literature, Burdick and Lipschuetz (1950) reported that very dilute ferrocyanide solutions become highly toxic to fish upon exposure to sunlight.

The interactive effects of UV and fire retardant chemicals were evaluated by exposing juvenile rainbow trout (*Onchorhyncus mykiss*) and Southern leopard frog (*Rana sphenocephala*) tadpoles to six fire retardant formulations with and without YPS and to YPS alone, under three simulated UV light treatments. The chemical concentrations tested were representative of what would occur naturally in the field following

application and the UV intensities applied were well below that of natural sunlight and were within tolerance limits for the species tested.

RESULTS

The following major results were determined during this investigation:

- Mortality of rainbow trout (Figure 1) and Southern leopard (Figure 2) frog tadpoles exposed to Fire-Trol GTS-R, Fire-Trol 300-F, Fire-Trol LCA-R, and Fire-Trol LCA-F was significantly increased in the presence of UV radiation. In tests with these chemicals free cyanide concentrations were much higher in UV light treatments than in treatments under dark and light control conditions. For both species, free cyanide concentrations at the high exposure concentration exceeded the criteria limit (5.2 µg/L) for freshwater organisms (USEPA, 1999).
- When sodium ferrocyanide (YPS) was not in the retardant formulation toxicity was significantly lower and survival of organisms was consistent with that observed in previous studies (Gaikowski et al., 1996) under laboratory lighting conditions. Un-ionized ammonia likely contributed to the decreased survival observed in tests with fire retardant chemicals without YPS in the formulation.
- The presence of colorant did not appreciably affect toxicity to rainbow trout or Southern leopard frog tadpoles.
- Rainbow trout were always more sensitive to exposure to all fire chemicals tested than the Southern leopard frog tadpoles. However, both species were equally affected by relatively low concentrations of YPS alone in the presence of UV.

- The UV levels applied during the laboratory exposures were well below those measured in a variety of natural habitats. The UV treatment (4 $\mu W/cm^2$) approximated 2-10% of sunlight penetrating 10 cm in various aquatic habitats. Therefore, photoenhancement of fire retardant chemicals can occur in a range of habitats and may be of concern even when optical clarity is low.

RECOMMENDATIONS

The assessment of the impacts of fire-fighting chemicals associated with aerial application of forest fires is important for the protection of aquatic resources and for establishing mitigation priorities and goals. The chemicals tested in this study have a high probability of entering the environment because of their widespread use. Given the extent of photoenhancement of these products, further evaluation of the persistence of toxicity, particularly under field conditions is warranted. Data are needed to confirm the photoenhanced toxicity of the chemicals in laboratory and *in-situ* field tests to determine how rapidly the chemical transformation occurs in sunlight and if toxicity persists over time. Such information may guide management decisions relative to application regime if the compounds are found to rapidly decline in toxicity after their release in the environment. On the other hand, there may be cause for concern about toxic runoff if toxicity remains high for long periods of time after field application.

Although contamination has been shown to cause fish kills, the avoidance of affected areas by fish has also been observed. The risk of environmental injury from the use of these substances is based not only on their toxicity and environmental persistence, but also on the tendency of organisms to avoid exposure. This apparent avoidance reaction may protect natural populations since by avoiding harmful concentrations the

fish minimize fire retardant exposure and subsequent injury. Since solar photoactivation significantly increases the toxicity of formulations containing sodium ferrocyanide, UV may also be important in inducing the avoidance of these chemicals.

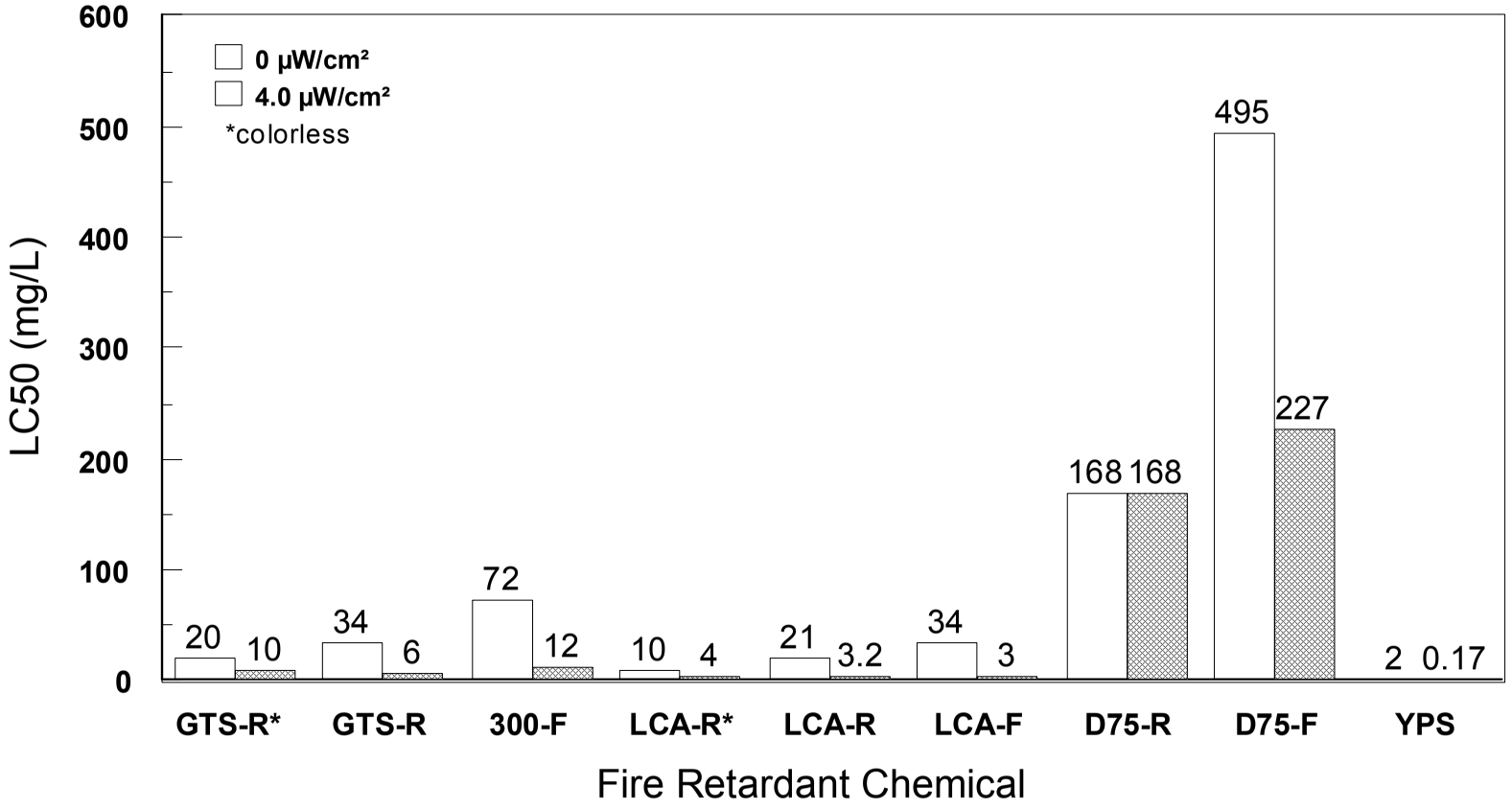

Figure 1. 96-h LC50s for rainbow trout exposed to seven fire retardant chemicals under the 0 and 4.0 µW/cm^2 light treatments. These are the complete formulations as applied in the field, including yellow prussiate of soda in those fire chemical products that have this component.

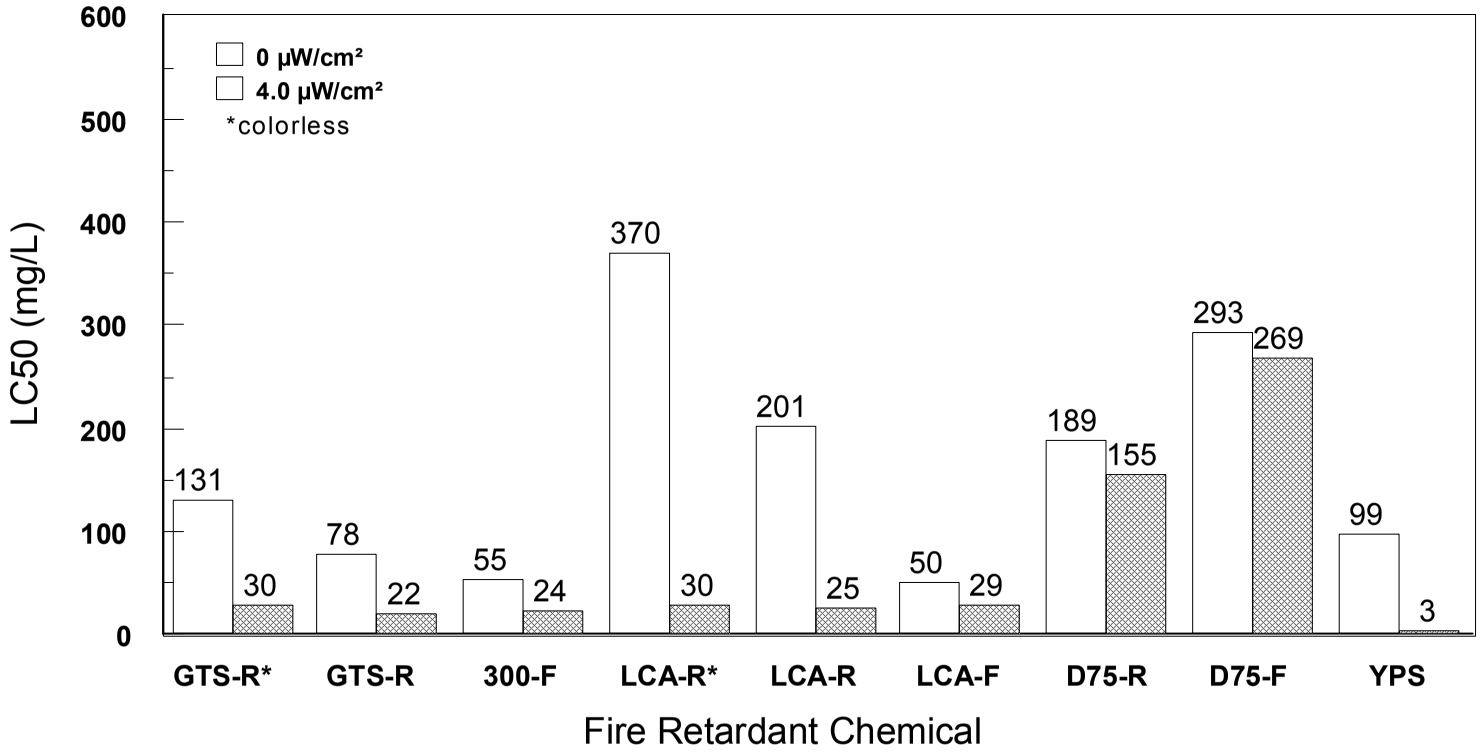

Figure 2. 96-h LC50s for Southern leopard frog tadpoles exposed to seven fire retardant chemicals under the 0 and 4.0 µW/cm^2 light treatments. These are the complete formulations as applied in the field, including yellow prussiate of soda in those fire chemical products that have this component.

INTRODUCTION

In the United States and Canada, forest fire managers and fire control agencies use a wide variety of chemicals to fight wildland fires. Different formulations of these chemicals may be used in relatively pristine areas potentially inhabited by endangered, threatened, or sensitive aquatic species. Aerial applications of these formulations can result in stream or lake contamination due to runoff and inaccurate drops. Such contamination has been implicated in fish kills. For example, considerable trout mortality occurred in Yellowstone National Park after the accidental release of fire retardant chemicals into the Little Firehole River in 1988 (Minshall and Brock, 1991). Approximately 127 million liters of ammonia-based fire retardants were applied in the United States in 1996 (Buhl and Hamilton 1998). In addition to ammonium compounds, retardant formulations also include one or more corrosion inhibitors to minimize damage to storage, transport, and delivery systems. Although ammonia is recognized as a potentially toxic component of these formulations, the corrosion inhibitor yellow prussiate of soda (YPS), which is sodium ferrocyanide, may also contribute to toxicity.

The toxicity of sodium ferrocyanide is relatively low when evaluated under standard laboratory lighting conditions (Degussa, 1995). However, the toxicity of certain chemicals including YPS increases in the presence of sunlight (Burdick and Lipschuetz, 1950). This is referred to as photoenhanced toxicity and is a reaction of the chemical to natural solar ultraviolet (UV) radiation. The ultraviolet (UV) region of the light spectrum spans the 280-400 nm wavelength range and includes both UVA and UVB radiation. UVB is defined as the range from 280-320 nm and UVA is defined as the range from 320-400 nm. Some chemicals are transformed in the presence of UV to more toxic

forms, which can have harmful effects on aquatic organisms (Zaga et al., 1999; Calfee et al., 1999; Cleveland et al., In Press). In earlier literature, Burdick and Lipschuetz (1950) reported that very dilute ferrocyanide solutions become highly toxic to fish upon exposure to sunlight.

Assessment of the potential impacts of chemicals associated with the aerial spraying of forest fires is important for the protection of aquatic resources and for establishing fire mitigation priorities and goals. The objective of this study was to investigate the interactive toxicity among YPS, ammonia, and UV radiation to a fish and an amphibian species. This report provides results of tests conducted with juvenile rainbow trout (*Onchorhynchus mykiss*) and Southern leopard frog tadpoles (*Rana sphenocephala*) exposed to fire fighting chemicals in the presence of light quality and intensity representative of sunlight conditions in natural habitats.

Specific objectives were as follows:

- To determine the influence of UV radiation on the survival of juvenile rainbow trout and Southern leopard frog tadpoles exposed to 6 fire chemical formulations and YPS.
- To determine the effects of color added to fire chemical formulations on the survival of rainbow trout and Southern leopard frog tadpoles in the presence of UV radiation.
- To determine the influence of UV radiation on the survival of juvenile rainbow trout and Southern leopard frog tadpoles exposed to technical grade sodium ferrocyanide or YPS.

METHODS

Experimental Design

A total of 30 exposures, were conducted to determine the effects of 6 fire-fighting chemicals (Fire-Trol GTS-R, Fire-Trol 300-F, Fire-Trol LCA-R, Fire-Trol LCA-F, Phos-Chek D75R, and Phos-Chek D75F), and YPS on the survival of rainbow trout and Southern leopard frog tadpoles. Both species were exposed to a range of chemical concentrations of under different simulated solar irradiance intensities. Tests were designed to evaluate any effects that the colorant and/or the corrosion inhibitor, YPS, might have on survival of each species. Therefore, toxicity tests were conducted using formulations of fire-fighting chemicals with and without the colorant as well as with and without YPS (where such additives were normally used in the applied formulation).

Test Organisms

The rainbow trout used in the studies were obtained from national hatcheries and cultured at the Columbia Environmental Research Center (CERC), Columbia, MO. The juvenile trout were tested at approximately 30-60 days after yolk sac absorption.

Southern leopard frog tadpoles were obtained from Charles Sullivan Company, Inc., Nashville, TN. The tadpoles (Gosner stage 25; Gosner, 1960) were shipped to CERC via overnight courier in plastic bags on ice. Upon receipt, the tadpoles were removed from the shipping cooler and allowed to warm to 18 °C. The tadpoles were held in well water (pH 7.0, hardness 283 mg/l $CaCO_3$) in 37.85 L aquariums until they were tested. The tests were started with Gosner stages 25-39 tadpoles.

Chemicals, Receipt and Handling

All fire retardant chemicals were shipped to CERC from the U.S. Forest Service Rocky Mountain Research Station (Missoula, Montana), via overnight courier in sealed 18.93-liter plastic containers. Upon receipt, the shipping container was inspected for damage and the security seals were inspected for evidence of tampering. The chemicals were stored in their shipping containers at room temperature according to manufacturer recommendations in a secured laboratory at CERC. Various fire retardant chemical formulations were tested. Formulations included field use formulations with and without YPS, some colorless formulations with and without YPS, and YPS alone (Table 1). The addition of a coloring agent to the formulation helps pilots and ground fire fighters see the aerial applications. The term "colorless" indicates that there was no coloring agent added to the formulation.

Light Exposures and Test Conditions

Irradiance treatments applied during the toxicity tests were representative of the quality and intensity of natural sunlight measured in a variety of habitats in the western U.S. (Table 2). The test organisms were exposed to each chemical treatment in combination with three treatments of light or absence of light, including 0 $\mu W/cm^2$ (dark control), 0.002 $\mu W/cm^2$ (light control), and 4.0 $\mu W/cm^2$ (UV). The light treatments were achieved using various filters covering the testing vessels (Table 2). The test temperatures for rainbow trout exposures ranged from 7 to 12 °C and for tadpoles was 17 °C.

Exposures were conducted in a solar simulator (Little and Fabacher, 1996) with dimensions of approximately 1 m X 2 m long. The simulator was suspended over a water bath of similar dimensions (approximately 1 X 2 meter) and was enclosed with a highly UV-reflective specular aluminum (National Institute for Standards and Technology). The simulator was equipped with cool white, UVB fluorescent lamps, UVA fluorescent lamps, and halogen flood lamps. The cool white, halogen, and UVA fluorescent lamps were controlled by a timer to operate for 16 hours daily. The UVB lamps were activated with a second timer to operate for 5 hours per day. The UVB photoperiod started five hours after the onset of the white light and UVA photoperiod. The simulator was checked daily for lamp function, waterbath temperature, and photoperiod cycles. Temperatures used for rainbow trout test were within 3 ^{o}C of temperatures used in culture and ranged from 7 to 12 ^{o} C. Temperature and mortality were recorded daily, and pH and dissolved oxygen were measured in the control, low, medium, and high concentrations during the tests.

The light intensity and spectra applied during the laboratory studies were generally below those measured in a variety of natural aquatic habitats. UVB measured at a water depth of 10 cm in Glacier National Park ranged from 26.2 to 47.5 ΦW/cm^2 (Figure 1) compared to the 4 ΦW/cm^2 UVB applied in the present study. This irradiance fell well within the range of 0.42 to 72 ΦW/cm^2 UVB measured at 10 cm, or 11 to 155 ΦW/cm^2 UVB measured at subsurface depths in montane wetland habitats of the San Juan and Roosevelt National Forests in central Colorado in July, and was also less than the UVB measured at depths of 10 cm in estuarine habitats of central California (Barron et al., In press).

ACUTE TOXICITY TESTS

Through out this report, toxicity will be referred to as a LC50 value, or concentration lethal to 50% of the test organisms during 96-hour exposures to the test substance. In comparing LC50 values it is important to remember that the lower the LC50 value, the more toxic the substance. Thus a substance with an LC50 of 1 mg/L would be 100 times more toxic than a substance with an LC50 value of 100 mg/L.

The toxicity testing procedures were conducted in basic accordance with American Society for Testing and Materials Guideline E729, "Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians" (1998). Range-finding tests were conducted with individual fire chemicals formulations and UV alone and in combination with each other to select chemical concentrations and UV irradiance levels during 96-hour static acute toxicity tests. The test organisms were exposed to duplicate treatments of a well water control (pH 7.0, hardness 283 mg/L $CaCO_3$) and well water dilutions of the chemicals two orders of magnitude above and below the LC50 values reported by Gaikowski et al (1996).

During definitive toxicity tests rainbow trout and leopard frog tadpoles were exposed in 96-hour static acute toxicity tests to five dilutions of fire retardant chemical and a well water (pH 7.0, hardness 286 mg/l $CaCO_3$, alkalinity 258 mg/l $CaCO_3$) control treatment. Exposure to each treatment and control was performed under three different UV light treatments (0, 0.002, and 4 $\mu W/cm^2$). Two replicates of each chemical dilution/light treatment were tested.

Ten rainbow trout were exposed in 4-L glass beakers containing 3500 ml of the chemical solution and ten tadpoles were exposed in 600-ml glass beakers containing 500

ml of the chemical solution. Well water was used to prepare a stock solution of each chemical. Prior to placing test organisms in the exposure beakers appropriate volumes of the stock solution were then pipetted into the test vessel to obtain the desired exposure concentrations. Solutions were then mixed thoroughly with a glass stir rod. Test beakers containing the organisms were then randomly positioned in a temperature-controlled waterbath under the solar simulator. Test vessels were loosely covered with the appropriate light filters to obtain the desired light treatments as stated above.

Chemical Analysis

Ammonia was measured as total ammonia at 24 and 96 hours in the control, low, medium, and high concentrations to document changes over the duration of the exposure in tests with formulations containing colorant, with and without YPS, and YPS alone. Un-ionized ammonia concentrations in each treatment were calculated using the ammonia equilibrium equation described by Emerson et al. (1975). Water samples for free cyanide analyses were taken at 24 hours in the high concentration under each light treatment when mortality among test organisms was greatest. The samples were placed in 250 ml poly bottles pre-preserved with NaOH, and shipped at 4 ^{0}C via overnight courier to the analytical laboratory (Severn Trent Laboratories, Arvada, Colorado). The samples were analyzed for weak acid dissociable cyanide or free cyanide (ASTM, 1987).

Statistical Analysis

Standard ANOVA analyses were conducted on mortality data to determine if toxicity resulted from the interaction of YPS and UV light treatment. Probit Analysis was used to calculate LC50 values and 95% confidence intervals for each chemical based

on nominal concentrations. The criterion of non-overlapping 95% confidence intervals was used to determine significant differences (p[0.05) between LC50 values (APHA, 1989). Total ammonia concentrations were used in regression analysis to estimate the total ammonia concentration at the LC50 for each fire retardant chemical under the three light treatments.

RESULTS

Exposure temperatures, dissolved oxygen and pH remained within acceptable ranges throughout the study for rainbow trout (Table 3) and the Southern leopard frog (Table 4). Detailed information about total and un-ionized ammonia are reported in Table 5 for rainbow trout studies, and Table 6 for Southern frog studies. Detailed LC50 data for various products under different lighting conditions are reported in Table 7 for rainbow trout and Table 8 for the Southern leopard frog. Cyanide measures observed during this project are reported in Table 9.

Fire-Trol GTS-R Series

GTS-R was tested both as a colorless and a colored formulation (Table 1) with and without YPS to determine if the presence of YPS exhibited photoenhanced toxicity. ANOVA conducted on the mortality data revealed a highly significant interaction of the colorless and colored GTS-R formulations with UV light treatment (p=0.0001). Free cyanide was not detected in either the colored or colorless GTS-R formulations without YPS after 24 hour, whereas in tests of formulations with YPS the free cyanide concentration of the uncolored GTS-R formulation ranged from 18 µg/L in the dark

control to 66 µg/L under UV conditions (Table 9). Free cyanide concentrations of the colored GTS-R formulated with YPS ranged from not detectable under the dark control condition to 22 µg/L under the UV light treatment.

Rainbow Trout

The 96-hour LC50 for rainbow trout exposed to colorless GTS-R without YPS under the UV light treatment was 58.36 mg/L, compared to an estimated LC50 (due to no partial kills) between 10-20 mg/L for those fish exposed to colorless GTS-R with YPS (Figure 3). Thus, toxicity to rainbow trout increased 2.9-5.8 times in the presence of UV.

The 96-hour LC50 for rainbow trout exposed to colored GTS-R without YPS under the UV treatment was 46.91 mg/L, compared to 6.46 mg/L for fish exposed to colored GTS-R with YPS (Figure 4). Thus, toxicity of colored GTS-R with YPS to rainbow trout increased 7.3 times in the presence of UV compared to toxicity of the formulation without YPS. The presence of color had no significant effect on toxicity.

Total ammonia concentrations of colored and colorless formulations with YPS ranged from 1.61-7.71 mg/L. Total ammonia concentrations for colored and uncolored formulations without YPS ranged from 7.48-43.77 mg/L. The higher concentrations of ammonia in tests with GTS-R without YPS were probably related to higher exposure concentrations that were necessary to induce toxicity. Un-ionized ammonia concentrations ranged from 0.03-0.05 mg/L in tests with GTS-R with YPS in formulation, and ranged from 0.08-0.14 mg/L in tests with GTS-R without YPS in formulation. The un-ionized ammonia concentrations observed in tests without YPS in

formulation were well within the range of concentrations that are acutely toxic (0.08-1.1 mg/L) to rainbow trout (Russo, 1985). Thus, mortality of rainbow trout exposed to colored GTS-R without YPS suggests that un-ionized ammonia was the toxic component in this formulation.

Southern Leopard Frog Tadpoles

The 96-hour LC50 for tadpoles exposed to colorless GTS-R without YPS under the UV light treatment was 38.15 mg/L, compared to the LC50 of 30.34 mg/L for tadpoles exposed to colorless GTS-R with YPS (Figure 5). The toxicity of the formulation with YPS to the tadpoles increased 1.3 times in the presence of UV compared to the formulation without YPS.

The 96-hour LC50 for tadpoles exposed to colored GTS-R formulation without YPS under the UV light treatment was 159.37 mg/L compared to 21.90 mg/L for those tadpoles exposed to the colored GTS-R formulation with YPS (Figure 6). This was a 7.3 fold increase in toxicity in the presence of UV.

Total ammonia concentrations from formulations with YPS ranged from 4.51-9.03 mg/L. Total ammonia concentrations for the formulation without YPS ranged from 6.14-25.48 mg/ L. The higher concentrations of ammonia in GTS-R exposures without YPS reflect the higher exposure concentrations that were needed to induce toxicity. Un-ionized ammonia concentrations ranged from 0.06-0.11 mg/L in tests with GTS-R with YPS in formulation, and ranged from 0.37-0.96 mg/L in tests with GTS-R without YPS in formulation. The concentrations observed in tests without YPS in formulation were well within the range of concentrations that are acutely toxic (0.28-0.88 mg/L) to amphibians (Schuytema and Nebeker, 1999). Thus, mortality of tadpoles exposed to

colorless GTS-R without YPS suggests that un-ionized ammonia was the toxic component in this formulation.

Fire-Trol LCA-R Series

LCA-R was tested both as a colorless and colored formulation (Table 1) with and without YPS to determine if the presence of YPS exhibited photoenhanced toxicity. ANOVA conducted on the mortality data revealed a highly significant interaction ($p=0.0003$) between LCA-R formulations and UV light treatment when YPS was present.

Rainbow Trout

The 96-hour LC50s for rainbow trout exposed to LCA-R without YPS under the 4 $\mu W/cm^2$ UV light treatment was 233.45 mg/L (Figure 7) for the colorless formulation and 251.06 mg/L (Figure 8) for the colored formulation. These LC50s were significantly higher than the LC50s of 3.58 mg/L (Figure 7) and 3.19 mg/L (Figure 8), respectively for the colorless and colored LCA-R formulations with YPS. A comparison of the LC50 values across light treatments shows that toxicity of the colorless LCA-R formulations with YPS significantly increased as UV irradiance increased. For example, the 96-hour LC50 for rainbow trout exposed to colored LCA-R without YPS under the light control treatment was 276.05 mg/L compared to an LC50 of 17.38 mg/L for colored LCA-R with YPS (Figure 8).

LC50 values did not differ significantly across light treatments for the colorless LCA-R formulation without YPS, however toxicity of LCA-R without YPS to rainbow trout also increased significantly under the UV light treatment (LC50=251.06 mg/L) compared to the dark control treatment (LC50=436.02 mg/L). These results indicate that

the colored LCA-R formulation may contain ingredients other than YPS that are photoactive.

Total ammonia concentrations of colorless LCA-R with YPS ranged from 0.45 to >0.91 mg/L (Table 5). In the absence of YPS, total ammonia ranged from 16.51-18.87 mg/L. Un-ionized ammonia concentrations were below detection limits (0.008 mg/L) in tests with colorless LCA-R with YPS in formulation, and ranged from 0.37-0.43 mg/L in tests with colorless LCA-R without YPS in formulation. The higher concentrations of ammonia in tests with colorless LCA-R without YPS reflect higher exposure concentrations that were needed to induce mortality and are well within the range of concentrations that are acutely toxic (0.08-1.1 mg/L) to rainbow trout (Russo, 1985). Thus, un-ionized ammonia was likely the toxic component in colorless LCA-R without YPS.

In tests with the colored LCA-R formulation with YPS total ammonia concentrations ranged from 0.69-2.54 mg/L and un-ionized ammonia concentration was within sublethal range of 0.03-0.05 mg/L for rainbow trout, (Table 6) (Thurston and Russo, 1983). In the absence of YPS, the ammonia of the colored LCA-R formulation ranged from 27.29-45.12 mg/L as total ammonia and 0.11-0.12 mg/L as un-ionized ammonia concentrations, which is within the range of toxicity for rainbow trout (Thurston and Russo, 1983). Thus, mortality of rainbow trout exposed to colored LCA-R without YPS was likely caused by un-ionized ammonia.

After 24 hours no free cyanide was detected in either the colorless or colored LCA-R formulations in the absence of YPS. In contrast, free cyanide concentrations in the colorless formulation with YPS ranged from 7 µg/L in the dark control to 10 µg/L in

the UV treatment. Free cyanide in the formulation with YPS ranged from 36 µg/L in the dark control treatment to 370 µg/L in the UV treatment.

Southern Leopard frog tadpoles

The 96-hour LC50 for Southern leopard frog tadpoles exposed to colorless LCA-R without YPS in the presence of the 4µW/cm^2 UV light treatment was 169.12 mg/L compared to 30.42 mg/L for tadpoles exposed to the colorless LCA-R formulation with YPS (Figure 9). Thus, the LC50 values show a 5.5 fold increase in toxicity of colorless LCA-R with YPS to Southern leopard frog tadpoles in the presence of UV compared to the formulation without YPS.

The 96-hour LC50 for tadpoles exposed to the colorless LCA-R formulation without YPS under the dark control light treatment was 223.8 mg/L compared to 169.1 mg/L among tadpoles exposed in the presence of the 0.002 and 4µW/cm^2 UV light treatments (Figure 9). The LC50 values were the same for both the 0.002 and 4µW/cm^2 UV light treatments due to the same amount of mortality and no partial kills in the intermediate concentrations of chemical. Although the difference in magnitude of response is less than two fold, these results represent a significant increase in toxicity and imply that the colorless LCA-R formulation elicits photoenhanced toxicity to tadpoles even in the absence of the ferrocyanide component. Photoenhanced toxicity occurred for tadpoles exposed to the colorless LCA-R formulation containing YPS as indicated by the increase in toxicity as the UV irradiance increased.

The 96-hour LC50 for Southern leopard frog tadpoles exposed to colored LCA-R without YPS under the UV light treatment was 202.04 mg/L. The LC50 for tadpoles

exposed to colored LCA-R with YPS was 24.50 mg/L, thus, toxicity of the LCA-R formulation containing YPS to the tadpoles was 8.2 times greater than the formulation with no YPS in the presence of UV (Figure 10).

Comparison of the LC50s across light treatment reveals that toxicity of the colored LCA-R formulation with YPS is photoenhanced. No significant differences in the LC50 values were observed for tadpoles exposed to the colored LCA-R formulation without YPS across all light treatments. However, the LC50 for the colored LCA-R formulation with YPS was significantly lower in the presence of 4 $\mu W/cm^2$ UV compared to the dark and light control treatments.

Total ammonia concentrations in LCA-R colored and colorless formulations with YPS ranged from 2.70-32.25 mg/L (Table 6). Total ammonia concentrations for the LCA-R formulations without YPS ranged from 14.01-32.59 mg/L. The higher concentrations of ammonia in tests with colorless or colored LCA-R without YPS were probably related to higher exposure concentrations required to induce mortality. Un-ionized ammonia concentrations ranged from 0.09-0.11 mg/L in tests with colorless LCA-R with YPS in formulation, and ranged from 0.53-0.61 mg/L in tests with colorless LCA-R without YPS in formulation. Thus, mortality of tadpoles exposed to colorless LCA-R without YPS was likely induced by un-ionized ammonia. In tests with the colored LCA-R formulation, un-ionized ammonia concentrations ranged from 0.01-0.30 mg/L with YPS in formulation, and from 0.20-0.24 mg/L without YPS in formulation, which is well below the range of concentrations that are acutely toxic (0.28-0.88 mg/L) to amphibians (Schuytema and Nebeker, 1999).

Boreal toad tadpoles

Boreal toad (*Bufo boreas*) tadpoles were tested once using the LCA-R colorless formulation to determine the relative sensitivity of this endangered. The 96-hour LC50 for tadpoles exposed to the colorless LCA-R formulation with YPS under the dark control light treatment was 177.62 mg/L compared to 12.74 mg/L among tadpoles exposed in the presence of the 4 $\mu W/cm^2$ UV light treatment. Photoenhanced toxicity occurred for tadpoles exposed to the colorless LCA-R formulation containing YPS as evidenced by an increase in toxicity as the UV irradiance increased.

Ammonia was not measured during this exposure, however, preliminary chemical analyses after 96 hours of exposure revealed a free cyanide concentration of 210 µg/L in the highest LCA-R treatment under the 4 $\mu W/cm^2$ UV light, a concentration well above the threshold of tolerance for fish and amphibians (Eisler, 1991).

Fire-Trol 300-F Series

Rainbow trout and Southern leopard frog tadpoles were exposed to formulations of 300-F (Table 1) with and without YPS under the three light treatments. ANOVA conducted on the mortality data revealed a highly significant interaction of the 300-F formulation containing YPS and UVB light treatment ($p=0.001$). After 24 hours, there was no detectable concentration of free cyanide in formulations without YPS, whereas free cyanide ranged from 10 µg/L in the dark control to 37 µg/L in the UV treatment when YPS was included in the Fire-Trol 300-F formulation.

Rainbow Trout

The 96-hour LC50s for rainbow trout exposed to 300-F with and without YPS were 160.87 mg/L and 12.45 mg/L respectively, under the UV light treatment (Figure 11). Thus, toxicity to rainbow trout of the 300-F formulation containing YPS was 13 times greater in the presence of UVB compared to the formulation with no YPS.

Total ammonia concentrations at the 96-hour LC50s were relatively low in test solutions with the 300-F formulation containing YPS and ranged from 3.72-14.84 mg/L (Table 5). Un-ionized ammonia concentrations in test solutions from exposures with 300-F with YPS ranged from <0.01-0.09 mg/L and were well below the LC50 values for rainbow trout (Thurston and Russo, 1983). The ammonia concentrations were much higher in the test solutions of 300-F formulation without YPS than with YPS. Total ammonia concentrations at the 96-hour LC50s for each light treatment ranged form 29.70-33.23 mg/L. The un-ionized ammonia concentrations ranged from <0.01-0.27 mg/L, which encompassed the range of tolerance for rainbow trout (Thurston and Russo, 1983). Thus, mortality of rainbow trout exposed to 300-F without YPS suggests that un-ionized ammonia was the toxic component in this formulation.

Southern Leopard frog tadpoles

The 96-hour LC50s for Southern Leopard frog tadpoles exposed to 300-F with and without YPS were 109.49 mg/L and 24.10 mg/l respectively, under the UV light treatment (Figure 12). Thus, toxicity to tadpoles of 300-F containing YPS increased 4.5 times in the presence of UV compared to the formulation without YPS. Comparison of LC50s across light treatment, indicate, that toxicity of the 300-F formulation with YPS increases with UV irradiance.

Ammonia concentrations were not measured in test solutions for the tadpole exposure with 300-F formulation containing YPS due to equipment malfunction. The total ammonia concentrations were relatively low in test solutions with 300-F formulation without YPS and ranged from 2.50-9.06 mg/L (Table 6). The un-ionized ammonia concentrations across all light treatments (0.03-0.05 mg/L) were well below the tolerance limits for amphibians (Schuytema and Nebeker, 1999).

Fire-Trol LCA-F Series

Rainbow trout and Southern leopard frog tadpoles were exposed to a colored formulation of LCA-F with and without YPS, under the three light treatments. ANOVA conducted on the mortality data revealed a highly significant interaction of fire retardant chemical containing YPS and UV light treatment ($p<0.05$). After 24 hours no free cyanide was detected under any lighting condition when YPS was excluded from the formulation, whereas in the UV treatment free cyanide concentrations of 270 µg/L were measured in the LCA-F product with YPS.

Rainbow Trout

The 96-hour LC50 for rainbow trout exposed to colored LCA-F without YPS under the UV light treatment was 240.86 mg/L compared to 3.05 mg/L LCA-F with YPS (Figure 13). The 96-hour LC50 for rainbow trout exposed to LCA-F with YPS under the light control treatment was 14.19 mg/L and 34.32 mg/L for the dark control treatment (Figure 13). Under all light treatments, the LC50s show that toxicity of LCA-F with YPS to rainbow trout significantly increases by orders of magnitude in the presence of UV.

The toxicity of LCA-F formulation to rainbow trout was similar to that of the liquid concentrate LCA-R described above.

Total ammonia concentrations at the 96-hour LC50s in test solutions with LCA-F formulation containing YPS and ranged from 1.25-3.82 mg/L (Table 5). Un-ionized ammonia concentrations in test solutions from exposures with LCA-F with YPS ranged from 0.02-0.04 mg/L. The un-ionized ammonia concentrations were well below the LC50 values for rainbow trout (Thurston and Russo, 1983). In contrast total ammonia concentrations ranged from 16.21-34.64 mg/L were much higher in the test solutions with LCA-F formulation without YPS. The un-ionized ammonia concentrations ranged from 0.05-0.06 mg/L, which were well below the range of concentrations that are acutely toxic (0.08-1.1 mg/L) to rainbow trout (Russo, 1985).

Southern Leopard frog tadpoles

The 96-hour LC50 for Southern leopard frog tadpoles exposed to LCA-F without YPS under the UV light treatment was 177.29 mg/L (Figure 14). The LC50 for tadpoles exposed to LCA-F with YPS was 29.41 mg/L (Figure 14) thus toxicity of the LCA-F formulation containing YPS to the tadpoles was 6 times greater to tadpoles than the formulation with no YPS in the presence of UV.

Comparison of the LC50s across light treatment reveals that toxicity of the colored LCA-F formulation with YPS is photoenhanced. However, the LC50 for the LCA-R formulation with YPS was significantly lower in the presence of 4 $\mu W/cm^2$ UV compared to the dark and light control treatments. No significant differences in the LC50 values were observed for tadpoles exposed to the LCA-F formulation without YPS across all light treatments.

Total ammonia concentrations were lower in the tests with YPS in the formulation and ranged from 3.98- >6.72 mg/L (Table 6). Total ammonia concentrations for the formulation without YPS ranged from 22.79-30.20 mg/ L. The higher concentrations of ammonia in tests with LCA-F without YPS were probably related to higher exposure concentrations that were used to induce toxicity. Un-ionized ammonia concentrations were <0.01 mg/L in tests with LCA-F with YPS in formulation, and ranged from 0.31-0.34 mg/L in tests with LCA-F without YPS in formulation (Table 6). The un-ionized ammonia concentrations observed in tests without YPS in formulation were within the range of concentrations that are acutely toxic (0.28-0.88 mg/L) to amphibians (Schuytema and Nebeker, 1999). Thus, mortality of tadpoles exposed to LCA-F without YPS suggests that un-ionized ammonia was the toxic component in this formulation.

Phos-Chek Series

Rainbow trout and Southern leopard frog tadpoles were exposed to two Phos-Chek formulations, D75-R and D75-F (Table 1), under the three light treatments. Neither formulation contains YPS as part of the corrosion inhibitor. No free cyanide was detected for either Phos-Chek D75-R or Phos-Chek D75-F under any lighting condition. ANOVA conducted on the mortality data revealed no significant interaction of fire retardant chemical with UV light treatment ($p>0.05$), except for rainbow trout exposed to D75-F ($p<0.05$).

Rainbow Trout

The 96-hour LC50 for rainbow trout was 168.21 mg/L across all light treatments for the D75-R formulation, indicating there was no photoenhanced toxicity (Figure 15).

For the D75-F formulation, the 96-hour LC50 under the control and UV light treatment were 495.25 mg/L and 227.48 mg/L, respectively (Figure 15). Photoenhanced toxicity was evident but minimal. A doubling of the LC50 is very significant, however since both toxicities are relatively low, the environmental impact is presumed to be low.

Total ammonia and un-ionized ammonia concentrations were at lethal concentrations for rainbow trout. Total ammonia concentrations at the 96-hour LC50 for D75-R ranged from 26.96-31.50 mg/L. Un-ionized ammonia concentrations for D75-R ranged from 0.11-0.14 mg/L, which fell within an acutely range of concentrations that are acutely toxic (0.08-1.1 mg/L) for rainbow trout (Russo, 1985).

Total ammonia concentrations at the 96-hour LC50 for D75-F ranged form 20.62-35.49 mg/L (Table 5). Un-ionized ammonia concentrations for D75-F ranged form 0.39-0.53 mg/L. Thurston and Russo (1983) reported a 96-hour un-ionized ammonia LC50 for rainbow trout in the range of 0.23-0.77 mg/L, so mortality was probably a result of ammonia toxicity.

Southern Leopard frog tadpoles

The 96-hour LC50 for tadpoles were very similar across light treatments ranging from 154.99-189.26 mg/L for the D75-R formulation and from 268.62-292.59 mg/L for the D75-F formulation (Figure 16). The LC50s did not significantly differ with light treatment therefore there was no indication of photoenhanced toxicity.

Total ammonia concentrations at the 96-hour LC50 for D75-R ranged from 22.64-33.16 mg/L (Table 6b). Un-ionized ammonia concentrations for D75-R ranged from 0.42-0.49 mg/L, which are within the range of concentrations that are acutely toxic (0.28-0.88 mg/L) to amphibians (Schuytema and Nebeker, 1999).

Total ammonia concentrations at the 96-hour LC50 for D75-F ranged from 24.97-37.85 mg/L Un-ionized ammonia concentrations for D75-F ranged from 0.27-0.38 mg/L which is within the range of concentrations that are acutely toxic (0.28-0.88 mg/L) to amphibians (Schuytema and Nebeker, 1999). Thus, mortality of tadpoles exposed to both D75-R and D75-F Phos-Chek formulations was likely caused by un-ionized ammonia.

Sodium ferrocyanide

Rainbow trout and Southern Leopard frog tadpoles were exposed to technical grade sodium ferrocyanide or yellow prussiate of soda (YPS). YPS is commonly used as a corrosion inhibitor in many fire retardant chemicals. ANOVA conducted on the mortality data for both rainbow trout and Southern leopard frog tadpoles revealed a highly significant ($p<0.05$) interaction of YPS and UV light treatment. After 24 hours, the free cyanide concentration ranged from 35 µg/L in the dark control to 270 µg/L under the UV light treatment.

Rainbow trout

The 96-hour LC50s for rainbow trout exposed to YPS under the dark control was 2.42 mg/L, under the light control was 0.977 mg/L, and under the UV light treatment was 0.168 mg/L (Figure 17). The toxicity of YPS increased with increasing light treatment. Comparing LC50s from the dark control and UV light treatment and was 14-fold greater in the presence of UV light.

Total ammonia and un-ionized ammonia concentrations (Table 5) were well below the toxicity range for rainbow trout (Thurston and Russo, 1983).

Southern Leopard frog tadpoles

The toxicity of YPS increased with increasing light treatment. The 96-hour LC50s for the tadpoles exposed to YPS was 99.27 mg/L under the dark control, 62.84 mg/L under the light control, and 2.63 mg/L under the UV light treatment (Figure 18). Thus toxicity of YPS increased 38-fold in the presence of UV light.

Total ammonia and un-ionized ammonia concentrations (Table 6) were well below the toxicity range for amphibians (Schuytema and Nebeker, 1999).

DISCUSSION

The toxicity of all fire retardant chemicals containing the corrosion inhibitor, YPS, significantly increased when exposed to UV. Rainbow trout were more sensitive than the Southern leopard frog tadpoles, however both species were adversely affected by relatively low concentrations of YPS in the presence of the simulated solar UV light treatment.

It appears likely that cyanide was responsible for the photoenhanced toxicity. Early studies indicate that free cyanide, the most toxic form of cyanide, is lethal to rainbow trout in low µg/L concentrations (40-75 µg/L) (Eisler, 1991). The free cyanide concentration range (10-370 µg/L) over all light treatments (Table 9), measured in the present study often exceeded these reported values. Free cyanide was not detected in formulations without YPS (Table 9). The chemical analyses indicated that the irradiance condition influenced free cyanide concentrations. Free cyanide ranged from below detections limits up to 36 µg/L under dark and light control conditions but increased up to

370 µg/L under UV irradiance conditions. The chemical analyses also indicate that the highest concentration of free cyanide was measured within 24 hours of exposure, and corresponded to mortality that occurred within the first 24 hours of exposure. In preliminary tests, after 96 hours of irradiance exposure relatively high concentrations of free cyanide (19-120 µg/L) were present in the formulations containing YPS that would be toxic to rainbow trout (Eisler, 1991).

Toxicity of all fire retardant chemicals without YPS in the formulation was consistent with previous studies conducted by Buhl and Hamilton (In Press) under UV-limited conditions. Under these test conditions, un-ionized ammonia was likely the major source of toxicity in formulations without YPS.

Photoenhanced toxicity of contaminants can occur through photoactivation or photosensitization. In photoactivation a substance is modified as a result of the energy absorbed by the parent compound that can result in a photoproduct that is more toxic than the parent compound (Ren et al. 1994, Zepp and Schlotzhauer 1979). Whereas, photosensitization occurs when the chemical (often tissue-bound) passes absorbed energy on to endogenous chemicals forming reactive species such as free radicals that cause cellular injury (Landrum et al. 1987, Newsted and Giesy 1987). The toxicity we observed was consistent with a photoactivation mode of action because organisms exposed to YPS-containing formulations receiving UV irradiance prior to exposure were more toxic than non-irradiated solutions.

A number of factors will influence photoenhanced toxicity in natural habitats. Solar angle associated with time of day, season, air pollution, clouds, and surface reflection will influence UV irradiance levels (Little and Fabacher 1996). Water quality,

especially humic acid concentration will limit the amount of UV penetrating the water column (Skully and Lean 1994) and may also influence the availability of chemical substances to the organism by binding them (Oris et al. 1990).

RECOMMENDATIONS FOR FURTHER WORK

Persistence of Fire Retardant Chemicals in the Environment

A variety of chemicals are used to fight fires in the United States and Canada. Fish kills have been associated with the contamination of water by release of these products from overspray and runoff. Although the toxicity of these products is generally low in the natural environment these products are subjected to photolytic processes by natural sunlight that may alter their chemical characteristics and increase toxicity. Significant amounts of these materials are applied in montane wilderness areas, including habitats of the boreal toad (*Bufo boreas*) as a species listed as endangered by the State of Colorado. Preliminary studies indicate that the sensitivity of this endangered species to these products is similar to that of the Southern leopard frog and rainbow trout.

Our laboratory studies with fire retardant chemicals indicate a significant photoenhanced toxicity of products containing ferrocyanide corrosion inhibitors, with up to a 100-fold increase in the toxicity to rainbow trout and a 10-fold increase to southern leopard frogs in the presence of ultraviolet (UV) light. In contrast, compounds without the corrosion inhibitor were either unaffected in the presence of UV or photoenhanced to a lesser extent than those containing the corrosion inhibitor. Although mortality appeared to occur within the first 48 hours of exposure, free cyanide concentrations of up

to 120 µg/L were evident after 96 hours. Given the extent of toxicity of cyanide photoenhancement as a result of these products, further evaluation of the persistence of this toxicity is warranted. Tests are needed to determine how rapidly the chemical transformations occur in sunlight and to determine how long the toxicity persists over time to understand the probability of biological injury from the application of these substances. Such information would support resource management decisions about application regime relative to weather conditions that may increase photo-transformation or runoff. For example, there may be cause for concern about toxic runoff if toxicity remains high for long periods of time after field application. This information could also guide selection of alternative fire retardant formulations

Fish Avoidance

Aquatic habitats can be contaminated by misplaced drops, drift, and by runoff of fire retardant chemicals during application by aircraft. Although the resulting contamination has been shown to cause fish kills, the avoidance of affected areas by fish has also been observed. This apparent avoidance reaction may protect natural populations since by avoiding harmful concentrations the fish minimize fire retardant chemical exposure and subsequent injury. Formulations inducing such responses, in the short term, may be safer than chemicals that are not avoided. Thus, the risk of environmental injury from the use of these substances is based not only on their toxicity and environmental persistence, but also on the tendency of organisms to temporarily avoid exposure to them. It is unknown which of the components of fire retardant formulations (e.g. ammonia compound, coloring agents, and corrosion inhibitors) induce

this aversive reaction. If a non-toxic component of the formulation is found to be responsible for causing avoidance, then its concentration could be manipulated to increase the probability of avoidance in the field. Since solar photoactivation significantly increases the toxicity of formulations containing sodium ferrocyanide, UV may also be important in inducing the avoidance of these chemicals.

REFERENCES

APHA (American Public Health Association), American Water Works Association and Water Pollution Control Federation. (1989). *Standard Methods for the Examination of Water and Wastewater.* 17th Edition. APHA, Washington, D.C.

American Society for Testing and Materials. (1987). Research Rep. D2036:19-1131. American Society for Testing and Materials, Philadelphia, PA.

American Society for Testing and Materials. (1998). ASTM E729, Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. 11.05:218-238 American Society for Testing and Materials, West Conshohochen, PA.

Barron MG, Little EE, Hurtubise RD, Diamond S. (In Press). Quantifying solar actinic radiation in aquatic habitats for assessment of photoenhanced toxicity. *Environmental Toxicology and Chemistry.*

Burdick, G.E. and M. Lipschuetz. (1950). Toxicity of ferro- and ferricyanide solutions to fish and determination of the cause of mortality. *Transactions of the American Fisheries Society*. **78**:192.

Buhl, K.J. and S.J. Hamilton. (In Press). Acute toxicity of fire control chemicals, nitrogenous chemicals, and surfactants to rainbow trout (*Oncorhynchus mykiss*), *Transactions of the American Fisheries Society.*

Buhl, K.J., and S.J. Hamilton. (1998). Acute toxicity of fire-retardant and foam-suppressant chemicals to early life stages of Chinook salmon (*Oncorhynchus tshawytscha*). *Environmental Toxicology and Chemistry* **17(8):**1589-1599.

Calfee, R.D., E.E. Little, L.Cleveland, and M.G. Barron. (1999). Photoenhanced toxicity of a weathered oil on *Ceriodaphnia dubia* reproduction. *Environmental Science and Pollution Research* **6(4)**:207-212.

Cleveland, L., E.E. Little, R.D. Calfee, and M.G. Barron. (In Press). Photoenhanced toxicity of weathered oil to *Mysidopsis bahia*. *Aquatic Toxicology*.

Chemonics Industries, Inc. (1993a). Fire-Trol GTS-R material safety data sheet. Chemonics Industries, Inc., Phoenix, AZ.

Chemonics Industries, Inc. (1993c). Fire-Trol LCA-R material safety data sheet. Chemonics Industries, Inc., Phoenix, AZ.

Chemonics Industries, Inc. (1993d). Fire-Trol LCA-F material safety data sheet. Chemonics Industries, Inc., Phoenix, AZ.

Chemonics Industries, Inc. (1995). Fire-Trol 300-F material safety data sheet. Chemonics Industries, Inc., Phoenix, AZ.

Degussa Corporation, (1995). Sodium ferrocyanide material safety data sheet. Degussa Corporation, Ridgefield Park, NJ.

Emerson, K.E., R.C. Russo, R.E. Lund, and R.V. Thurston. (1975). Aqueous ammonia Equilibrium calculations: Effects of pH and temperature. *Journal of the Fisheries Research Board of Canada* **32**:2379-2383.

Eisler, R. (1991). Cyanide hazards to fish, wildlife, and invertebrates: A synoptic Review. U.S. Fish and Wildlife Service, Biological Report 85(1.23). 55pp.

Gaikowski, M.P., S.J. Hamilton, K.J. Buhl, S.F. McDonald, and C.H. Summers. (1996). Acute toxicity of three fire-retardant and two fire-suppressant foam formulations to the early life stages of rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry* **15(8):**1365-1374.

Gosner, K.L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetelogica* **16**:183-190.

Landrum P.F., Giesy J.P., Oris J.T., and Allred P.M. (1987). Photo-induced toxicity of polycyclic aromatic hydrocarbons to aquatic organisms. In J.H. Vandermeulen JH, Hrudey SE. Eds. *Oil In Freshwater: Chemistry, Biology, Countermeasure Technology*. Proceedings of a symposium of oil in freshwater, Edmonton Alberta, Canada. Pergamon Press, New York, NY, USA. pp. 314-328.

Little, E.E, and D. Fabacher. (1996). Exposure of freshwater fish to simulated solar UVB radiation. In G.K. Ostrander, ed., *Techniques in Aquatic Toxicology*. CRC Press, Boca Raton, FL, pp. 141-158.

Minshall, G.W. and J.T. Brock. (1991). Observed and anticipated effects of forest fire on Yellowstone stream ecosystems. In R.B. Keiter and M.S. Boyce, eds., *Greater Yellowstone Ecosystem:Redefining America's Wilderness Heritage.*

Yale University Press, New Haven, CT, pp. 123-135.

Newsted JL, Giesy JP,Jr. (1987). Predictive models for photoinduced acute toxicity of polycyclic aromatic hydrocarbons to *Daphnia magna*, Strauss (Cladocera, Crustacea). *Environmental Toxicology and Chemistry* **6**:445-461.

Oris JT, Hall AT, Tylka, JD. (1990). Humic acids reduce the photo-induced toxicity of anthracene to fish and daphnia. *Environmental Toxicology and Chemistry* **9**:575-583.

Oris, J.T. and J.P. Giesy Jr. (1985). Photoenhanced toxicity of anthracene to juvenile sunfish (*Lepomis spp.*). *Aquatic Toxicology* **6**:133-146.

Pelletier, M.C., et al. (1997). Phototoxicity of individual polycyclic aromatic hydrocarbons and petroleum to marine inverteberate larvae and juveniles. *Environmental Toxicology and Chemistry* **16(10)**:2190-2199.

Ren L, Huang XD, McConkey BJ, Dixon DG, Greenberg BM. (1994). Photoinduced toxicity of three polycyclic aromatic hydrocarbons (fluoranthene, pyrene, naphthalene) to duckweed *Lemna gibba L*. G-3. *Ecotoxicol. Environ. Saf*. **28**:160-177.

Schuytema, G.S. and A.V. Nebeker. (1999). Comparative toxicity of ammonium and nitrate compounds to Pacific treefrog and African clawed frog tadpoles. *Environmental Toxicology and Chemistry* **18(10)**:2251-2257.

Thurston, R.V. and R. Russo. (1983). Acute toxicity of ammonia to rainbow trout. *Transactions of the American Fisheries Society* **112**:696-704.

U.S. Environmental Protection Agency (USEPA) (1980). Ambient water quality criteria for cyanides. U.S. Environmental Protection Agency Report 440/5-80/037, pp.1-72.

Zaga, A., E.E. Little, C.F. Rabeni, and M.R. Ellersieck. (1998). Photoenhanced toxicity of a carbamate insecticide to early life stage anuran amphibians. *Environmental Toxicology and Chemistry* **17(12)**:2543-2553.

Zepp RG, Schlotzhauer PF. 1979. Photoreactivity of selected aromatic hydrocarbons in water. In Jones PW, Leber P. Eds. *Polynuclear Aromatic Hydrocarbons. Third International Symposium On Chemistry And Biology-- Carcinogenesis and Mutagenesis*. Ann Arbor Science Publishers, Inc. Ann Arbor, MI, USA. Pp 141-158.

List of Tables

List of Figures

Figure 17. 96-hour LC50 for rainbow trout exposed to sodium ferrocyanide.

Figure 18. 96-hour LC50 for Southern leopard frog tadpoles exposed to sodium ferrocyanide.

Table 1. Composition of fire retardant chemicals tested with juvenile rainbow trout and Southern Leopard frog tadpoles

Chemical	Formulation	Concentrations tested (mg/L)	Ingredients	Reference
Fire-Trol GTS-R	powder w/yps, colorless	1.25-50.0	Ammonium sulfate, diammonium phosphate, gum-thickener, preservative, corrosion inhibitor (yps)	(Chemonics, 1992)
	powder w/o yps, colorless	15.63-250.0	Ammonium sulfate, diammonium phosphate, gum-thickener, preservative	(Chemonics, 1992)
Fire-Trol LCA-R	Liquid concentrate w/yps, colorless	3.13-50.0	Ammonium polyphosphate, clay thickener, corrosion inhibitor (yps)	(Chemonics, 1992)
	Liquid concentrate w/o yps, colorless	62.5-1000.0	Ammonium polyphosphate, clay thickener	(Chemonics, 1992)

Table 1 Cont'd. Composition of fire retardant chemicals tested with juvenile rainbow trout and Southern Leopard frog tadpoles.

Chemical	Formulation	Concentrations tested (mg/L)	Ingredients	Reference
Fire-Trol GTS-R	powder w/yps, color	1.25-50.0	Ammonium sulfate, diammonium phosphate, gum-thickener, preservative, corrosion inhibitor (yps), colorant	(Chemonics, 1992)
	powder w/o yps, color	15.63-250.0	Ammonium sulfate, diammonium phosphate, gum-thickener, preservative, colorant	(Chemonics, 1992)
Fire-Trol 300-F	powder w/yps; color	3.13-50.0	Ammonium sulfate, diammonium phosphate, gum-thickener, corrosion inhibitor (yps), preservative, colorant	(Chemonics, 1995)
	powder w/o yps; color	15.6-1000.0	Ammonium sulfate, diammonium phosphate, gum-thickener, preservative, colorant	(Chemonics, 1995)

Table 1. Cont'd. Composition of fire retardant chemicals tested with juvenile rainbow trout and Southern Leopard frog tadpoles.

Chemical	Formulation	Concentrations tested (mg/L)	Ingredients	Reference
Fire-Trol LCA-R	Liquid concentrate w/yps, color	3.13-50.0	Ammonium polyphosphate, clay thickener, corrosion inhibitor (yps), colorant	(Chemonics, 1993)
	Liquid concentrate w/o yps, color	62.5-1000.0	Ammonium polyphosphate, clay thickener, colorant	(Chemonics, 1993)
Fire-Trol LCA-F	Liquid concentrate w/yps; color	3.13-50.0	Ammonium polyphosphate, clay thickener, corrosion inhibitor (yps), colorant	(Chemonics, 1993)
	Liquid concentrate w/o yps; color	62.5-1000.0	Ammonium polyphosphate, clay thickener, colorant	(Chemonics, 1993)

Table 1. Cont'd. Composition of fire retardant chemicals tested with juvenile rainbow trout and Southern Leopard frog tadpoles.

Chemical	Formulation	Concentrations tested (mg/L)	Ingredients	Reference
Phos-Chek D-75R	powder color	62.5-1000.0	Diammonium sufate, monoammonium phosphate, diammonium phosphate, guar gum, performance additives, colorant	(Solutia, 1998)
Phos-Chek D-75F	powder color	62.5-1000.0	Diammonium sufate, monoammonium phosphate, diammonium phosphate, guar gum, performance additives, colorant	(Solutia, 1998)
Sodium ferrocyanide (YPS)	Yellow powder	0.06-50.00	Sodium ferrocyanide	(Degussa, 1995)

Table 2. Nominal UV and visible irradiance provided by various filter treatments applied during exposures of rainbow trout and Southern leopard frog tadpoles to fire retardant chemicals compared to the intensity of natural solar radiation measured in a variety of Western habitats.

Light Treatment	Nominal Irradiance ($\mu W/cm^2$)			Filter Combinations
	UV-B	UV-A	Visible	
Dark Control	0	0	0	Side: Aluminum foil wrap Top: Cardboard layer
Light Control	0.002	3.0	260.0	Side: Polycarbonate (0.79 mm), mylar (0.13 mm) wrap Top: Two polycarbonate (0.79), shade cloth (50%) layer
UV	3.85	114.4	2785.3	Side: Mylar (0.13 mm) wrap Top: Mylar (0.13) layer
Glacier National Park	26.2-47.5	1104-1449	5591-15,304	Measurements taken in water at 10 cm depth at various sites within the Park.
Colorado (subsurface at various sites)	11-155	4450-6425	-----	Measurements taken in water at just below the surface at various sites within central Colorado .
Colorado (10 cm depth at various sites)	0.42-72	124-1785	-----	Measurements taken in water at 10 cm depth at various sites within central Colorado

Table 3. Water quality parameters measured during the rainbow trout exposures to 7 fire retardant chemicals.

Chemical and formulations tested	Water Quality Parameters		
	Temperature (°C)	Dissolved Oxygen (mg/L)	pH
Fire-Trol GTS-R			
Powder w/yps; colorless	9.5-10	8.8-9.4	7.5-7.7
Powder w/o yps; colorless	10	7.6-8.5	7.7-7.9
Powder w/yps; color	10	8.0-9.1	8.1-8.3
Powder w/o yps; color	10	7.3-8.4	8.2-8.4
Fire-Trol 300-F			
Powder w/yps; color	11	6.8-7.3	7.9-8.2
Powder w/o yps; color	11.2-12.0	6.5-8.0	8.1-8.2
Fire-Trol LCA-R			
Liquid w/yps; colorless	8-10	7.7-9.1	8.0-8.3
Liquid w/o yps; colorless	10	7.7-8.4	7.7-8.1
Liquid w/yps; color	7.2-8.4	9.5-11.1	8.1-8.3
Liquid w/o yps; color	9.6-10	7.3-8.6	7.4-7.5
Fire-Trol LCA-F			
Liquid w/yps; color	7.2-7.5	7.3-9.0	7.9-8.2
Liquid w/o yps; color	10	7.6-8.4	7.1-7.7
Phos-Chek D75-R			
Powder w/o yps; color	10	7.1-7.9	7.2-7.9
Phos-Chek D75-F			
Powder w/o yps; color	10	7.1-7.8	7.9-8.0
Sodium ferrocyanide	7.6-8.0	7.4-9.7	8.1-8.2

Table 4. Water quality parameters measured during the Southern leopard frog tadpole exposures to 7 fire retardant chemicals.

Chemical and formulations tested	Water Quality Parameters		
	Temperature (°C)	Dissolved Oxygen (mg/L)	pH
Fire-Trol GTS-R			
Powder w/yps; colorless	17	7.3-7.6	8.2-8.4
Powder w/o yps; colorless	17	7.2-7.8	8.1-8.4
Powder w/yps; color	17	7.5-7.6	7.9-8.2
Powder w/o yps; color	17	7.1-7.7	7.8-8.0
Fire-Trol 300-F			
Powder w/yps; color	17	7.1-7.4	8.0-8.4
Powder w/o yps; color	17	6.7-7.5	6.5-7.4
Fire-Trol LCA-R			
Liquid w/yps; colorless	17	7.2-7.7	7.9-8.1
Liquid w/o yps; colorless	17	5.2-7.0	7.5-7.9
Liquid w/yps; color	17	7.2-7.6	7.9-8.1
Liquid w/o yps; color	17	8.2-8.7	7.9-8.4
Fire-Trol LCA-F			
Liquid w/yps; color	17	7.6-7.8	6.8-7.2
Liquid w/o yps; color	17	6.5-7.3	7.7-7.8
Phos-Chek D75-R			
Powder w/o yps; color	17	6.8-7.2	7.5-7.8
Phos-Chek D75-F			
Powder w/o yps; color	17	5.5-6.8	7.5-7.7
Sodium ferrocyanide	17	6.8-7.6	7.9-8.1

Table 5. Rainbow Trout– Total ammonia (TA) concentrations measured during exposure of rainbow trout to 7 fire retardant chemicals under three UV light treatments and the estimated range of unionized ammonia (UA) at the 96-hour LC50. Asterixes * indicate concentrations of unionized ammonia that are lethal to fish

Chemical and formulations tested	**Total ammonia concentrations (mg/L) at the LC50 and range of un-ionized ammonia concentrations (mg/L)**					
	Dark Control (0 μW/cm²)		**Light Control (0.002 μW/cm²)**		**UV (4.0 μW/cm²)**	
	TA	**UA**	**TA**	**UA**	**TA**	**UA**
Fire-Trol GTS-R						
Powder w/yps; colorless	>4.88	0-0.03	>5.13	0-0.05	2.49-4.55	0-0.04
Powder w/o yps; colorless	19.92	0-0.16*	24.99	0-0.17*	7.48	0-0.08*
Powder w/yps; color	7.68	0-0.03	7.71	0-0.03	1.61	0-0.03
Powder w/o yps; color	11.92	0-0.12*	10.87	0-0.14*	43.77	0-0.14*
Fire-Trol 300-F						
Powder w/yps; color	14.84	0-0.07	9.55	0-0.07	3.72	0-0.09
Powder w/o yps; color	33.23	0-0.24*	30.74	0-0.20*	29.70	0-0.27*
Fire-Trol LCA-R						
Liquid w/yps; colorless	>0.91	<0.006	>0.84	<0.008	0.45	<0.004
Liquid w/o yps; colorless	18.87	0-0.37*	17.25	0-0.43*	16.51	0-0.41*
Liquid w/yps; color	2.54	0-0.04	2.18	0-0.05	0.69	0-0.03
Liquid w/o yps; color	45.12	0-0.11*	29.74	0-0.11*	27.29	0-0.12*
Fire-Trol LCA-F						
Liquid w/yps; color	3.82	0-0.03	2.02	0-0.02	1.25	0-0.04
Liquid w/o yps; color	34.64	0-0.05	23.18	0-0.05	16.21	0-0.06
Phos-Chek D75-R						
Powder w/o yps; color	31.21	0-0.14*	26.96	0-0.14*	31.50	0-0.11*
Phos-Chek D75-F						
Powder w/o yps; color	35.49	0-0.39*	33.78	0-0.51*	20.62	0-0.53*
Sodium ferrocyanide	<0.1	0-0.01	1.41	0-0.01	0.21	0-0.01

Table 6. Southern Leopard frog tadpoles– Total ammonia (TA) concentrations measured during exposure of rainbow trout to 7 fire retardant chemicals under three UV light treatments and the estmated range of unionized ammonia (UA) at the 96-hour LC50. Asterixes * indicate concentrations of unionized ammonia that are lethal to fish

Chemical and formulations tested	**Total ammonia (TA) concentrations (mg/L) at the LC50 and range of un-ionized ammonia (UA) concentrations (mg/L)**					
	Dark Control (0 μW/cm²)		**Light Control (0.002 μW/cm²)**		**UV (4.0 μW/cm²)**	
	TA	**UA**	**TA**	**UA**	**TA**	**UA**
Fire-Trol GTS-R						
Powder w/yps; colorless	23.75	0-0.09	12.57	0-0.11	6.14	0-0.10
Powder w/o yps; colorless	9.03	0-0.37*	7.76	0-0.43*	5.74	0-0.41*
Powder w/yps; color	15.38	0-0.06	9.14	0-0.07	4.51	0-0.06
Powder w/o yps; color	22.95	0-0.81*	25.48	0-0.96*	20.78	0-0.81*
Fire-Trol 300-F						
Powder w/yps; color	9.06	0-0.04	2.50	0-0.03	4.78	0-0.05
Powder w/o yps; color	N/A	N/A	N/A	N/A	N/A	N/A
Fire-Trol LCA-R						
Liquid w/yps; colorless	32.25	0-0.09	9.91	0-0.10	3.33	0-0.11
Liquid w/o yps; colorless	23.15	0-0.53*	14.01	0-0.57*	16.07	0-0.61*
Liquid w/yps; color	21.17	0-0.03	15.62	0-0.03	2.70	0-0.01
Liquid w/o yps; color	32.59	0-0.22*	29.02	0-0.20*	24.21	0-0.24*
Fire-Trol LCA-F						
Liquid w/yps; color	>6.72	<0.01	6.59	<0.01	3.98	<0.01
Liquid w/o yps; color	30.20	0-0.32*	30.10	0-0.31*	22.79	0-0.34*
Phos-Chek D75-R						
Powder w/o yps; color	29.61	0-0.49*	33.16	0-0.43*	22.64	0-0.42*
Phos-Chek D75-F						
Powder w/o yps; color	24.97	0-0.27*	37.85	0-0.35*	31.43	0-0.38*
Sodium ferrocyanide	0.19	<0.01	0.51	<0.01	1.03	<0.01

Table 7. Rainbow trout - Acute toxicity (LC50 mg/L), measured during exposure to 7 fire retardant chemicals under three UV light treatments.

Chemical and formulations tested	96-h LC50 (mg/L of formulation)		
	Dark Control (0 μW/cm²)	**Light Control (0.002 μW/cm²)**	**UV (4.0 μW/cm²)**
Fire-Trol GTS-R			
Powder w/yps; colorless	>20	>20	10-20
Powder w/o yps; colorless	90	92	58
Powder w/yps; color	34	33	6
Powder w/o yps; color	64	54	47
Fire-Trol 300-F			
Powder w/yps; color	72	43	12
Powder w/o yps; color	166	166	161
Fire-Trol LCA-R			
Liquid w/yps; colorless	>10	>10	4
Liquid w/o yps; colorless	296	249	233
Liquid w/yps; color	21	17	3.19
Liquid w/o yps; color	436	276	251
Fire-Trol LCA-F			
Liquid w/yps; color	34	14	3
Liquid w/o yps; color	336	326	241
Phos-Chek D75-R			
Powder w/o yps; color	168	168	168
Phos-Chek D75-F			
Powder w/o yps; color	495	351	227
Sodium ferrocyanide	2	.98	.17

Table 8. Southern Leopard Frog - Acute toxicity (LC50 mg/L), measured during exposure of 7 fire retardant chemicals under three UV light treatments.

Chemical and formulations tested	96-h LC50 (mg/L of formulation)		
	Dark Control (0 μW/cm^2)	**Light Control (0.002 μW/cm^2)**	**UV (4.0 μW/cm^2)**
Fire-Trol GTS-R			
Powder w/yps; colorless	131	58	30
Powder w/o yps; colorless	61	47	38
Powder w/yps; color	78	40	22
Powder w/o yps; color	153	168	159
Fire-Trol 300-F			
Powder w/yps; color	55	33	24
Powder w/o yps; color	114	113	109
Fire-Trol LCA-R			
Liquid w/yps; colorless	370	105	30
Liquid w/o yps; colorless	224	169	169
Liquid w/yps; color	201	141	25
Liquid w/o yps; color	241	228	202
Fire-Trol LCA-F			
Liquid w/yps; color	>50	49	29
Liquid w/o yps; color	237	237	177
Phos-Chek D75-R			
Powder w/o yps; color	189	178	155
Phos-Chek D75-F			
Powder w/o yps; color	293	269	269
Sodium ferrocyanide	99	63	3

Table 9. Free cyanide concentrations measured in 7 fire retardant chemicals after 24 hours of exposure to three UV light treatments. Non-detection limit for free cyanide in water is 0.01 mg/L. ND = non-detectable-- detection limits for free cyanide in water is 0.01 mg/L.

	Free cyanide concentration at 24 hrs (µg/L)		
Chemical and formulations tested	**Dark Control (0 µW/cm^2)**	**Light Control (0.002 µW/cm^2)**	**UV (4.0 µW/cm^2)**
Fire-Trol GTS-R			
Powder w/yps; colorless	18	50	66
Powder w/o yps; colorless	ND	ND	ND
Powder w/yps; color	ND	21	22
Powder w/o yps; color	ND	ND	ND
Fire-Trol 300-F			
Powder w/yps; color	10	34	37
Powder w/o yps; color	ND	ND	ND
Fire-Trol LCA-R			
Liquid w/yps; colorless	7	46	100
Liquid w/o yps; colorless	ND	ND	ND
Liquid w/yps; color	36	160	370
Liquid w/o yps; color	ND	ND	ND
Fire-Trol LCA-F			
Liquid w/yps; color	ND	ND	270
Liquid w/o yps; color	ND	ND	ND
Phos-Chek D75-R			
Powder w/o yps; color	ND	ND	ND
Phos-Chek D75-F			
Powder w/o yps; color	ND	ND	ND
Sodium ferrocyanide	35	100	270

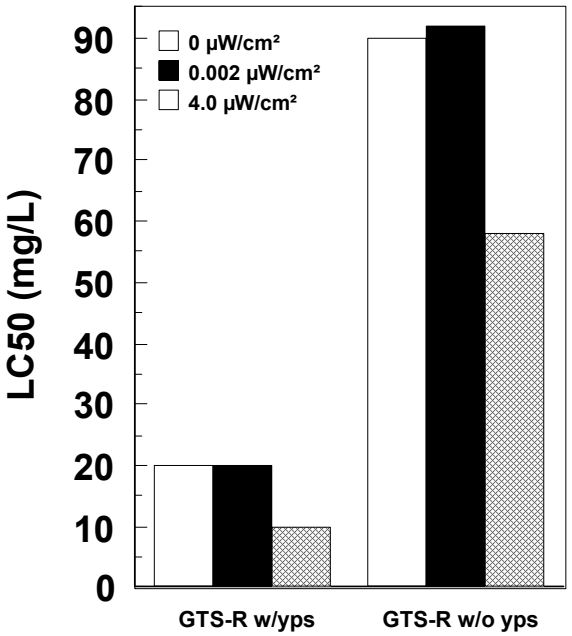

Figure 3. 96-hr LC50s for rainbow trout exposed to colorless Fire-Trol GTS-R.

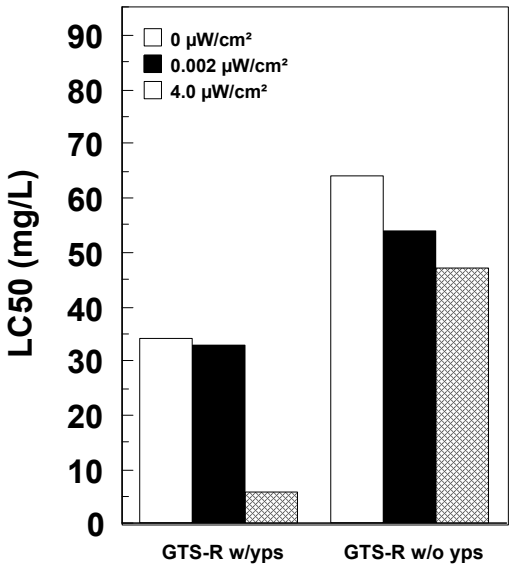

Figure 4. 96-hr LC50s for rainbow trout exposed to colored Fire-Trol GTS-R.

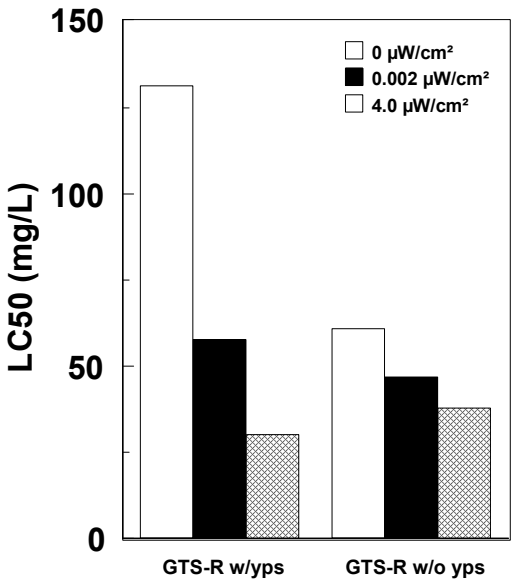

Figure 5. 96-hr LC50s for Southern leopard frog tadpoles exposed to colorless Fire-Trol GTS-R.

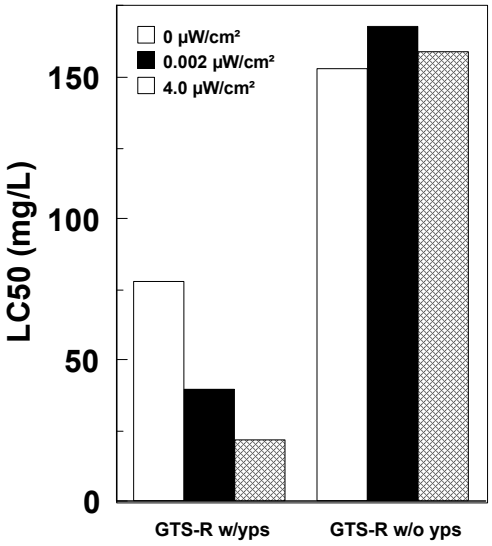

Figure 6. 96-hr LC50s for Southern leopard frog tadpoles exposed to colored Fire-Trol GTS-R.

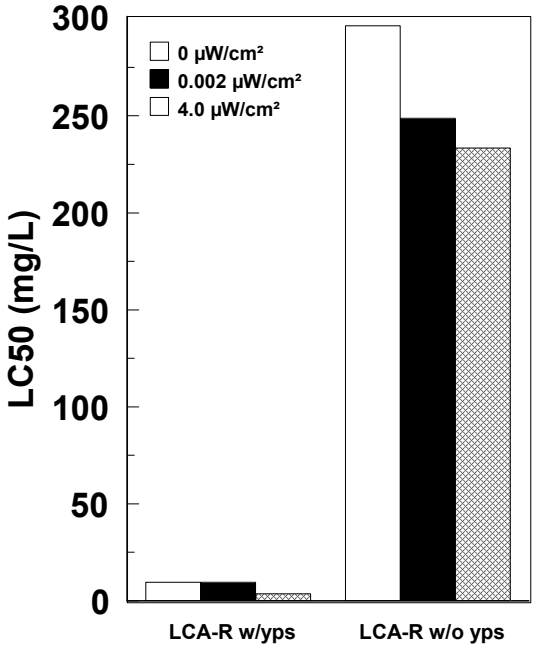

Figure 7. 96-hr LC50s for rainbow trout exposed to colorless Fire-Trol LCA-R.

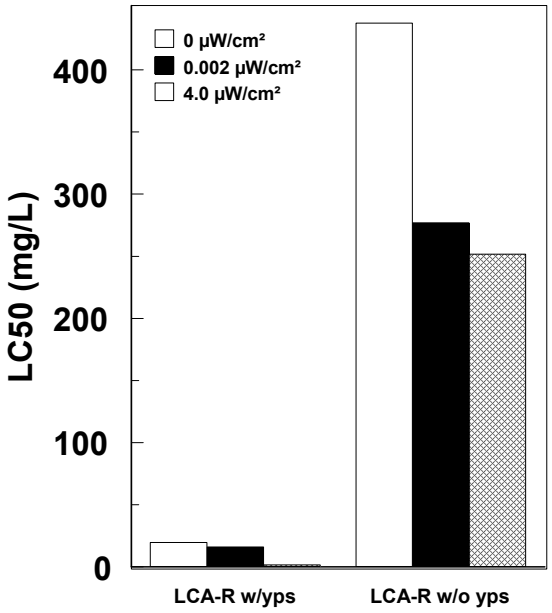

Figure 8. 96-hr LC50s for rainbow trout exposed to colored Fire-Trol LCA-R.

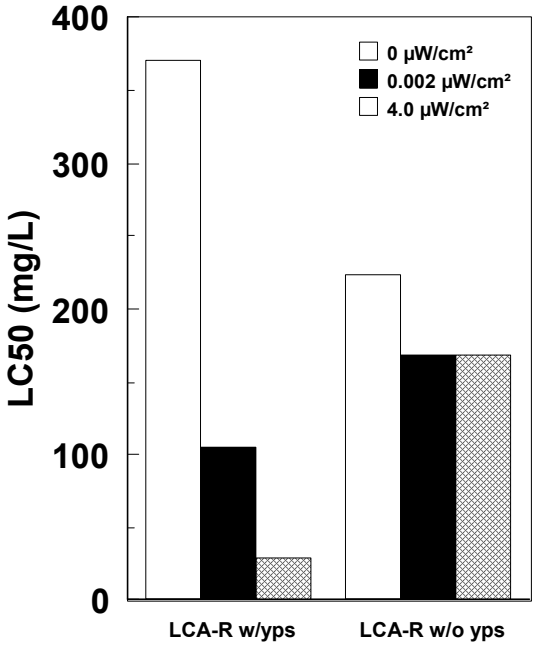

Figure 9. 96-hr LC50s for Southern leopard frog tadpoles exposed to colorless Fire-Trol LCA-R.

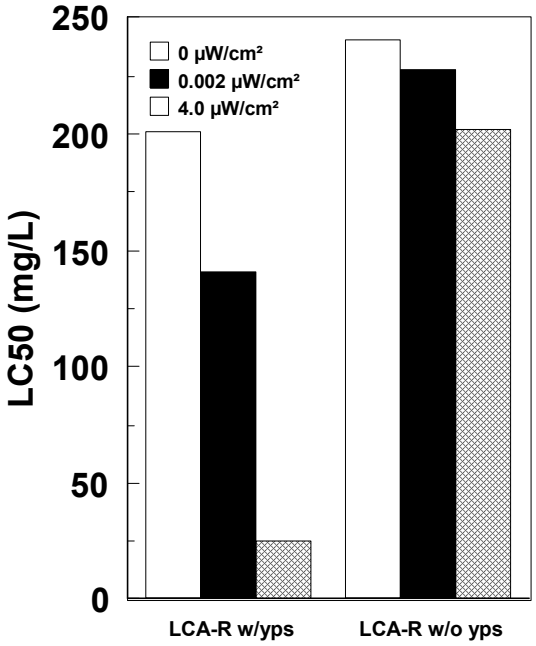

Figure 10. 96-hr LC50s for Southern leopard frog tadpoles exposed to colored Fire-Trol LCA-R.

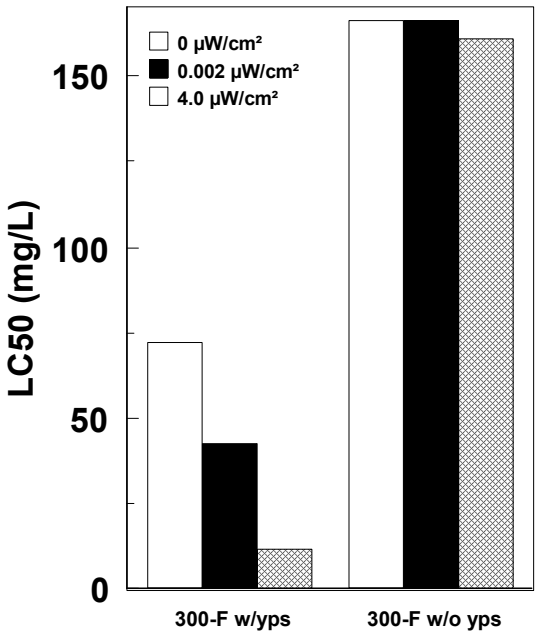

Figure 11. 96-hr LC50s for rainbow trout exposed to colored Fire-Trol 300-F.

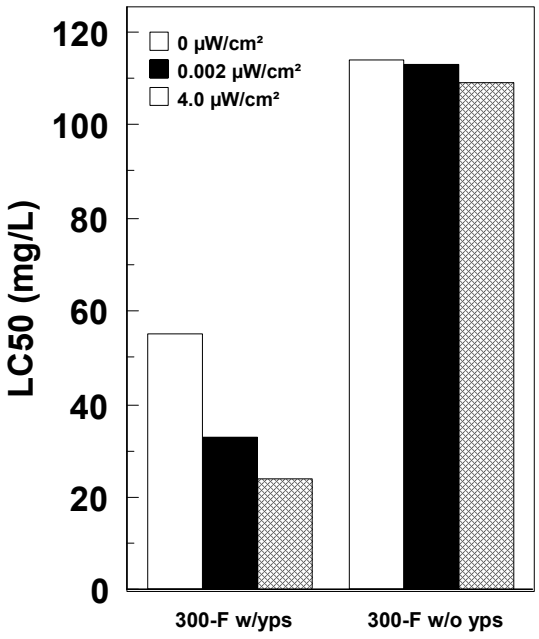

Figure 12. 96-hr LC50s for Southern leopard frog tadpoles exposed to colored Fire-Trol 300-F.

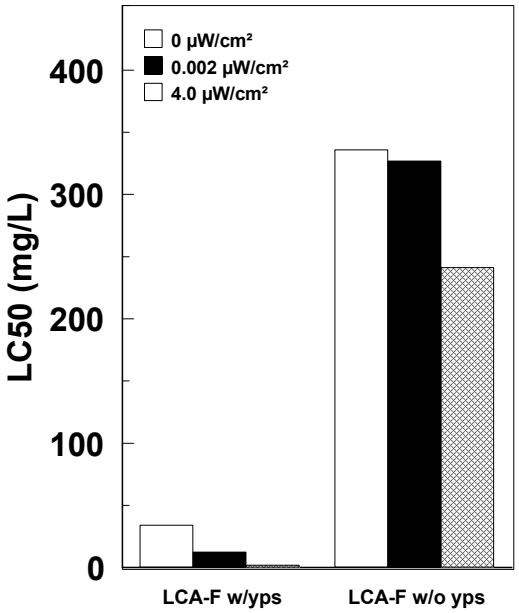

Figure 13. 96-hr LC50s for rainbow trout exposed to colored Fire-Trol LCA-F.

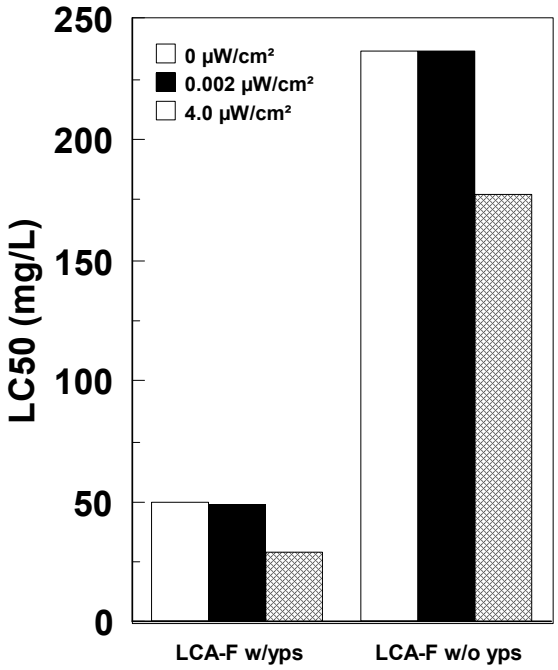

Figure 14. 96-hr LC50s for Southern leopard frog tadpoles exposed to colored Fire-Trol LCA-F.

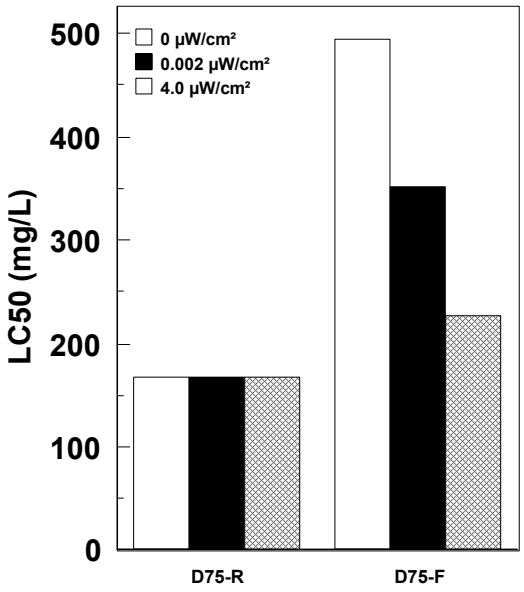

Figure 15. 96-hr LC50s for rainbow trout exposed to colored Phos-Chek D75-R and D75-F.

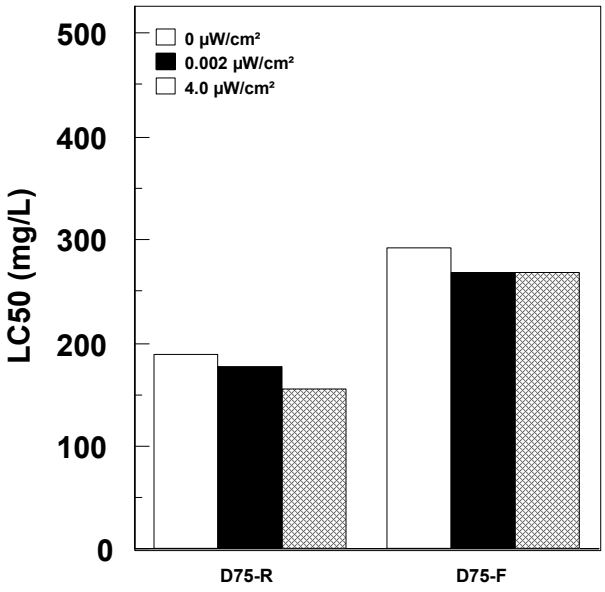

Figure 16. 96-hr LC50s for Southern leopard frog tadpoles exposed to colored Phos-Chek D75R and D75-F.

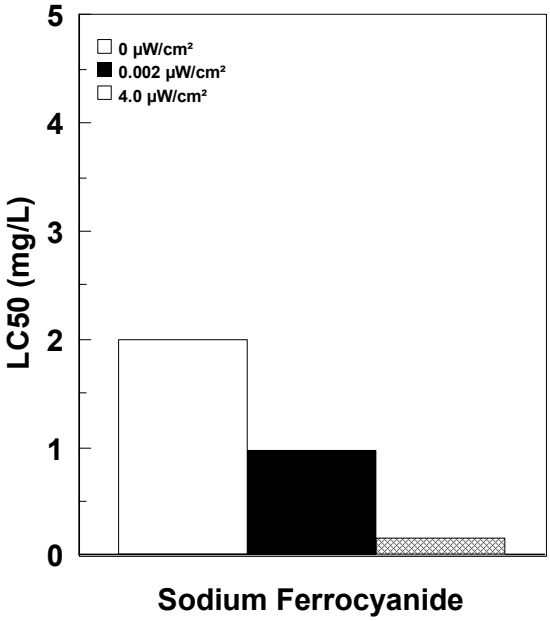

Figure 17. 96-hr LC50s for rainbow trout exposed to sodium ferrocyanide.

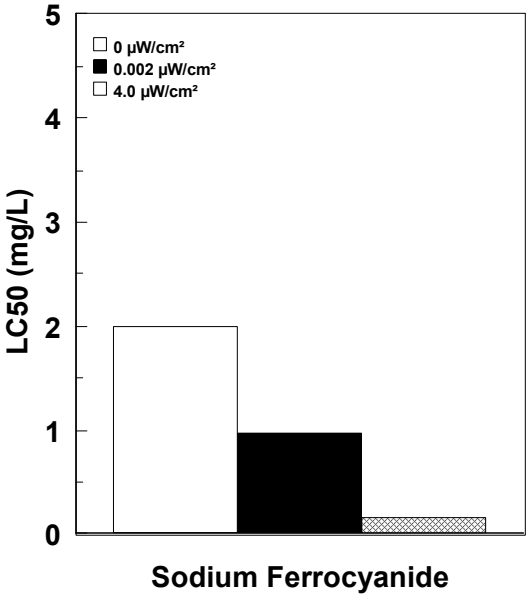

Figure 18. 96-hr LC50s for Southern leopard frog tadpoles exposed to sodium ferrocyanide.

ERRATA

Page 4:	The 1999 EPA criterion for free cyanide in water is 5.2 ug/L, rather than the previous criterion of < 3 ug/L that we cite in this report.
Pages 8 and 9:	Figures 1 and 2 denote toxicity of the full fire chemical formulation as applied in the field, including yellow prussiate of soda in those fire chemical products that include this component.
Page 14:	Temperatures used for rainbow trout studies ranged from 7 to 12^{o} C depending on the temperature used in culture, such that test temperatures were within 3^{o} of the culture temperature.
Page 15:	The toxicity testing procedures were conducted in basic accordance with American Society for Testing and Materials Guideline ASTM E729, "Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians".
Page 16:	Water samples were taken at 24 hours when mortality among test organisms was greatest.
Page 50:	Table legend should read "Total ammonia (TA) concentrations measured during exposure of rainbow trout to 7 fire retardant chemicals under three UV light treatments and the estimated range of unionized ammonia (UA) at the 96-hour LC50."
Pages 50 and 51	Tables 5 and 6. Asterixes * indicate concentrations of un-ionized ammonia that are lethal to fish and amphibians.
Page 55	Table 9. Non-detection limits for free cyanide in water is 0.01 mg/L.

Outlines of test procedures for rainbow trout and Southern leopard frogs are included.

ATTACHMENT 1

Rana sphenocephala Test Procedures

Definitive toxicity tests with various amphibian larvae are conducted according to procedures described in ASTM Guide E 729 - 88a, "Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians." Definitive test procedures, including alterations from ASTM Guide E 729 - 88a, are summarized below.

Test type and duration. static acute: 96 hours.

Test solution volume. 600 mL.

Animals per vessel. 10 tadpoles/ vessel.

Feeding. No feeding

Test substances.
GTS-R with and without yps, with and without colorant
LCG-R with and without yps, with and without colorant
LCA-F with and without yps, with colorant
300-F with and without yps, with colorant
D75-R
D75-F
Sodium ferrocyanide

Replication. Two replications per treatment are tested

Treatments. 5 chemical dilutions (50%) and a control, 3 light regimes.

Dilution water. Deep well water.

Test conditions. No aeration, 17 ± 2^{o} C.

Test Monitoring. Daily measurements in 12 randomly selected exposure chambers for dissolved oxygen, temperature, and pH. Ammonia measured at 24 and 96 hours.

Definitive test endpoints. Daily survival,

Chemistry sampling. Samples for chemical analysis of test solutions are collected according to Attachment 5.

ATTACHMENT 2

Onchorhyncus mykiss Test Procedures

Definitive toxicity tests with *Onchorhyncus mykiss* are conducted according to procedures described in ASTM Guide E 729 - 88a, "Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians." *Onchorhyncus mykiss* definitive test procedures, including alterations from ASTM Guide E 729 - 88a, are summarized below.

Test type and duration. static acute, 96 hours

Test solution volume. 3500 mL.

Animals per vessel. 10 fish / vessel.

Feeding. No feeding

Test substances.
GTS-R with and without yps, with and without colorant
LCG-R with and without yps, with and without colorant
LCA-F with and without yps, with colorant
300-F with and without yps, with colorant
D75-R
D75-F
Sodium ferrocyanide

Replication. Two replications per treatment are tested

Treatments. 5 chemical dilutions (50%) and a control, 3 light regimes.

Dilution water. Deep well water.

Test conditions. No aeration, 10 ± 3^{o} C.

Test Monitoring. Daily measurements in 12 randomly selected exposure chambers for dissolved oxygen, temperature, and pH. Ammonia measured at 24 and 96 hours.

Definitive test endpoints. Daily survival,

Chemistry sampling. Samples for chemical analysis of test solutions are collected according to Attachment 5.