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**TOXICITY OF FIRE
RETARDANT
AND FOAM SUPPRESSANT
CHEMICALS
TO PLANT AND ANIMAL
COMMUNITIES**

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EXECUTIVE SUMMARY

TOXICITY OF FIRE RETARDANT AND FOAM SUPPRESSANT CHEMICALS TO PLANT AND ANIMAL COMMUNITIES

Fire retardants and suppressants are used extensively in the United States for suppression and control of range and forest fires. Each year, fire control agencies utilize millions of gallons of these mixtures on a wide array of ecosystems. These chemicals are often applied in environmentally sensitive areas which may contain endangered, threatened, or economically significant plant and animal species. Historically, little information was available on the toxicity of these chemicals to aquatic and terrestrial life; less information was available concerning impacts at the community and ecosystem level.

The extensively used ammonium compounds - essentially dry or liquid fertilizer formulations - have long been considered to have minimal toxicological or ecological impact. Research has been mostly confined to effects on aquatic organisms. Several authors have reported on the toxicity of the active ammonium salts found in most fire retardants (Pramanik and Sarkar 1987, Sheehan and Lewis 1987, Ram and Sathyanesan 1986, Singh et. al. 1985). Limited studies concerning nitrate poisoning to aquatic animals (Johnson and Sanders 1977) from fire retardant formulations have been conducted. Even less information is available on foam products. Although the risk associated with fire fighting chemicals has generally been accepted as minimal, extensive fish kills have been documented after accidental drops of chemicals directly in a stream. For example, many trout were killed in the Little Firehole River during the major 1988 fire in Yellowstone National Park (Minshall and Brock 1991). Specific

concerns over potential fire chemical effects on endangered and threatened fish has underscored the need to better understand the potential response of these aquatic and terrestrial species and their associated vegetative environments to chemical application.

Research presented in the attached final report includes results from standard laboratory testing of select chemicals (Fire-Trol GTS-R, Phos-Chek D75-F, Fire-Trol LCG-R, Silv-Ex, and Phos-Chek WD-881) to determine their toxicity to two fish, two aquatic invertebrates, an algae, three birds, a mammal, and a terrestrial invertebrate. This basic information was then used to design and implement studies to evaluate potential ecological consequences resulting from fire chemical application. These ecological studies were conducted during separate years in a prairie wetland habitat in North Dakota and in an area in the Great Basin region of northern Nevada. Research from these studies should provide information to fire managers and policy developers to assist in formulating sound decisions concerning fire-fighting activities on private, state, and federal lands. In the text below, bullets have been used to highlight the major results from these studies.

Laboratory toxicity testing:

- ! All five chemicals were of comparatively low order of toxicity to terrestrial species. For all test species, the LD₅₀ exceeded the limit criteria for significant acute toxicity suggesting that no mortality should result from direct chemical application or from dietary exposure to fire chemicals. However, results from avian tests suggest that dietary exposure to Silv-Ex may result in temporary lethargy and loss of equilibrium in birds.
- ! Tests with aquatic organisms indicated the two foam suppressants (Silv-Ex and Phos-Chek WD-881) were similar in toxicity and were significantly more toxic than were the

three non-foam chemicals. Water quality did not modify toxicity in a consistent manner for all species.

- ! The egg life stage of fish species was more tolerant of chemical exposure than other life stages; swim-up (larval) stage was most sensitive. These results imply that accidental introduction of these chemicals into an aquatic system during the salmonid swim-up period could cause significant mortality and be catastrophic to a local population, especially if that population were threatened or endangered.
- ! Degradation of all five chemicals was more rapid in soils with high organic content than in soils with low organic content. The overall rapid degradation of both foam and non-foam chemicals that was documented during these studies suggests that long term effects from the chemicals tested would be unlikely.

Mixed grass prairie wetland ecosystem:

- ! Fire chemical application resulted in changes in growth, including biomass accumulation, and changes in species diversity in a mixed grass prairie.
- ! Phos-Chek produced a pronounced fertilization effect causing increases in herbaceous biomass, but depressing species diversity. This provided a competitive advantage for the exotic grass, *Poa pratensis*, over other species.
- ! Foams such as Silv-Ex did not affect growth, but did depress species diversity.
- ! Terrestrial field studies documented no measurable effects on small mammal populations in the mixed grass prairie ecosystem.
- ! Of the two foams tested in the aquatic environment, Silv-Ex was more toxic than was Phos-Chek WD-881. Sensitivity of fathead minnows was similar between in-situ field

exposures and standard laboratory tests. The most dramatic decrease in survival occurred during the first 24 hours.

- ! No effects on the macroinvertebrate community in the wetland resulted from either the Silv-Ex or Phos-Chek foams.
- ! Based on information derived from this field exposure, a spill of 1% Silv-Ex into a closed aquatic system such as a pond or terminal wetland would require an estimated 41,600-fold dilution. Thus, in a one-acre pond with an average depth of 10 feet, (and use of a safety factor of 100) about 78 gallons of 1% Silv-Ex spilled directly into the pond should represent no appreciable threat to aquatic organisms.

Great Basin ecosystem (northern Nevada):

- ! The majority of plant species demonstrated no response to chemical application over the course of the growing season.
- ! In riparian zones, burning obscured responses of vegetation to chemicals that were observed in unburned sites.
- ! Vegetation in riparian zones was more responsive to June chemical treatments than to July treatments; upland zones responded only to July treatments.
- ! Although the number of plant species declined significantly after the application of Phos-Chek, eleven weeks later, the number of species in chemically treated plots were statistically indistinguishable from the number in control plots.
- ! Similar to results from studies in the mixed grass prairie, small mammals and insects showed no measurable response to application of either Phos-Chek or Silv-Ex.

- ! Aquatic invertebrates and fish responded similarly to Silv-Ex and Phos-Chek exposure in both field (dilutions with stream water) and laboratory (dilutions with reconstituted water) tests, with Silv-Ex being more toxic than Phos-Chek D75F.
- ! Studies with indigenous aquatic invertebrates indicated that mayflies were consistently more sensitive than stoneflies to both Silv-Ex and Phos-Chek.
- ! Substantial increases in drift of aquatic invertebrates resulted from in-stream application of Silv-Ex to the North Fork of the Little Humboldt River. A similar degree of response was not observed following application of Phos-Chek D75F.
- ! Lahontan cutthroat and rainbow trout were less sensitive to Phos-Chek D75F and Silv-Ex than were daphnids and fathead minnows, yet more sensitive than mayflies and stoneflies.
- ! When trout were returned to fresh stream water after a 30-minute exposure to Silv-Ex, mortality of both species continued to occur suggesting that short-term exposure to Silv-Ex may result in mortality, even after the chemical has been eliminated from the stream by dilution or flushing. A similar result did not occur after exposure to Phos-Chek D75F.
- ! Fish exposed to Silv-Ex and Phos-Chek D75F during in-stream studies responded similarly to fish exposed to similar chemical concentrations in laboratory, on-site, and artificial channel tests. As expected, no mortality of fish occurred from exposure to the low concentrations of chemicals used in the stream studies.
- ! Sub-lethal effects, including increased opercular movement and erratic swimming behavior, were apparent in trout exposed to Silv-Ex, but were not observed in Phos-Chek exposed fish. In contrast to artificial channel studies, all fish recovered fully from the in-stream exposures. However, this may not be the case in an actual fire situation where

elevated stream temperatures and turbidity could result in increased stress in fish, thus causing mortality.

- ! In a hypothetical case involving the application of Silv-Ex directly into the North Fork of the Little Humboldt River, calculations with data generated during this study suggested that the coverage rate would have to be reduced to 5.3 gal/100ft² *or* the percent mixture would have to be reduced to 0.53% to achieve a level where no appreciable mortality would be expected. This represents a worst-case scenario by assuming complete stream coverage and uniform mixing; the simultaneous occurrence of both of these assumptions is highly improbable. Risk would also be reduced with increasing stream size (order) or application of the chemical perpendicular rather than parallel to the stream.

Results from this research effort confirm that the current policy of exercising caution when applying fire chemicals near streams with threatened or endangered species is appropriate. Most importantly, for protection of fish populations, the time of application as it coincides with fish development is a decisive factor in estimating potential effects. Accidental introduction of these chemicals into an aquatic system during a salmonid swim-up period could cause significant mortality and be catastrophic to a local population, especially if that population were threatened or endangered. Mortality of terrestrial organisms is not anticipated from direct exposure to any of these fire-fighting chemicals. However, lethargic behavior or loss of equilibrium in birds may result from dietary exposure to foams such as Silv-Ex. In general, long-term vegetative effects from chemical application are not expected, but the objectives of the land manager are very important in decisions concerning fire chemical use in vegetated areas. If the objective is to halt an uncontrolled fire, subtle changes caused by Silv-Ex and Phos-Chek may be of little

importance. On the other hand, if the objective is to aid in the control of prescribed burns, the potential effect on species diversity should be considered. In particular, if the control of exotic, robust grasses such as *Poa pratensis* is important, these results suggest that use of these chemicals should be minimized or avoided. In summary, information from the fire chemical evaluation studies presented in this report must be combined with existing knowledge of ecological effects of fire on the environment to insure that the best possible management alternative is exercised.

Literature Cited

- Johnson, W.W., and H.O. Sanders. 1977. Chemical forest fire retardants: Acute toxicity to five freshwater fishes and a scud. Technical U.S.Department of Interior, Fish and Wildlife Service, Number 91.
- 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.
- Pramanik, A., and S.K. Sarkar. 1987. Comparative study of the sensitivity of egg spawn and fry of *Cyprinus carpio* exposed to ammonium sulfate at different temperature. *Geobios* 14:229-230.
- Ram, R.N., and A.G. Sathyanesan. 1986. Inclusion bodies formation and degeneration of the oocytes in the fish channel-punctatus in response to ammonium sulfate treatment. *Ecotoxicology and Environmental Safety* 11:272-276.
- Sheehan, R.J., and W.M. Lewis. 1987. Influence of pH and ammonia salts on ammonia toxicity and water balance in young channel catfish. *Transactions of the American Fisheries Society* 115:891-899.
- Singh, H.R., Dobriyal, A.K., and R.C. Pokhriyal. 1985. Toxicity of diammonium phosphate of the hill stream minor carp *Barilius benedelis*. *Uhar Pradesh Journal of Zoology* 5:89-92.

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INTRODUCTION

Millions of liters of fire control chemicals are used each year in the United States to control and suppress range and forest fires. These chemicals are often applied to environmentally sensitive areas that may contain endangered, threatened, and economically important plant and animal species. Aquatic habitats adjacent to areas treated with fire control chemicals are subject to inputs of these chemicals via accidental aerial drops and runoff. Historically, most fire retardant formulations were thought to have minimal toxicity, however, fish kills have occurred in streams accidentally contaminated by fire retardant chemicals (Dodge 1970). Almost complete mortality of trout was reported in a section of the Little Firehole River following a fire retardant drop during the major fire in Yellowstone National Park in 1988 (Minshall and Brock 1991). Aside from these reports, little information is available on the toxicity of these chemical formulations to aquatic biota and their impacts on community structure and function.

There are two general types of fire control chemicals typically used in fire fighting; short-term fire-suppressant foams and long-term fire retardant chemicals. Fire-suppressant foams are wetting agents that enhance the extinguishing ability of water by increased retention on fuel sources and/or reduced evaporation. Typically, fire-suppressant foams are composed of a mixture of surfactants, foam stabilizers, inhibiting agents, and solvents. The use of fire-suppressant foams in fire fighting is becoming more prevalent because the amount of water required can be reduced by 60% (Schlobohm and Rochna 1988). However, fire-suppressant foams do not reduce combustion after the water has evaporated from the fuel source.

Long-term fire-retardant formulations are typically composed of ammonium salts with an attapulgite clay thickener or diammonium phosphate with a guar gum-derivative thickener. These formulations may also contain rust inhibitors and trace amounts of colorants, such as ferric oxide, to mark drop sites. Fire-retardant chemicals form a long-term combustion barrier after the

water carrier has evaporated and their effectiveness can be increased by using highly concentrated solutions (Dodge 1970).

Several studies have reported on the toxicity of the ammonium salts and other constituent chemicals of fire control formulations to fish (Thurston and Russo 1983, Singh *et al.* 1985, Sheehan and Lewis 1987). However, relatively few studies have been conducted to determine the toxicity of the actual fire-fighting formulations to fish (Blahm and Snyder 1973, Johnson and Sanders 1977). In addition, most of these studies were conducted with formulations no longer in use. Information on the toxicity of fire-suppressant foams to aquatic biota is limited to reports by the manufacturer.

Lack of published toxicity information for fire control chemicals has made it difficult to assess their effects on aquatic organisms. Studies on the biological effects of these chemicals to important aquatic species provide information required by fire managers and policy developers to make sound decisions regarding their use on private and public lands.

I. PROJECT OBJECTIVE 1. TOXICITY OF FIRE RETARDANT CHEMICALS AND FIRE SUPPRESSANT FOAMS TO AQUATIC SPECIES

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Objective:

- (1) Determine the acute toxicity of five commercially available and commonly used wildland fire retardant and foam products on specific aquatic species.**

General Procedures:

The toxicity of five fire retardant chemicals and foams were determined for three species of fish, two aquatic invertebrates, and one algae. The test organisms were rainbow trout (*Oncorhynchus mykiss*), fathead minnows (*Pimephales promelas*), chinook salmon, (*Oncorhynchus tshawytscha*) daphnids (*Daphnia magna*), amphipods (*Hyalella azteca*), and the algae (*Selenastrum capricornutum*). The specific chemicals tested were determined based on a critical review of the literature and interactions with qualified personnel familiar with use of various chemical and foam retardants. Studies were conducted with Fire-Trol GTS-R (powder), Fire-Trol LCG-R (liquid), Phos-Chek D-75-F (powder) retardants, and Phos-Chek WD-881 (liquid) and Silv-Ex (liquid) foams.

The fire control chemicals were obtained from the U.S. Forest Service, Intermountain Fire Sciences Laboratory, Missoula, MT. A description of the chemical formulations tested is given in Table 1. All test concentrations and subsequent LC₅₀ values were based on the total formulation of each chemical.

Test Methods:

All tests were conducted under the existing quality assurance program of the Environmental and Contaminants Research Center (ECRC, formerly the Midwest Science

Center-Columbia MO). Eyed-eggs and juvenile fish were handled so as to minimize stress in accordance with the ECRC-Animal Welfare Plan Animal Welfare Committee 1991). This research involved conducting 96-hour acute toxicity studies with fish, algae, adult amphipods, and 48-hour studies with < 24-h-old *Daphnia magna*.

Acute toxicity tests with fish and aquatic invertebrates were conducted according to established methods (ASTM 1989). In each test, ten organisms were exposed to a geometric series of seven to eight toxicant concentrations plus a control treatment for a total of 80-90 organisms per test. The exposures were conducted in test solutions under static conditions in glass containers and continued for either 48 or 96 hours duration. The test temperature was maintained at 25° C for fathead minnow, 20° C for *Hyalella azteca*, 20° C for *Daphnia magna*, and 12° C for rainbow trout and chinook salmon. Organisms were tested in standardized reconstituted hard water and soft water (ASTM 1989) (Tables 2-6). Test water was prepared by addition of reagent grade salts (CaSO₄ • 2H₂O, MgSO₄, NaHCO₃, and KCl) to ultra-pure water prepared by reverse osmosis and deionization. The test waters were analyzed using standard methods (APHA et al. 1989) to insure that the water quality met the criteria of the experimental design in terms of hardness, alkalinity, and concentrations of major cations (calcium, magnesium) and anions (chloride, sulfate) before it was used in tests with fish and aquatic invertebrates. For all organisms, observations on mortality were recorded daily. For fish, mortality and abnormal behavior were recorded and any dead eggs and fish were removed at 24-h intervals. At the end of each test, control eggs and fish were weighed and the volume of eggs and the total length of individual fish were measured.

Test solutions of Fire-Trol LCG-R, Fire-Trol GTS-R, and Phos-Chek D75-F were prepared by adding appropriate amounts of the chemical directly to the test vessel. Each test vessel solution was and mixed twice for 1 minute with a polyethylene stirrer attached to an electric drill. Phos-Chek WD-881 and Silv-Ex solutions were prepared by pipetting appropriate

aliquants of stock solution (prepared in DI water) into the test vessel. These solutions were hand mixed with a teflon stir rod to prevent excessive foaming.

Ammonia, dissolved oxygen, and pH were measured in the control, low, medium, and high test concentrations at 0, 48, and 96 h of exposure. A regression equation was derived for each set of tests to estimate the concentration of ammonia as nitrogen (NH₃-N) at the 48-h or 96-h LC₅₀ for each chemical. Equations were determined by regressing the initial (0-h) NH₃-N concentrations in the low, medium, and high treatments against the appropriate concentrations of the chemical being tested. The range of un-ionized ammonia (NH₃) concentrations at the LC₅₀ concentration for each chemical was estimated by the method of Emerson *et al.* (1975) using the high and low pH values at test initiation.

Nitrate and nitrite were measured in solutions of the non-foam retardants (Fire-Trol GTS-R, Fire-Trol LCG-R, and Phos-Chek D75-F) that were aged for 96 h without organisms. The two foam suppressants (Phos-Chek WD-881 and Silv-Ex) were not tested in this manner. Two concentrations of each non-foam retardant that simulated the 96-h LC₅₀ for the most sensitive life stage of chinook salmon and rainbow trout were prepared in hard water and soft water. Nitrate and nitrite concentrations were determined colorimetrically (Hach Company 1992). A regression equation was used to estimate the nitrate and nitrite concentrations at the 96-h LC₅₀ for the other life stages.

The severity of an aquatic organism's response to a chemical is often dependent upon its life stage. Therefore, five life stages of rainbow trout were tested (eggs, sac-fry, swimup, 60 days post hatch, and 90 days post hatch) to determine which developmental stage was most sensitive to fire retardant chemicals (Table 7). In addition, four life stages of fathead minnow (Table 8) and chinook salmon (Table 9) were tested with each of the five test chemicals.

Acute toxicity tests with algae were conducted according to established methods (ASTM 1990). In each test, 2×10^4 cells/mL were exposed to each of five to six toxicant concentrations plus a control treatment. The exposures were conducted in test solutions under static conditions

in glass jars and continued for 96 hours duration. The test temperature was maintained at 24° C. Observations on cell counts, biomass dry weight, and chlorophyll a were recorded at the end of the test. The moving average-angle method (Peltier and Weber 1985) was used to calculate 96-hour IC₅₀ values for reduced cell counts, biomass dry weight, and chlorophyll a.

Statistical Analysis:

The LC₅₀ values and their 95% confidence intervals (C.I.) were calculated using the moving-average angle method (Peltier and Weber 1985). In tests where no partial kills occurred, the 95% C.I. were estimated as follows: the lower limit was the highest concentration with 0% mortality and the upper limit was the lowest concentration with 100% mortality. The criterion of nonoverlapping 95% C.I. was used to determine significant differences (P=0.05) between LC₅₀ values (APHA *et al.*, 1989). All LC₅₀ values are expressed as nominal concentrations of the fire control formulation.

Results:

Fish. The egg life stage of all fish species was the least sensitive to the five fire retardants tested and the swim-up stage was the most sensitive for rainbow trout and fathead minnows (Tables 10 and 11). However, the 60- and 90-day-old rainbow trout and the 30- and 60-day old fathead minnow were only slightly less sensitive than their respective swim-up life stage. For chinook, all three post-embryonic life stages exhibited similar sensitivities to all five chemicals (Table 12). In general, no consistent difference in sensitivity to the five fire retardant chemicals among the three species of fish was observed.

The five fire retardants were generally more toxic in hard water than in soft water, which is unusual. Typically, the toxicity of contaminants, especially inorganics, is greater in soft water than in hard water (Rand and Petrocelli 1985). However, regardless of water type, the two foam suppressants (Phos-Chek WD-881 and Silv-Ex) were at least an order of magnitude more toxic

to all life stages of all life stages of chinook salmon than the three non-foam retardants (Fire-Trol GTS-R, Fire-Trol LCG-R, and Phos-Chek D75-F; Table 12). Fire-Trol LCG-R was the least toxic chemical to the three post-embryonic life stages in both water types. Based on the acute toxicity rating scale for chemicals given in Passino and Smith (1987), Phos-Chek WD-881 and Silv-Ex were rated as moderately to slightly toxic, Fire-Trol GTS-R and Phos-Chek D75-F were rated as practically harmless, and Fire-Trol LCG-R was rated as relatively harmless to the post-embryonic life stages.

The rank order from most toxic to least toxic of the chemicals tested for fish species was: Silv-Ex = Phos-Chek WD-881 > Phos-Chek D-75-F = Fire-Trol GTS-R > Fire-Trol LCG-R. The two foam suppressants (Phos-Chek WD881 and Silv-Ex) were substantially more toxic than the three non-foam retardants (Phos-Chek D75-F, Fire-Trol GTS-R, and Fire-Trol LCG-R).

Ammonia concentrations in the low, medium, and high test concentrations were measured and used in regression analysis to determine the total ammonia concentration as nitrogen that would have been present at the 96-hour LC₅₀ concentration (Tables 13 -15). The three non-foam chemicals (Fire-Trol LCG-R, Fire-Trol GTS-R, and Phos-Chek D-75-F) had substantially more ammonia than the two foam chemicals (Phos-Chek WD-881 and Silv-Ex) as expected. Fire-Trol LCG-R had the highest total ammonia concentration (as nitrogen) of the three non-foam chemicals. For the foam retardants, concentrations of unionized ammonia were higher in Silv-Ex than in Phos-Chek WD-881. In test solutions of Fire-Trol LCG-R and Fire-Trol GTS-R, concentrations of nitrite-nitrogen (NO₂-N) at the 96-h LC₅₀ were considerably higher than those of nitrate-nitrogen (NO₃-N; Tables 13-15). Conversely, in test solutions of Phos-Chek D75-F concentrations of NO₃-N were considerably higher than those of NO₂-N.

Daphnia magna. No consistent effect of water quality on the toxicity of the five fire retardant chemicals was observed in tests with *Daphnia magna*. Toxicity did not differ for Fire-Trol LCG-R, Fire-Trol GTS-R, or Silv-Ex in soft and hard water. Soft water increased the toxicity of

Phos-Chek D-75-F and soft water decreased the toxicity of Phos-Chek WD-881 (Table 16). From most toxic to least toxic the rank order of the five chemicals was: Silv-Ex = Phos-Chek WD-881 > Phos-Chek D-75-F > Fire-Trol GTS-R > Fire-Trol LCG-R. This rank order was similar to that for fish.

The concentration of total ammonia was measured in the low, medium, and high test chemical concentrations and the amount of unionized ammonia calculated (Table 17). Results were similar to those reported for fish tests.

Hyaella azteca. All five fire retardant chemicals were consistently more toxic in soft water than in hard water (Table 18). For the three non-foam chemicals the increase in toxicity in soft water was substantial. The rank order from most toxic to least toxic in soft water was: Phos-Chek WD-881 > Silv-Ex > Phos-Chek D-75-F = Fire-Trol LCG-R = Fire-Trol GTS-R. In hard water the rank order was: Phos-Chek WD-881 = Silv-Ex > Fire-Trol GTS-R = Phos-Chek D-75-F = Fire-Trol LCG-R. The concentration of total ammonia was measured in the low, medium, and high test chemical concentrations and the amount of unionized ammonia calculated (Table 19).

Discussion:

Surfactants. Toxicity of the foam suppressants, Phos-Chek WD-881 and Silv-Ex, may be partly due to the anionic surfactant portion of their formulation. The 96-h LC₅₀ values we obtained for Phos-Chek WD-881 and Silv-Ex are comparable to toxicity values reported by other investigators for surfactants. Müller (1980) reported a 24-h LC₅₀ of 8.5 mg/L for a commercial, non-ionic surfactant using 8-g rainbow trout. Müller (1980) determined that the mode of surfactant toxicity was related to the reduction in surface tension caused by the surfactant. Holman and Macek (1980) tested three different chain length linear alkylbenzene sulfonate (LAS) anionic surfactants using 2-3-month old fathead minnow (*Pimephales promelas*) and found that toxicity increased with increasing alkyl chain length. The 96-h LC₅₀ they reported for

C_{11.7} chain length LAS (12.3 mg/L) was within the range of 96-h LC₅₀s we determined in this study for Phos-Chek WD-881 and Silv-Ex.

Un-ionized ammonia. The toxicity of the three non-foam retardants was probably related to NH₃ derived from the formulations. Little or no NH₃ was present in solutions of the two foam suppressants. Concentrations of NH₃ estimated at the 96-h LC₅₀ for the three non-foam retardants are close to those reported to be acutely lethal to salmonids. The 96-h LC₅₀s of NH₃ reported by Thurston and Russo (1983) for 0.12-1.8-g rainbow trout ranged from 0.23 to 0.77 mg/L. Their toxicity values overlap the range of NH₃ concentrations we estimated at the 96-h LC₅₀ for the three non-foam retardants with 0.28-2.86-g chinook salmon (0.03-1.45 mg/L). Russo (1985) reported a similar range of 96-h LC₅₀s for NH₃ (0.08-1.1 mg/L) for rainbow trout and pink salmon (*O. gorbuscha*). The similarity between the estimated NH₃ concentrations at the 96-h LC₅₀ for the three non-foam retardants and the acutely lethal concentrations of NH₃, suggests that NH₃ was the primary toxic component in these chemicals.

Nitrate and nitrite. Nitrate-nitrogen concentrations at the 96-h LC₅₀ concentrations of the three non-foam retardants (0.73-4.08 mg/L for swim-up fry and juveniles) are considerably lower than the 96-h LC₅₀ of NO₃-N for rainbow trout (1,362 mg/L) reported by Westin (1974). These findings suggest that NO₃-N probably did not have a significant influence on the toxicity of the non-foam retardants.

Nitrite-nitrogen probably influenced the toxicity of Fire-Trol LCG-R and Fire-Trol GTS-R to chinook salmon. Estimated NO₂-N concentrations at the 96-h LC₅₀ of Fire-Trol LCG-R and Fire-Trol GTS-R (Table 5) are considerably higher than those reported to be acutely lethal to rainbow trout (0.19-1.05 mg/L; Russo *et al.* 1974).

Intralaboratory comparisons. Toxicity results for chinook salmon were compared with those for similar life stages of rainbow trout tested in our laboratory (Table 20). Differences in sensitivity between rainbow trout and chinook salmon for each chemical-life stage combination are within a factor of two, which is within the expected intralaboratory variation for acute toxicity tests (Lemke 1981, Schimmel 1981).

Comparison to published/manufacturer data. The only published information found on the toxicity of these fire control chemicals to salmonids is from studies conducted by the manufacturer or by their contract laboratory. Toxicity data are available for all five chemicals and rainbow trout (Table 21). The toxicity values we obtained for chinook salmon are lower than those reported by the manufacturer or contract laboratory, except for Fire-Trol LCG-R. The largest difference in results between studies is for Fire-Trol GTS-R and Phos-Chek D75-R. The 96-h LC₅₀s reported for Fire-Trol GTS-R and Phos-Chek D75-R with rainbow trout are at least two times higher than those we determined for chinook salmon.

Relation to environmental considerations. To assess the potential impacts of these chemicals on aquatic ecosystems, toxicity data must be related to expected or measured environmental concentrations. Due to the lack of data on measured concentrations of these chemicals in aquatic systems, toxicity values were compared to their field application concentrations in tank mixtures (Table 22). The ratio of the tank mixture concentration to its 96-h LC₅₀ value indicates the amount of dilution required to achieve a concentration that is lethal to 50% of the fish. For example, an accidental drop of Phos-Chek D75-F in an aquatic environment would require a dilution of 660-fold to reach a concentration lethal to 50% of the chinook salmon. Based on these ratios, Fire-Trol GTS-R requires the lowest dilution (184-290-fold) and Fire-Trol LCG-R and Phos-Chek WD-881 require the highest dilution (745-1429-fold).

A more conservative approach would be to apply a safety factor to the toxicity data to estimate safe concentrations for aquatic organisms. A safety factor is the inverse of an application factor, which is the ratio of the maximum acceptable toxicant concentration (MATC) derived from chronic tests to its acute toxicity value (96-h LC₅₀; Rand and Petrocelli 1985). An application factor of 0.01 (safety factor=100) is typically used in estimating the MATC if chronic toxicity data are not available. Applying a safety factor of 100 to the Phos-Chek D75-F toxicity data indicates that a 66,000-fold dilution of the tank mixture is required to approach a safe concentration, i.e., MATC. The same approach can be used to estimate the dilutions required to achieve safe concentrations of the other fire control chemicals.

Summary:

Overall, the toxicity of the five fire retardant chemicals to these five species is remarkably similar. The two foams, Silv-Ex and Phos-Chek WD-881, have very similar toxicity and are substantially more toxic than the three non-foams. Of the non-foams, Fire-Trol GTS-R and Phos-Chek D-75-F have similar toxicity, which was substantially higher than Fire-Trol LCG-R except for the amphipod *Hyaella azteca*. The major toxic component in the three non-foam retardants was probably unionized ammonia, whereas in the foams it was the surfactants.

Water quality did not seem to modify the toxicity of the five fire retardant chemicals in a consistent manner. For the three non-foam chemicals, *Hyaella* was the most sensitive species in soft water, whereas fathead minnow was the most sensitive species in hard water (Table 15). For the two foam chemicals, *Daphnia* in three tests and *Hyaella* in one soft water test were the most sensitive species. In 8 out of 10 tests *Daphnia* were more sensitive than the swim-up life stage of rainbow trout. Overall, *Hyaella* showed the greatest difference in sensitivity associated with water quality. In 4 out of 5 soft water tests *Hyaella* was the most sensitive species, but in 4 out of 5 hard water tests it was the least sensitive species.

Comparing the acute toxicity values for chinook salmon to those for rainbow trout tested in our laboratory showed that the two salmonids are similar in their sensitivities to the five fire control chemicals. Comparisons of our results to those reported by the manufacturers (or the contract laboratories that conducted the toxicity tests) for these chemicals indicated that three of the chemicals (Fire-Trol GTS-R, Phos-Chek D75-R, and Phos-Chek WD-881) are more toxic to salmonids than previously reported.

Literature Cited:

- ABC Laboratories (Analytical Bio-Chemistry Laboratories, Inc.). 1986. Acute toxicity of PC-D75R to rainbow trout (*Salmo gairdneri*). Report No. 35089. ABC Laboratories, Columbia, MO. Contract report submitted to Monsanto Co., St. Louis, MO.
- ABC Laboratories (Analytical Bio-Chemistry Laboratories, Inc.). 1988. Acute toxicity of PC-WD881 to rainbow trout (*Salmo gairdneri*). Report No. 36856. ABC Laboratories, Columbia, MO. Contract report submitted to Monsanto Co., St. Louis, MO.
- Animal Care and Use Committee. 1991. Animal Welfare Plan. U.S. Fish and Wildlife Service National Fisheries Contaminant Research Center, Columbia, MO.
- Ansul Fire Protection. 1991. Extinguishing Agent Data Sheet, Silv-Ex "Class A" fire control concentrate. Ansul Fire Protection, Marinette, WI.
- 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. American Public Health Association, Washington, DC.
- ASTM (American Society for Testing and Materials). 1989. Standard guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. In 1990 Annual Book of ASTM Standards Vol. 11.04, Philadelphia PA, pp 360-379.
- Blahm, T.H., and G.R. Snyder. 1973. Effect of chemical fire retardants on the survival of juvenile salmonids. National Marine Fisheries Service, Environmental Facility. Prescott, Oregon. Research Contract Number: 53500-CT2-85(N) 1973, U.S. Bureau of Land Management.
- Chemonics Industries, Inc. 1992a. Fire-Trol LCG-R material safety data sheet. Chemonics Industries, Inc., Phoenix, AZ.
- Chemonics Industries, Inc. 1992b. Fire-Trol GTS-R material safety data sheet. Chemonics Industries, Inc., Phoenix, AZ.
- Dodge, M. 1970. Nitrate poisoning, fire retardants, and fertilizers - any connection? *Journal of Range Management* 23:244-247.
- 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.
- Gaikowski, M.P. 1994. The acute toxicity of three fire-retardant and two fire-suppressant foam formulations to the early life stages of rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*). Master's thesis, University of South Dakota, Vermillion, SD.

- Hach Company. 1992. Hach Water Analysis Handbook, 2nd ed. Loveland, CO. 831 pages.
- Holman, W.F., and K.J. Macek. 1980. An aquatic safety assessment of linear alkylbenzene sulfonate (LAS): Chronic effects on fathead minnows. *Transactions of the American Fisheries Society* 109:122-131.
- Johnson, W.W., and H.O. Sanders. 1977. Chemical forest fire retardants: Acute toxicity to five freshwater fishes and a scud. U.S. Department of the Interior, Fish and Wildlife Service, Technical Paper 91, Washington, DC.
- Lemke, A.E. 1981. Interlaboratory comparison acute testing set. U.S. Environmental Protection Agency Publication EPA-600/3-81-005. Duluth, MN.
- 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.
- Monsanto Company. 1990. Phos-Chek WD-881 fire suppressant foam fact sheet. Monsanto Wildfire Division, Monsanto Co., Ontario, CA.
- Müller, R. 1980. Fish toxicity and surface tension of non-ionic surfactants: Investigations of antifoam agents. *Journal of Fish Biology* 16:585-589.
- Passino, D.R., and S.B. Smith. 1987. Acute bioassays and hazard evaluation of representative contaminants detected in Great Lakes fish. *Environmental Toxicology and Chemistry* 6:901-907.
- Peltier, W.H., and C.I. Weber. 1985. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, 3rd Edition. U.S. Environmental Protection Agency Publication EPA-600/4-85-013. Cincinnati, OH.
- Rand, G.M., and S.R. Petrocelli. 1985. Fundamentals of Aquatic Toxicology. Hemisphere Publishing, Washington, D.C. 666 pages.
- Russo, R.C. 1985. Ammonia, nitrite, and nitrate. In G.M. Rand and S.R. Petrocelli, eds., Fundamentals of Aquatic Toxicology. Hemisphere Publishing, Washington, D.C., pp. 455-469.
- Russo, R.C., C.E. Smith, and R.V. Thurston. 1974. Acute toxicity of nitrite to rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* 31:1653-1655.
- Schimmel, S.C. 1981. Results: Interlaboratory comparison--acute toxicity tests using estuarine animals. U.S. Environmental Protection Agency Publication EPA-600/4-81-003. Gulf Breeze, FL.

- Schlobohm, P., and R. Rochna. 1988. An evaluation of foam as a fire suppressant is available. National Wildlife Coordinating Group. Foam Applications for Wildland and Urban Fire Management 1: 6-7.
- Sheehan, R.J., and W.M. Lewis. 1987. Influence of pH and ammonia salts on ammonia toxicity and water balance in young channel catfish. Transactions of the American Fisheries Society 115:891-899.
- Singh, H.R., A.K. Dobriyal, and R.C. Pokhriyal. 1985. Toxicity of diammonium phosphate of the hill stream minor carp *Barilius bendelisis*. Uhar Pradesh Journal of Zoology 5:89-92.
- Springborn Bionomics. 1986. 96-hour acute (LC50) with rainbow trout. Report No. BW-86-10-2044. Springborn Bionomics, Inc., Wareham, MA. Contract report submitted to Wormald CDN, Thurso, Quebec, Canada.
- Thurston, R.V., and R.C. Russo. 1983. Acute toxicity of ammonia to rainbow trout. Transactions of the American Fisheries Society 112:696-704.
- Westin, D.T. 1974. Nitrate and nitrite toxicity to salmonoid fishes. The Progressive Fish-Culturist 36:86-89.

Table 1. Fire control chemicals used in toxicity tests.

Name	Category of Fire Control	Manufacturer	Lot number	Ingredients
Fire-Trol GTS-R	Fire-retardant powder	Chemonics Industries, Inc.	84FT232	Ammonium sulfate, diammonium phosphate, guar gum thickener, spoilage and corrosion inhibitors, and iron oxide
Fire-Trol LCG-R	Fire-retardant liquid	Chemonics Industries, Inc.	91FT11	Ammonium polyphosphate, attapulgite clay thickener, corrosion inhibitor, and iron oxide
Phos-Chek D75-F	Fire-retardant powder	Monsanto Co.	2468762A	Ammonium sulfate, ammonium phosphate, guar gum thickener, orange coloring agent, and other additives
Phos-Chek WD-881	Fire-suppressant foam liquid	Monsanto Co.	3616836A	Anionic surfactants, foam stabilizers, and solvents (hexylene glycol)
Silv-Ex	Fire-suppressant foam liquid	Ansul Fire Protection	75451	Anionic surfactants, stabilizers, inhibitors and solvents (diethylene glycol monobutylether)

Table 2. Summary of **water quality** characteristics (mean and range in parentheses) of test water used in acute toxicity tests with five life stages of **rainbow trout** exposed to five fire retardant chemicals in ASTM soft and hard waters.

Characteristic	Water Type	
	Soft (N=7)	Hard (N=7)
pH	7.5 (7.3 – 7.6)	8.19 (8.03 – 8.29)
Conductivity (μhos/cm @ 25° C)	164 (157 – 168)	542 (531 – 556)
Hardness (mg/L as CaCO₃)	42 (41 – 43)	163 (160 – 165)
Alkalinity (mg/L as CaCO₃)	32 (32 – 34)	112 (110 – 115)
Calcium (mg/L)	8 (7 – 8)	28 (27 – 29)
Magnesium (mg/L)	5 (5 – 6)	23 (21 – 24)
Chloride (mg/L)	0.5 (0.1 – 1.0)	2.8 (2.0 – 3.4)
Sulfate (mg/L)	40 (39 – 40)	160 (145 – 203)

Table 3. Summary of **water quality** characteristics (mean and range in parentheses) of test water used in acute toxicity tests with five life stages of **fathead minnows** exposed to five fire retardant chemicals in ASTM soft and hard waters.

Characteristic	Water Type	
	Soft (N=7)	Hard (N=7)
pH	7.5 (7.5 – 7.5)	8.2 (8.0 – 8.3)
Conductivity (μhos/cm @ 25° C)	163 (161 – 168)	544 (531 – 557)
Hardness (mg/L as CaCO₃)	42 (41 – 45)	163 (160 – 165)
Alkalinity (mg/L as CaCO₃)	33 (32 – 34)	112 (110 – 113)
Calcium (mg/L)	7 (7 – 8)	28 (28– 29)
Magnesium (mg/L)	6 (6 - 7)	23 (22 – 23)
Chloride (mg/L)	1.0 (1.0 – 1.0)	3.0 (3.0 – 3.0)
Sulfate (mg/L)	39 (35 – 41)	175 (156 – 200)

Table 4. Summary of **water quality** characteristics (mean and range in parentheses) of test water used in acute toxicity tests with five life stages of *Daphnia magna* exposed to five fire retardant chemicals in ASTM soft and hard waters.

Characteristic	Water Type	
	Soft (N=7)	Hard (N=7)
pH	7.6 (7.55 – 7.66)	8.2 (8.21 – 8.25)
Conductivity (μhos/cm @ 25° C)	166 (159 – 172)	552 (537 – 556)
Hardness (mg/L as CaCO ₃)	40 (40 – 41)	162 (160 – 162)
Alkalinity (mg/L as CaCO ₃)	32 (31 – 32)	112 (110 – 115)
Calcium (mg/L)	8 (7 – 97)	27 (27 – 27)
Magnesium (mg/L)	6 (5 – 6)	23 (23 – 23)
Chloride (mg/L)	< 0.1 (–)	3 (3 – 3)
Sulfate (mg/L)	41 (40 – 42))	150 (145 – 155)

Table 5. Summary of **water quality** characteristics (mean and range in parentheses) of test water used in acute toxicity tests with five life stages of *Hyalella azteca* exposed to five fire retardant chemicals in ASTM soft and hard waters.

Characteristic	Water Type	
	Soft (N=7)	Hard (N=7)
pH	7.45 (7.31 – 7.50)	8.36 (8.31 – 8.40)
Conductivity (μ hos/cm @ 25° C)	162 (157 – 166)	560 (557 – 562)
Hardness (mg/L as CaCO ₃)	41 (40 – 42)	162 (162 – 164)
Alkalinity (mg/L as CaCO ₃)	32 (31 – 32)	111 (110 – 112)
Calcium (mg/L)	7 (7 – 7)	28 (27 – 28)
Magnesium (mg/L)	6 (5 – 6)	24 (23 – 24)
Chloride (mg/L)	< 0.1 (–)	3 (3 – 3)
Sulfate (mg/L)	35 (34 – 36)	171 (164 – 178)

Table 6. Mean **water quality** characteristics (range in parentheses) of test waters used in acute toxicity tests with **chinook salmon**.

Characteristic	Water Type	
	Soft (N=7)	Hard (N=7)
pH	7.4 (7.3 – 7.5)	8.2 (8.1 – 8.2)
Conductivity ($\mu\text{hos/cm @ } 25^\circ \text{ C}$)	162 (159 – 168)	545 (537 – 552)
Hardness (mg/L as CaCO_3)	40 (40 – 41)	162 (160 – 164)
Alkalinity (mg/L as CaCO_3)	32 (32 – 32)	110 (110 – 113)
Calcium (mg/L)	7 (7 – 7)	27 (27 – 27)
Magnesium (mg/L)	6 (6 – 6)	23 (22 – 23)
Chloride (mg/L)	<0.1 (–)	172 (166 – 184)
Sulfate (mg/L)	4 (3 - 4)	40 (38 - 40)

Table 7. Life stages of **rainbow trout** tested with five fire retardant chemicals in ASTM soft and hard waters. Sizes are mean (range in parenthesis) of 10 control fish.

Life Stage	Water Type	Age ^a	Weight(g)	Length (mm)
Egg	Soft	373 ^b	0.0898 (0.0896-0.0899)	0.095 ml ^d d=5.66 mm
	Hard	373 ^b	0.0898 (0.0896-0.0899) ^c	0.095 ml ^d d=5.66 mm
Embryo-larval	Soft	527 ^b	-	-
	Hard	527 ^b	-	-
Sac-fry	Soft	5	0.101 (0.076-0.120) ^e	21 (19-22) ^e
	Hard	5	0.101 (0.076-0.120) ^e	21 (19-22) ^e
Swim-up	Soft	21	0.094 (0.069-0.114) ^e	25 (23-26) ^e
	Hard	21	0.094 (0.069-0.114) ^e	25 (23-26) ^e
60 day	Soft	65	0.622 (0.252-1.002) ^f	44 (34-52) ^f
	Hard	58	0.415 (0.220-0.663) ^f	39 (33-45) ^f
90 day	Soft	90	1.496 (0.798-2.296) ^g	57 (47-64) ^g
	Hard	83	1.189 (0.813-1.735) ^g	53 (49-60) ^g
Fish source: Ennis NFH, Ennis, MT. Strain: McConaghy ^a = Days post median hatch day to test initiation; ^b = Daily temperature units (DTU) to test initiation. 1 DTU = 1F for 24 hours ^c = Average of 2 pools of 10 eggs each ^d = Volume displacement; d = diameter ^e N = 20 (pool of hard and soft control treatments) ^f N = 20 ^g N = 30				

Table 8. Life stages of **fathead minnow** tested with five fire retardant chemicals in ASTM soft and hard waters. Sizes are mean (range in parentheses) of control fish.

Life Stage	Water type	Lot no.	Age ^a	Weight (g)	Length (mm)
Egg	Soft	1	3 ^b	0.009 ^c	-
	Hard	1	3 ^b	0.009 ^c	-
Swim-up	Soft	2	1	0.008 ^d	6 (5-6) ^e
	Hard	3	1	0.003 ^d	5 (4-5) ^e
30 day	Soft	4	32-38	0.041 (0.023-0.076) ^e	18 (16-22) ^e
	Hard	4	30-36	0.032 (0.011-0.050) ^e	16 (13-19) ^e
60 day	Soft	4	53-59	0.118 (0.034-0.298) ^f	24 (17-32) ^f
	Hard	4	53-59	0.118 (0.034-0.298) ^f	24 (17-32) ^f

Fish source: NFCRC, Columbia, MO.
^a =Days post hatch day to test initiation;
^b =Days post fertilization to test initiation;
^c =Pooled weight of 10 eggs;
^d =Pooled weight of 10 fry;
^e N=10;
^f N=20 (pool of hard and soft treatments).

Table 9. Life stages of **chinook salmon** tested with five fire control chemicals in ASTM soft and hard waters. Sizes are mean (range in parentheses) of control fish.

Life Stage	Water type	Age ^a	Weight (g)	Length (mm)	n
Egg	Soft	647-659 ^b	0.205 ^c	0.19 mL ^d	10
	Hard	647-659 ^b	0.193 ^c	0.18 mL ^d	10
Swim-up	Soft	29	0.284	35	20
			(0.196-0.356)	(31-38)	
	Hard	291	0.291	35	20
			(0.210-0.368)	(33-37)	
60 day	Soft	57	0.850	51	20
			(0.670-0.962)	(45-53)	
	Hard	57	0.032	50	20
			(0.603-0.868)	(46-53)	
90 day	Soft	101	2.863	74	30
			(2.118-3.549)	(66-78)	
	Hard	92	2.555	71	29
			(1.960-3.168)	(67-77)	

^a = Days post median hatch to test initiation.
^b = Daily temperature units to test initiation.
^c = Calculated from pooled weight of 10 eggs.
^d = Calculated from pooled volume displacement of 10 eggs

Table 10. Acute toxicity (48-hour LC₅₀; mg/L; 95% confidence interval in parentheses) of five fire retardant chemicals to **rainbow trout** exposed at 12° C in ASTM soft and hard waters at five life stages.

Water type	Life stage				
	Egg	Embryo-larval	Swim-up	60 day	90 day
Fire-Trol LCG-R					
Soft	> 10,000	> 3600	954 (764-1173)	1481 (1171-1810)	1676 ^d (1300-2160)
Hard	> 10,000	> 6000	1011 (817-1252)	1676 ^d (1300-2160)	1682 (1362-2081)
Fire-Trol GTS-R					
Soft	> 3600	> 3600	412 (336-523)	432 (352-555)	490 (378-597)
Hard	> 6000	> 6000	218 ^b (170-280)	269 (221-347)	246 (202-310)
Phos-Chek D-75-F					
Soft	> 1700	316 ^c (258-400)	279 ^d (216-360)	234 (191-291)	218 ^d (170-280)
Hard	> 3600	435 ^c (348-534)	218 ^d (170-280)	218 ^d (170-280)	218 ^d (170-280)
Phos-Chek WD-881					
Soft	>47	20 (16-25)	13 ^d (12-17)	16 (13-20)	20 ^d (17-28)
Hard	32 (25-39)	20 (16-25)	11 (9-14)	13 ^d (10-17)	13 ^d (10-17)
Silv-Ex					
Soft	> 78	38 (31-47)	20 (16-25)	22 ^d (17-28)	22 ^d (17-28)
Hard	69 (56-87)	43 (35-56)	14 (11-18)	16 (13-20)	22 ^d (17-28)

Table 10 cont. Acute toxicity (96-hour LC₅₀; mg/L; 95% confidence interval in parentheses) of five fire retardant chemicals to **rainbow trout** exposed at 12° C in ASTM soft and hard waters at five life stages.

Chemical	Water type	Life stage				
		Egg	Embryo-larval	Swim-up	60 day	90 day
Fire-Trol LCG-R	Soft	> 10,000	> 3600	910 (722-1115)	1080 (880-1353)	1413 (1105-1724)
	Hard	> 10,000	2642 ^a (2117-3249)	827 (685-1066)	1413 (1105-1724)	1006 ^d (780-1300)
Fire-Trol GTS-R	Soft	> 3600	718 (589-918)	363 ^d (280-470)	390 (316-489)	363 ^d (280-470)
	Hard	> 6000	606 (490-749)	207 ^b (170-280)	234 (191-291)	234 ^d (191-291)
Phos-Chek D-75-F	Soft	> 1700	266 ^c (213-327)	279 ^d (216-360)	234 (191-291)	218 ^d (170-280)
	Hard	> 3600	235 ^c (183-287)	218 ^d (170-280)	218 ^d (170-280)	218 ^d (170-280)
Phos Chek WD-881	Soft	44 ^e	13 ^d (10-17)	13 ^d (10-17)	15 (12-19)	20 (16-25)
	Hard	22 (18-27)	10 (8-13)	11 (9-14)	13 (10-17)	13 ^d (10-17)
Silv-Ex	Soft	> 78	15 (12-20)	20 (16-25)	22 ^d (17-28)	22 ^d (17-28)
	Hard	47 (38-62)	11 (9-14)	13 ^d (10-17)	14 (11-18)	15 (12-19)

^a LC₅₀ calculated with 1300 mg/L test concentration omitted.

^b LC₅₀ calculated using binomial test.

^c Tests were started with true sac-fry.

^d= No partial kills; 95% confidence interval: lower limit = highest test concentration with 0% mortality, and upper limit = lowest test concentration with 100% mortality.

^e= LC₅₀ calculated by binomial test; less than 70% mortality in highest test concentration.

Table 11. Acute toxicity (48-hour LC₅₀; mg/L; 95% confidence interval in parentheses) of five fire retardant chemicals to fathead minnow exposed in ASTM soft and hard waters at four life stages.

Chemical	Water Type	Life Stage			
		Egg ^a	Swim-Up	30-day	60 day
Fire-Trol LCG-R	Soft	3233 (2646-4118)	1394 (1086-1700)	1676 ^c (1300-2160)	1797 (1466-2247)
	Hard	>10,000	2569 (2047-3151)	1569 (1255-1924)	1676 ^c (1300-2160)
Fire-Trol GTS-R	Soft	1413 (1105-1724)	363 ^c (280-470)	528 ^b (280-780)	605 ^c (470-780)
	Hard	2463 (1945-3013)	222 (191-272)	193 (153-235)	320 (252-392)
Phos-Chek D-75-F	Soft	2789 (2253-3451)	652 (509-797)	572 (455-780)	746 (576-952)
	Hard	2789 (2160-3600)	545 (433-731)	262 (2116-336)	535 (424-654)
Phos Chek WD-881	Soft	36 ^b (28-47)	16 (13-20)	22 ^c (17-28)	22 ^c (17-28)
	Hard	28 (23-37)	14 (11-17)	13 ^c (10-17)	13 ^c (10-17)
Silv-Ex	Soft	36 ^c (28-47)	22 ^c (17-28)	20 (16-25)	22 ^c (17-28)
	Hard	30 (14-37)	20 (16-25)	19 (15-24)	22 ^c (17-28)

^a Test temperature was 20° C for all tests with eggs; all other life stages were tested at 25° C.

^b LC₅₀ calculated using binomial test.

^c = No partial kills; 95% CI ; lower limit = highest test concentration with 0% mortality, and upper limit = lowest test concentration with 100% mortality.

Table 11 cont. Acute toxicity (96-hour LC₅₀; mg/L; 95% confidence interval in parentheses) of five fire retardant chemicals to **fathead minnow** exposed in ASTM soft and hard waters at four life stages.

Chemical	Water Type	Life Stage			
		Egg ^a	Swim-Up	30-day	60 day
Fire-Trol LCG-R	Soft	2317 (1802-2830)	1080 (880-1353)	1676 ^c (1300-2160)	1797 (1466-2247)
	Hard	7037 ^b (3600-10000)	519 (389-654)	1181 (937-1584)	1676 ^c (1300-2160)
Fire-Trol GTS-R	Soft	787 (529-1025)	233 (184-301)	528 ^b (280-780)	605 ^c (470-780)
	Hard	363 ^b (280-470)	135 (105-165)	193 (153-235)	320 (252-392)
Phos-Chek D-75-F	Soft	2250 (1736-2748)	420 (320-532)	572 (455-780)	612 (444-770)
	Hard	1569 (1255-1924)	168 (136-207)	237 (194-296)	490 (378-597)
Phos Chek WD-881	Soft	32 ^b (10-47)	14 (11-17)	22 ^c (17-28)	22 ^c (17-28)
	Hard	26 (21-33)	14 (11-17)	13 ^c (10-17)	13 ^c (10-17)
Silv-Ex	Soft	32 (25-39)	22 ^c (17-28)	20 (16-25)	22 ^c (17-28)
	Hard	28 (23-37)	20 (16-25)	19 (15-24)	22 ^c (17-28)

^a Test temperature was 20° C for all tests with eggs; all other life stages were tested at 25° C.
^b LC₅₀ calculated using binomial test.
^c = No partial kills; 95% CI ; lower limit = highest test concentration with 0% mortality, and upper limit = lowest test concentration with 100% mortality.

Table 12. Acute toxicity (**48-hour LC₅₀**; mg/L; 95% confidence interval in parentheses) of five fire retardant chemicals to early life stages of **chinook salmon** exposed in ASTM soft and hard waters at 12° C.

Chemical	Water Type	Life Stage			
		Egg ^a	Swim-Up	60-day	90 day
Fire-Trol LCG-R	Soft	> 10,000	1141 (933-1445)	1195 (979-1532)	1141 (933-1445)
	Hard	> 10,000	1195 (979-1532)	1481 (1171-1810)	1007 ^a (780-1300)
Fire-Trol GTS-R	Soft	> 3600	404 (329-511)	470 (383-619)	390 (316-489)
	Hard	> 6000	258 (212-329)	280 (230-365)	234 (191-291)
Phos-Chek D-75-F	Soft	> 1700	218 ^a (170-280)	305 (212-401)	218 ^a (170-280)
	Hard	> 3600	218 ^a (170-280)	269 (221-347)	218 ^a (170-280)
Phos Chek WD-881	Soft	> 47	13 ^a (10-17)	16 (13-20)	17 (14-23)
	Hard	44 (36-57)	12 (10-15)	13 ^a (10-17)	13 ^a (10-17)
Silv-Ex	Soft	> 78	22 ^a (17-28)	22 ^a (17-28)	22 ^a (17-28)
	Hard	> 130	19 (15-24)	22 ^a (17-28)	14 (11-17)

^a No partial kills; 95% CI; lower limit = highest test concentration with 0% mortality, and upper limit = lowest test concentration with 100% mortality.

^b 50% mortality in highest test concentration.

Table 12 cont. Acute toxicity (96-hour LC₅₀; mg/L; 95% confidence interval in parentheses) of five fire retardant chemicals to early life stages of **chinook salmon** exposed in ASTM soft and hard waters at 12° C.

Chemical	Water Type	Life Stage			
		Egg ^a	Swim-Up	60-day	90 day
Fire-Trol LCG-R	Soft	> 10,000	1141 (933-1445)	1195 (979-1532)	1080 (880-1353)
	Hard	> 10,000	1007 ^a (780-1300)	969 (748-1237)	685 (561-866)
Fire-Trol GTS-R	Soft	> 3600	385 (312-482)	412 (336-523)	363 ^a (280-470)
	Hard	> 6000	218 ^b (170-280)	269 (221-347)	218 ^a (170-280)
Phos-Chek D-75-F	Soft	> 1700	218 ^b (170-280)	305 (212-404)	218 ^a (170-280)
	Hard	> 3600	218 ^b (170-280)	258 (212-329)	218 ^a (170-280)
Phos Chek WD-881	Soft	47 ^b	13 ^b (10-17)	13 ^b (10-17)	13 ^a (10-17)
	Hard	29 (21-36)	10 (8-13)	8 (6-10)	7 (6-9)
Silv-Ex	Soft	39 (32-49)	22 ^a (17-28)	22 ^a (17-28)	16 (13-21)
	Hard	43 (35-56)	17 (14-23)	22 ^a (17-28)	11 (9-14)
^a No partial kills; 95% CI; lower limit = highest test concentration with 0% mortality, and upper limit = lowest test concentration with 100% mortality. ^b 50% mortality in highest test concentration.					

Table 13. Summary of the **ammonia** characteristics of five fire retardant chemicals used in tests with **rainbow trout** exposed in ASTM soft and hard waters. An ammonia equation was fitted for each life stage tested using the total ammonia as nitrogen (NH₃-N) concentrations determined at test initiation. Each regression model was used to predict NH₃-N (mg/L) at the 96-hour LC50 (parentheses).

Chemical and Life Stage	Water type			
	Soft		Hard	
	Regression equation ^a	NH ₃ -H mg/L at LC ₅₀	Regression equation ^a	NH ₃ -H mg/L at LC ₅₀
Fire-Trol LCG-R				
Egg	NH ₃ -N = -2.954 + 0.110 * FC adj R ² =1.0000 N=3	>1097 mg/L (>10000)	NH ₃ -N = 1.700 + 0.114 * FC adj R ² =1.0000 N=3	>1142 mg/L (>10000)
Embryo-larval	NH ₃ -N = -0.834 + 0.107 * FC adj R ² =1.0000 N=3	>384 mg/L (>3600)	NH ₃ -N = -12.763 + 0.115 * FC adj R ² =0.9991 N=3	291 mg/L (2642)
Swim-up	NH ₃ -N = 0.326 + 0.133 * FC adj R ² =0.9999 N=3	121 mg/L (910)	NH ₃ -N = 0.337 + 0.139 * FC adj R ² =0.9999 N=3	122 mg/L (872)
60 day	NH ₃ -N = -18.983 + 0.209 * FC adj R ² =0.9993 N=3	207 mg/L (1080)	NH ₃ -N = -3.451 + 0.104 * FC adj R ² =0.9999 N=3	144 mg/L (1413)
90 day	NH ₃ -N = -5.295 + 0.119 * FC adj R ² =1.0000 N=3	163 mg/L (1413)	NH ₃ -N = -5.155 + 0.114 * FC adj R ² =0.9998 N=6	110 mg/L (1006)
Fire-Trol GTS-R				
Egg	NH ₃ -N = -2.726 + 0.213 * FC adj R ² =1.0000 N=3	>764 mg/L (>3600)	NH ₃ -N = -5.452 + 0.232 * FC adj R ² =1.0000 N=3	>1387 mg/L (>6000)
Embryo-larval	NH ₃ -N = -1.597 + 0.204 * FC adj R ² =1.0000 N=3	145 mg/L (718)	NH ₃ -N = -8.410 + 0.235 * FC adj R ² =0.9998 N=3	134 mg/L (606)
Swim-up	NH ₃ -N = -9.270 + 0.269 * FC adj R ² =0.9997 N=3	88 mg/L (363)	NH ₃ -N = -15.986 + 0.286 * FC adj R ² =0.9997 N=3	43 mg/L (207)
60 day	NH ₃ -N = -3.248 + 0.203 * FC adj R ² =0.9992 N=3	76 mg/L (390)	NH ₃ -N = -0.985 + 0.197 * FC adj R ² =0.9999 N=3	45 mg/L (234)
90 day	NH ₃ -N = -1.741 + 0.227 * FC adj R ² =1.0000 N=6	81 mg/L (363)	NH ₃ -N = -1.435 + 0.218 * FC adj R ² =1.0000 N=6	50 mg/L (234)

Table 13 cont. Summary of the **ammonia** characteristics of five fire retardant chemicals used in tests with **rainbow trout** exposed in ASTM soft and hard waters. A regression equation was fitted for each life stage tested using the total ammonia as nitrogen (NH₃-N) concentrations determined at test initiation. Each regression model was used to predict NH₃-N (mg/L) at the 96-hour LC₅₀ (parentheses).

Chemical and Life Stage	Water type			
	Soft		Hard	
	Regression equation ^a	NH ₃ -H mg/L at LC ₅₀	Regression equation ^a	NH ₃ -H mg/L at LC ₅₀
Phos-Chek D-75-F				
Egg	NH ₃ -N = 62.257 + 0.166 * FC adj R ² =0.6365 N=3	>344 mg/L (>1700)	NH ₃ -N = -2.617 + 0.205 * FC adj R ² =1.0000 N=3	>735 mg/L (>3600)
Embryo-larval	NH ₃ -N = -2.215 + 0.173 * FC adj R ² =1.0000 N=3	44 mg/L (266)	NH ₃ -N = -0.289 + 0.153 * FC adj R ² =1.0000 N=3	36 mg/L (235)
Swim-up	NH ₃ -N = -5.860 + 0.252 * FC adj R ² =0.9999 N=3	64 mg/L (279)	NH ₃ -N = 50.144 + 0.166 * FC adj R ² =0.9943 N=3	86 mg/L (218)
60 day	NH ₃ -N = -11.526 + 0.351 * FC adj R ² =0.9992 N=3	71 mg/L ((234)	NH ₃ -N = -4.111 + 0.186 * FC adj R ² =0.9994 N=3	36 mg/L (218)
90 day	NH ₃ -N = 0.465 + 0.199 * FC adj R ² =1.0000 N=6	44 mg/L (218)	NH ₃ -N = -0.0644 + 0.193 * FC adj R ² =1.0000 N=6	42 mg/L (218)
Phos-Chek WD-881				
Egg	NH ₃ -N = 0.0451 + 0.0000801 * FC adj R ² = -0.773 N=3	0.05 mg/L (44)	NH ₃ -N = 0.0388 + 0.000141 * FC adj R ² = 0.9318 N=3	0.04 mg/L (22)
Embryo-larval	NH ₃ -N = 0.0414 + -0.0000739 * FC adj R ² = -0.9357 N=3	0.04 mg/L (13)	NH ₃ -N =0.0351 + 0.0000476 * FC adj R ² = -0.7802 N=3	0.04 mg/L (10)
Swim-up	NH ₃ -N = 0.0463 + -0.000154 * FC adj R ² = -0.1624 N=3	0.04 mg/L (13)	NH ₃ -N =0.0312 + -0.000141 * FC adj R ² = 0.9318 N=3	0.03 mg/L (11)
60 day	NH ₃ -N =0.0412 + -0.000141 * FC adj R ² = 0.9318 N=3	0.04 mg/L (15)	NH ₃ -N = 0.03 + (no slope) adj R ² = -- N=3	0.03 mg/L (13)

Table 13 cont. Summary of the **ammonia** characteristics of five fire retardant chemicals used in tests with **rainbow trout** exposed in ASTM soft and hard waters. A regression equation was fitted for each life stage tested using the total ammonia as nitrogen (NH₃-N) concentrations determined at test initiation. Each regression model was used to predict NH₃-N (mg/L) at the 96-hour LC₅₀ (parentheses).

Chemical and Life Stage	Water type			
	Soft		Hard	
	Regression equation ^a	NH ₃ -H mg/L at LC ₅₀	Regression equation ^a	NH ₃ -H mg/L at LC ₅₀
Silv-Ex				
Egg	NH ₃ -N = 0.0492 + 0.00846 * FC adj R ² =0.9999 N=3	>0.71 mg/L (>78)	NH ₃ -N = -0.0238 + 0.00896 * FC adj R ² =0.9999 N=3	0.44 mg/L (47)
Embryo-larval	NH ₃ -N = 0.0494 + 0.00743 * FC adj R ² =0.9994 N=3	0.16 mg/L (15)	NH ₃ -N = 0.0373 + 0.00803 * FC adj R ² =0.9996 N=3	0.13 mg/L (11)
Swim-up	NH ₃ -N = 0.0439 + 0.0078 * FC adj R ² =0.9969 N=3	0.20 mg/L (20)	NH ₃ -N = 0.0351 + 0.00857 * FC adj R ² =0.9999 N=3	0.15 mg/L (13)
60 day	NH ₃ -N = 0.023 + 0.0107 * FC adj R ² =0.9983 N=3	0.26 mg/L (22)	NH ₃ -N = 0.0644 + 0.00524 * FC adj R ² =0.9887 N=3	0.14 mg/L (14)
90 day	NH ₃ -N = 0.0304 + 0.00775 * FC adj R ² =0.9985 N=6	0.20 mg/L (22)	NH ₃ -N = 0.0308 + 0.00794 * FC adj R ² =0.9992 N=6	0.15 mg/L (15)
^a NH ₃ -N = total ammonia as nitrogen (mg/L) used as dependent variable in regression model FC = fire chemical concentration (mg/L) used as independent variable in regression model Adjusted R ² = 1-(1-R ²) (n-1)/df error.				

Table 14. Summary of the **ammonia** characteristics of five fire retardant chemicals used in tests with **fathead minnow** exposed in ASTM soft and hard waters. A regression equation was fitted for each life stage tested using the total ammonia as nitrogen (NH₃-N) concentrations determined at test initiation. Each regression model was used to predict NH₃-N (mg/L) at the 96-hour LC₅₀ (in parentheses).

	Water type			
	Soft		Hard	
Chemical and Life Stage	Regression equation ^a	NH ₃ -H mg/L at LC ₅₀	Regression equation ^a	NH ₃ -H mg/L at LC ₅₀
Fire-Trol LCG-R				
Egg (Temp=20)	NH ₃ -N = 6.367 + 0.0979 * FC adj R ² =0.9999 N=3	233 mg/L (2317)	NH ₃ -N = -10.295 + 0.0938 * FC adj R ² =0.9994 N=3	670 mg/L (7037)
Fry	NH ₃ -N = 1.785 + 0.104 * FC adj R ² =0.9998 N=3	117 mg/L (1080)	NH ₃ -N = -0.845 + 0.105 * FC adj R ² =1.0000 N=3	54 mg/L (519)
30 day	NH ₃ -N = -2.623 + 0.0848 * FC adj R ² =0.9999 N=3	140 mg/L (1676)	NH ₃ -N = -1.879 + 0.110 * FC adj R ² =0.9999 N=3	128 mg/L (1181)
60 day	NH ₃ -N = 0.608 + 0.108 * FC adj R ² =1.0000 N=3	195 mg/L (1797)	NH ₃ -N = 1.028 + 0.103 * FC adj R ² =1.0000 N=3	174 mg/L (1676)
Fire-Trol GTS-R				
Egg (Temp=20)	NH ₃ -N = 73.069 + 0.134 * FC adj R ² =0.9829 N=3	179 mg/L (787)	NH ₃ -N = 71.589 + 0.129 * FC adj R ² =0.9834 N=3	118 mg/L (363)
Fry	NH ₃ -N = -4.160 + 0.211 * FC adj R ² =0.9999 N=3	45 mg/L (233)	NH ₃ -N = -5.600 + 0.216 * FC adj R ² =0.9997 N=3	24 mg/L (135)
30 day	NH ₃ -N = -0.666 + 0.159 * FC adj R ² =0.9998 N=3	86 mg/L (528)	NH ₃ -N = -0.251 + 0.202 * FC adj R ² =1.0000 N=3	39 mg/L (193)
60 day	NH ₃ -N = -0.594 + 0.207 * FC adj R ² =1.0000 N=3	125 mg/L (605)	NH ₃ -N = -1.746 + 0.216 * FC adj R ² =0.9997 N=3	67 mg/L (320)

Table 14 cont. Summary of the **ammonia** characteristics of five fire retardant chemicals used in tests with **fathead minnow** exposed in ASTM soft and hard waters. A regression equation was fitted for each life stage tested using the total ammonia as nitrogen (NH₃-N) concentrations determined at test initiation. Each regression model was used to predict NH₃-N (mg/L) at the 96-hour LC₅₀ (parentheses).

Chemical and Life Stage	Water type			
	Soft		Hard	
	Regression equation ^a	NH ₃ -H mg/L at LC ₅₀	Regression equation ^a	NH ₃ -H mg/L at LC ₅₀
Phos-Chek D-75-F				
Egg	NH ₃ -N = 66.657 + 0.105 * FC adj R ² =0.9784 N=3	303 mg/L (2250)	NH ₃ -N = 69.320 + 0.109 * FC adj R ² =0.9784 N=3	240 mg/L (1569)
Fry	NH ₃ -N = -0.106 + 0.188 * FC adj R ² =0.9999 N=3	79 mg/L (420)	NH ₃ -N = -1.709 + 0.182 * FC adj R ² =0.9999 N=3	29 mg/L (168)
30 day	NH ₃ -N = -0.232 + 0.144 * FC adj R ² =1.0000 N=3	82 mg/L (572)	NH ₃ -N = -0.062 + 0.179 * FC adj R ² =1.0000 N=3	42 mg/L (237)
60 day	NH ₃ -N = -1.181 + 0.186 * FC adj R ² =0.9998 N=3	113 mg/L (612)	NH ₃ -N = 1.882 + 0.168 * FC adj R ² =0.998 N=6	84 mg/L (490)
Phos-Chek WD-881				
Egg	NH ₃ -N = 0.0425 + 0.000388 * FC adj R ² = -0.7733 N=3	0.05 mg/L (32)	NH ₃ -N = 0.0364 + -0.0000936 * FC adj R ² = 0.9318 N=3	0.03 mg/L (26)
Fry	NH ₃ -N = 0.0463 + -0.000154 * FC adj R ² = -0.1624 N=3	0.04 mg/L (14)	NH ₃ -N = 0.0288 + -0.000141 * FC adj R ² = 0.9318 N=3	0.03 mg/L (14)
30 day	NH ₃ -N = 0.03 + (no slope) adj R ² = -- N=3	0.03 mg/L (22)	NH ₃ -N = 0.0414 + -0.000143 * FC adj R ² = 0.9429 N=3	0.04 mg/L (13)
60 day	NH ₃ -N = 0.0464 + -0.0000936 adj R ² = - 0.1517 N=3	0.04 mg/L (22)	NH ₃ -N = 0.0740 + -0.000328 * FC adj R ² =0.4923 N=3	0.07 mg/L (13)

Table 14 cont. Summary of the **ammonia** characteristics of five fire retardant chemicals used in tests with **fathead minnow** exposed in ASTM soft and hard waters. A regression equation was fitted for each life stage tested using the total ammonia as nitrogen (NH₃-N) concentrations determined at test initiation. Each regression model was used to predict NH₃-N (mg/L) at the 96-hour LC₅₀ (parentheses).

Chemical and Life Stage	Water type			
	Soft		Hard	
	Regression equation ^a	NH ₃ -H mg/L at LC ₅₀	Regression equation ^a	NH ₃ -H mg/L at LC ₅₀
Silv-Ex				
Egg	NH ₃ -N = 0.0542 + 0.00729 * FC adj R ² =0.9971 N=3	0.29 mg/L (32)	NH ₃ -N = -0.0564 + 0.00755 * FC adj R ² =0.9996 N=3	0.27 mg/L (28)
Fry	NH ₃ -N = 0.0468 + 0.00823 * FC adj R ² =0.9993 N=3	0.23 mg/L (22)	NH ₃ -N = 0.0401 + 0.00699 * FC adj R ² =0.9997 N=3	0.18 mg/L (20)
30 day	NH ₃ -N = 0.0294 + 0.00578 * FC adj R ² =0.9998 N=3	0.15 mg/L (20)	NH ₃ -N = 0.0325 + 0.00753 * FC adj R ² =1.0000 N=3	0.18 mg/L (19)
60 day	NH ₃ -N = 0.0570 + 0.00720 * FC adj R ² =0.9983 N=3	0.22 mg/L (22)	NH ₃ -N = 0.0604 + 0.00719 * FC adj R ² =0.9989 N=3	0.22 mg/L (22)
^a NH ₃ -N = total ammonia as nitrogen (mg/L) used as dependent variable in regression model FC = fire chemical concentration (mg/L) used as independent variable in regression model Adjusted R ² = 1-(1-R ²) (n-1)/df error.				

Table 15. Summary of total ammonia as nitrogen (NH₃-N), un-ionized ammonia (NH₃), nitrate as nitrogen (NO₃-N), and nitrite as nitrogen (NO₂-N) concentrations (mg/L) at the 96-h LC₅₀ of five fire control chemicals tested with **chinook salmon** in ASTM soft and hard water.

Chemical and Life Stage	Water type							
	Soft				Hard			
	NH ₃ -N	NH ₃	NO ₃ -N	NO ₂ -N	NH ₃ -N	NH ₃	NO ₃ -N	NO ₂ -N
Fire-Trol LCG-R								
Egg	>1067	>2.82	>60.1	>540	>985	>3.27	>60.1	>540
Swim Up	116	0.19 – 0.31	3.73	50.7	102	0.21 – 0.34	2.88	43.3
Juv. 60 Day	132	0.22 – 0.35	4.08	53.7	107	0.28 – 0.35	2.64	41.2
Juv 90 Day	133	0.28 – 0.35	3.35	47.4	70	0.18 – 0.37	0.84	25.5
Fire-Trol GTS-R								
Egg	>775	>6.45	>3.23	>67.6	>1307	>21.7	>5.41	>113
Swim Up	79	0.52 – 0.66	0.31	6.92	443	0.56 – 0.88	0.15	3.77
Juv. 60 Day	92	0.61 – 0.96	0.33	7.43	56	0.73 – 1.45	0.20	4.73
Juv 90 Day	75	0.62 – 0.78	0.29	6.51	44	0.58 – 1.44	0.15	3.77
Phos-Chek D75-F								
Egg	>321	> 0.85	--	--	>744	>6.19	--	--
Swim Up	40	0.03 – 0.11	--	--	40	0.05 – 0.42	--	--
Juv. 60 Day	61	0.06 – 0.20	0.73	0.04	46	0.06 – 0.60	0.70	0.04
Juv 90 Day	35	0.03 – 0.12	--	--	36	0.06 – 0.60	--	--

Table 16. Acute toxicity (EC₅₀; mg/L; 95% confidence interval in parentheses) of five fire retardant chemicals to neonate *Daphnia magna* exposed in ASTM soft and hard waters.

Chemical	Water Type	Exposure	
		24 hour	48 hour
Fire-Trol LCG-R	Soft	1007 ^a (780-1300)	848 (662-1036)
	Hard	1676 ^a (1300-2160)	813 (672-992)
Fire-Trol GTS-R	Soft	780 ^b	257 (211-327)
	Hard	>780 ^c	339 (270-418)
Phos-Chek D-75-F	Soft	188 (124-256)	140 (113-177)
	Hard	280 (230-365)	280 (223-386)
Phos Chek WD-881	Soft	15 (11-18)	11 (9-14)
	Hard	8 (4-13)	4 (3-5)
Silv-Ex	Soft	10 (8-15)	7 (6-9)
	Hard	7 (6-9)	7 (5-8)

^a No partial kills; 95% CI; lower limit = highest test concentration with 0% mortality, and upper limit = lowest test concentration with 100% mortality.

^b 50% of the animals in the highest concentration were immobilized.

^c 40% of the animals in the highest concentration were immobilized.

Table 17. Summary of ammonia characteristics of five fire retardant chemicals used in tests with neonate *Daphnia magna* exposed in ASTM soft and hard waters.

Water Type	Conc.	Total Ammonia (mg/L)		Unionized ^a Ammonia (mg/L)	
		0 Hour	48 Hour	0 Hour	48 Hour
Fire-Trol LCG-R					
Soft	Low		23.44	0.10	0.31
	Med	96.07	104.66	0.56	1.82
	High	475.34	495.19 ^a	2.37	2.52 ^b
Hard	Low	13.67	13.62	0.26	0.81
	Med	101.57	106.53	0.57	1.86
	High	485.34	518.51 ^b	2.15	3.32 ^b
Fire-Trol GTS-R					
Soft	Low	5.32	5.70	0.14	0.17
	Med	39.23	42.99	0.73	1.07
	High	194.09	209.77 ^b	2.70	2.98 ^b
Hard	Low	6.76	5.48	0.51	0.46
	Med	47.91	39.71	1.96	2.46
	High	227.73	201.62 ^b	4.86	6.89 ^b
Phos-Chek D-75-F					
Soft	Low	2.74	2.66	0.05	0.46
	Med	20.21	22.04	0.15	2.46
	High	99.99	104.46 ^b	0.32	6.89 ^b
Hard	Low	22.12	25.64	0.48	0.05
	Med	178.07	178.17	0.89	0.39
	High	785.34	833.57 ^b	1.88	0.73 ^b
Phos-Chek WD-881					
Soft	Low	0.13	0.15	0.00	0.00
	Med	0.08	0.09	0.00	0.00
	High	0.11	0.10 ^b	0.00	0.00 ^b
Hard	Low	1.13	0.09	0.08	0.01
	Med	1.13	0.07 ^b	0.08	0.00 ^b
	High	0.14	0.02 ^b	0.09	0.00 ^b

Table 17 cont. Summary of ammonia characteristics of five fire retardant chemicals used in tests with neonate *Daphnia magna* exposed in ASTM soft and hard waters.

Water Type	Conc	Total Ammonia (mg/L)		Unionized ^a Ammonia (mg/L)	
		0 Hour	48 Hour	0 Hour	48 Hour
Silv-Ex					
Soft	Low	0.12	0.13	0.00	0.0
	Med	0.35	0.38 ^b	0.01	0.01 ^b
	High	1.26	1.42 ^b	0.04	0.05 ^b
Hard	Low	0.11	0.20	0.01	0.02
	Med	0.33	0.39 ^b	0.02	0.03 ^b
	High	12.28	1.37 ^b	0.09	0.11 ^b
^a Unionized ammonia = total ammonia adjusted for temperature and pH. ^b Measurements were made although all test animal were dead.					

Table 18. Acute toxicity (LC₅₀; mg/L; 95% confidence interval in parentheses) of five fire retardant chemicals to adult *Hyalella azteca* exposed in ASTM soft and hard waters.

		Exposure			
Chemical	Water Type	24 Hour	48 Hour	72 Hour	96 hour
Fire-Trol LCG-R	Soft	417 (329-560)	182 (135-227)	93 (65-165)	73 (42-115)
	Hard	961 (771-1183)	685 (561-866)	606 ^a (470-780)	535 (424-654)
Fire-Trol GTS-R	Soft	385 (312-482)	314 (246-384)	192 (128-262)	127 (92-172)
	Hard	813 (627-992)	635 (517-790)	441 (349-603)	363 (292-450)
Phos-Chek D-75-F	Soft	421 (317-610)	94 (69-148)	74 (49-108)	53 (49-65)
	Hard	974 (752-1244)	450 (356-620)	421 (317-610)	394 (310-519)
Phos-Chek WD-881	Soft	45 (34-57)	36 (24-47)	30 (21-43)	10 (6-17)
	Hard	46 (38-58)	35 (28-45)	28 (23-35)	22 (17-28)
Silv-Ex	Soft	35 (27-45)	31 (23-39)	26 (20-34)	24 (20-30)
	Hard	35 (27-45)	31 (23-39)	26 (20-34)	24 (20-30)

^a No partial kills; 95% CI: lower limit = highest test concentration with 0% mortality, and upper limit = lowest test concentration with 100% mortality.

Table 19. Summary of ammonia characteristics of five fire retardant chemicals used in tests with adult *Hyalella azteca* exposed in ASTM soft and hard waters.

		Total Ammonia (mg/L)				Unionized ^a Ammonia (mg/L)			
Water Type	Conc	0 Hour ^b	48 Hour ^b	96 Hour ^b	96 Hour ^c Exposure	0 Hour ^b	48 Hour ^b	96 Hour ^b	96 Hour ^c Exposure
Fire-Trol LCG-R									
Soft	Low	4.5	4.62	4.49	4.31	0.02	0.02	0.03	0.03
	Med	35.17	35.13	35.99	--	0.08	0.10	0.12	--
	High	169.38	-- ^d	--	--	0.58	--	--	--
Hard	Low	7.03	6.76	7.17	6.35	0.10	0.23	0.33	0.30
	Med	56.89	63.14	58.99	54.4	0.24	0.73	0.82	0.45
	High	281.49	--	--	--	1.09	--	--	--
Fire-Trol GTS-R									
Soft	Low	8.52	10.32	8.64	8.30	0.13	0.20	0.17	0.14
	Med	65.44	79.67	65.68	--	0.79	1.34	1.12	--
	High	311.07	--	--	--	3.95	--	--	--
Hard	Low	13.91	13.52	13.67	12.12	0.53	0.47	0.49	0.63
	Med	117.41	129.63	114.55	105.72	2.40	3.71	4.38	3.78
	High	564.16	--	--	--	12.89	--	--	--
Phos-Chek D-75-F									
Soft	Low	8.52	9.53	7.05	7.35	0.09	0.13	0.11	0.11
	Med	65.44	69.81	--	--	0.25	0.50	--	--
	High	311.07	--	--	--	0.76	--	--	--
Hard	Low	14.29	12.11	13.11	12.6	0.38	0.56	0.79	0.85
	Med	131.79	96.09	112.69	--	1.01	1.75	3.69	--
	High	507.78	--	--	--	1.53	--	--	--

Table 19 cont. Summary of ammonia characteristics of five fire retardant chemicals used in tests with adult *Hyalomma azteca* exposed in ASTM soft and hard waters.

		Total Ammonia (mg/L)				Unionized ^a Ammonia (mg/L)			
Water Type	Conc	0 Hour	48 Hour	96 Hour	96 Hour Exposure	0 Hour	48 Hour	96 Hour	96 Hour Exposure
Phos-Chek WD-881									
Soft	Low	0.09	0.07	0.06	0.20	0.00	0.00	0.00	0.00
	Med	0.03	0.03	0.03	0.29	0.00	0.00	0.00	0.00
	High	0.08	--	--	--	0.00	--	--	--
Hard	Low	0.04	0.02	0.03	--	0.00	0.00	0.00	--
	Med	0.03	0.02	0.02	0.20	0.00	0.00	0.00	0.01
	High	0.04	--	--	--	0.00	--	--	--
Silv-Ex									
Soft	Low	0.10	0.06	0.07	0.24	0.00	0.00	0.00	0.00
	Med	0.45	0.45	0.44	--	0.01	0.01	0.00	--
	High	2.10	--	--	--	0.03	--	--	--
Hard	Low	0.08	0.07	0.06	0.25	0.00	0.00	0.00	0.01
	Med	0.44	0.41	--	--	0.02	0.02	--	--
	High	1.93	--	--	--	0.09	--	--	--
^a Unionized ammonia = total ammonia adjusted for temperature and pH. ^b Measure in vessels with no test animals. ^c Measured in exposure vessels with test animals. ^d No measurement made because all test animals were dead.									

Table 20. Comparisons of acute toxicity values for five fire control chemicals tested with **rainbow trout** (RBT) and **chinook salmon** (FCS).

Chemical	Life stage	Water Type	96-h LC ₅₀ (mg/L)		Ratio: High / Low 96-h LC ₅₀	
			RBT ^a	FCS ^b		
Fire-Trol LCG-R	Swim-Up	Soft	910	1141	1.3	
		Hard	872	1007	1.2	
	Juvenile (60-day)	Soft	1080	1195	1.1	
		Hard	1413	969	1.5	
	Juvenile (90-Day)	Soft	1413	1080	1.3	
		Hard	1006	685	1.5	
	Fire-Trol GTS-R	Swim-Up	Soft	363	385	1.1
			Hard	207	218	1.1
Juvenile (60-day)		Soft	390	412	1.1	
		Hard	234	269	1.1	
Juvenile (90-Day)		Soft	363	363	1.0	
		Hard	234	218	1.1	
Phos-Chek D75-F	Swim-Up	Soft	279	218	1.3	
		Hard	218	218	1.0	
	Juvenile (60-day)	Soft	234	305	1.3	
		Hard	218	258	1.2	
	Juvenile (90-Day)	Soft	218	218	1.0	
		Hard	218	218	1.0	

Table 20 cont. Comparisons of acute toxicity values for five fire control chemicals tested with rainbow trout (RBT) and chinook salmon (FCS).

Chemical	Life stage	Water Type	96-h LC ₅₀ (mg/L)		Ratio: High / Low 96-h LC ₅₀	
			RBT ^a	FCS ^b		
Phos-Chek WD-881	Swim-Up	Soft	13	13	1.1	
		Hard	11	10	1.3	
	Juvenile (60-day)	Soft	15	13	1.0	
		Hard	13	8	1.6	
	Juvenile (90-Day)	Soft	20	13	1.4	
		Hard	13	7	1.4	
	Silv-Ex	Swim-Up	Soft	20	22	1.1
			Hard	13	17	1.3
Juvenile (60 Day)		Soft	22	22	1.0	
		Hard	14	22	1.6	
Juvenile (90 Day)		Soft	22	16	1.4	
		Hard	15	11	1.4	
^a Gaikowske 1994. ^b This study.						

Table 21. Acute toxicity values for five fire control chemicals reported by manufacturers or their contract laboratories. Acute toxicity values from this study are included for comparison.

Chemical	Species ^a	Weight (g)	Water Type	96-h LC ₅₀ (mg/L)	Reference
Fire-Trol LCG-R	RBT	NR ^b	NR	790	Chemonics (1992a)
	FCS	0.3-2.9	Soft	1082-1195	This Study
	FCS	0.3-2.6	Hard	685-1007	This Study
Fire-Trol GTS-R	RBT	0.5	Soft	899	C. Chang, Chemonics (pers. comm..)
	RBT	NR	NR	1000	Chemonics (1992b)
	FCS	0.3-2.9	Soft	363-412	This Study
	FCS	0.3-2.6	Hard	218-269	This Study
Phos-Chek D75-R	RBT	0.4	Soft	> 1000	ABC Laboratories (1986)
	FCS	0.3-2.9	Soft	218-305	This Study
	FCS	0.3-2.6	Hard	218-258	This Study
Phos-Chek WD-881	RBT	0.6	Soft	22	ABC Laboratories (1988)
	FCS	0.3-2.9	Soft	13	This Study
	FCS	0.3-2.6	Hard	7-10	This Study
Silv-Ex	RBT	0.4	Soft	25	Springborn Bionomics (1986)
	FCS	0.3-2.9	Soft	16-22	This Study
	FCS	0.3-2.6	Hard	11-22	This Study
^a RBT = rainbow trout, FCS = Chinook salmon. ^b NR = not reported.					

Table 22. Concentrations of five fire control chemicals used in tank mixtures and the ratio of the mixture concentration to its acute toxicity value.

Chemical	Tank mixture ^a (mg/L)		Water Type	Ratio: Tank mixture/ 96-h LC ₅₀ ^b
Fire-Trol GTS-R	1.66 lb/gal	198,930	Soft	184
			Hard	290
Fire-Trol LCG-R	1 gal/4.5 gal	270,400	Soft	745
			Hard	1240
Phos-Chek D75-F	1.2 lb/gal	143,8000	Soft	660
			Hard	660
Phos-Chek WD-881	1%	10,000	Soft	769
			Hard	1429
Silv-Ex	1%	10,000	Soft	625
			Hard	909
^a Weight or volume of chemical concentrate combined with water to produce application mixture. ^b 96-h LC ₅₀ for the most sensitive life stage of Chinook salmon.				

II. PROJECT OBJECTIVE 2: TOXICITY OF FIRE RETARDANT CHEMICALS AND FIRE SUPPRESSANT FOAMS TO VERTEBRATE AND INVERTEBRATE WILDLIFE SPECIES

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Objective:

- (1) Determine the toxicity of five commercially available and commonly used wildland fire retardant and foam products to specific vertebrate and invertebrate species.**

General Procedures:

Acute oral, and subacute and subchronic (as indicated from results of subacute tests) dietary toxicity tests on selected representative terrestrial vertebrates and earthworms were conducted with three commonly used fire retardants (Fire-Trol GTS-R, Phos-Chek D75-F, and Fire-Trol LCG-R) and two fire suppressant foams (Silv-Ex and Phos-Chek WD-881) which were also tested for aquatic toxicity in Objective 1. Chemical selection was based on consultation with the Bureau of Land Management, the Interior Fire Coordination Committee and results of preliminary aquatic toxicity tests. Northern bobwhite (*Colinus virginianus*), American kestrel (*Falco sparverius*), red-winged blackbird (*Agelaius phoeniceus*) and white-footed mouse (*Peromyscus leucopus*) were selected as primary vertebrate models and the earthworm (*Eisenia foetida*) as the invertebrate model.

Test Methods:

Acute Oral Testing. Acute toxicity tests with Fire-Trol GTS-R, Phos-Chek D75-F, Fire-Trol LCG-R, Silv-Ex and Phos-Chek WD-881 were conducted with northern bobwhite, American kestrel, and white-footed mouse. The standardized acute oral toxicity testing was designed to determine the single-dose 24-h median lethal dosage (LD₅₀). For the acute oral toxicity testing, chemicals were administered orally by gavage. Animals were carefully observed for evidence of toxicity for 24 hours and then all survivors were euthanized and critical tissues collected for chemical and

biochemical analysis. Procedures for administering test compounds in these experiments followed previously described protocols (American kestrel: Wiemeyer and Sparling 1991; red-winged blackbird: Grue 1982; and white-footed mouse: Rattner and Hoffman 1984).

Subacute Dietary Testing. For the subacute dietary toxicity testing of the white-footed mouse in 1994, chemicals were administered via feed for 8 consecutive days. Animals were observed for evidence of toxicity for the 8 days and then survivors were euthanized and critical tissues sampled for chemical and biochemical analysis. This test deviated from the standard 5-day subacute test to allow collection of blood samples from animals consuming contaminated feed and to generate information to define the acceptable interval between chemical application and small mammal population surveys during the field test in Nevada (summer 1994). In 1995, subacute dietary tests with bobwhite quail and the white-footed mouse followed the standardized subacute dietary toxicity testing to determine the dietary median lethal dosage (LD₅₀). Animals were exposed to the chemicals in feed for 5 consecutive days and then fed a clean (control) diet for 3 additional days. Animals were carefully observed for evidence of toxicity for the 8 days. Procedures are described in detail by Hill and Camardese (1986). All survivors were euthanized and critical tissues were collected for chemical and biochemical analysis. The limit test methodology was employed during testing and the exposure level was 5,000 mg chemical/kg feed (ppm).

Embryotoxicity / Hatchability. The effects of Phos-Chek G75-F and Silv-Ex on embryo survival, hatching success and chick survival of Northern bobwhite and red-winged blackbirds were evaluated by exposing eggs to the chemicals during either early or late stage of embryo development. Bobwhite eggs in early embryonic stage of development were treated with Silv-Ex 0.1%, 1.0%, or 10% solutions or with 0.11, 1.12, or 3.36 lbs of Phos-Chek G75-F /gallon of water. The late embryonic stage of development eggs were treated with Silv-Ex 10% solution, 3.36 lbs of Phos-Chek G75-F/gallon of water, or a mixture (Silv-Ex 5% + Phos-Chek G75F 1.68 lbs of Phos-Chek G75-F/gallon of water). Early and late embryonic development stage eggs of the blackbird were exposed to Silv-Ex 0.5% or 1.0% solutions or 0.56 or 1.12 lbs of Phos-Chek G75-F per gallon of water.

All chemicals were mixed in distilled water at room temperature. Eggs were dipped into either distilled water (control) or the treatment solutions for ten seconds. Procedures for this test followed previously described protocol by Hoffman (1988). Eggs were returned to the incubator after treatment. Eggs were candled weekly and eggs with embryo mortality and poor development were marked. Hatched birds were placed in brooder units and had access to feed and water ad libitum. They were checked each day for treatment effects and general health. All survivors were euthanized on day 14 post hatch. Data on embryo survival, embryo deformity, hatching success, and survival to 14 days post hatch and body weight were collected.

Biomarker Evaluation. In the biomarker test, chemicals were administered orally by gavage at 2,000 mg chemical/kg body weight (mg/kg). Animals were carefully observed for evidence of toxicity for a 14-day period. All survivors were euthanized and critical tissues were collected for chemical and biochemical analysis. Procedures for administering test compounds in these experiments followed previously described protocols (Hill and Camardese, 1984; Rattner and Hoffman, 1984). Blood was collected and body weights were measured on days 1, 4, and 8 days post dosage for the quail and on days 1 and 4 post dosage for the rats.

Earthworm Toxicity. Testing on earthworms was conducted using standardized methods as established by the European Economic Community for estimating the toxicity of chemicals to earthworms (Beyer et al., 1990). The earthworm bioassay was conducted in artificial soil consisting of 10% peat, 20% kaolin clay, 69% fine sand, and about 1% calcium carbonate. The calcium carbonate was added to adjust the pH to between 6 and 7, and water was added to give a 35% moisture content. The soil was added to one-liter glass jars. Half of the jars had the fire retardants mixed into the soil and half had the fire retardants spread on top. Surviving earthworms were counted after two weeks.

Results:

Acute Tests. The single-dose 24-h median lethal dosage (LD₅₀) for all five chemicals to adult northern bobwhite was determined to be above the pre-determined 2000 mg (active ingredient) per

kg body mass limit criteria for significant acute toxicity (Bascietto, 1985). No mortalities were observed and all animals appeared alert and active at all times post dosage (Table 1).

Initial testing of all chemicals on red-winged blackbird was conducted in outdoor pens at doses of 2,000 mg (active ingredient) per kg body mass. Tests resulted in mortality of 1 of 10 Phos-Chek D75-F treated birds and 3 of 10 Fire-Trol GTS-R treated birds. No mortalities occurred for the remaining three chemicals. Based on this study, we could not determine whether the mortalities resulted from the chemical substance, the cold ambient temperatures, or both. To eliminate cold temperature effects, additional testing on red-winged blackbirds was conducted outdoors during warmer weather. Birds were exposed to the following treatment levels: 1,300, 1,580, 1,900, 2,300 and 2,800 mg (active ingredient) per kg body mass. All 10 birds exposed to 1,300 mg (active ingredient) per kg body mass survived while 9 of 10 birds exposed to 2,800 mg (active ingredient) per kg body mass died. Thus, the LD₅₀ using the probit method is 2,197 mg (active ingredient) per kg body mass [95% CI: 1892 - 2574 mg (active ingredient) per kg body mass] and the slope of the dose response is 8.52 (95% CI: 3.5 - 13.5). The LD₅₀ for all five chemicals was greater than 2,000 mg (active ingredient) per kg body mass (Table 1) indicating that all of these chemicals are of a comparatively low order of acute toxicity (Smith 1987).

During 1993, initial limit tests on American kestrel were inconclusive because they regurgitated the chemical capsules. Further testing indicated that the cause of regurgitation was the time of dosing rather than the chemical substance. In 1994, tests revealed that kestrels at Patuxent Environmental Science Center were conditioned to being fed in the morning. Initial testing (1993) had been conducted in the morning. Kestrels and other birds of prey normally regurgitate (pellet) bones, feathers, and fur to make room for their next meal. The Patuxent Environmental Science Center kestrels were regurgitating the chemical in response to their routine morning feeding. Future testing on kestrels was conducted in the afternoon. The limit test uses one dose level of 2,000 mg (active ingredient) per kg body mass. In a series of tests, kestrels regurgitated all chemicals except Silv-Ex. This test demonstrated that the chemicals Fire-Trol GTS-R, Phos-Chek D75-F, and Fire-Trol LCG-R and Phos-Chek WD-881 induced regurgitation. Birds exposed to Silv-Ex exhibited

signs of toxicity similar to those in the initial testing including periods of stupor and lack of coordination. Since no mortalities were observed during the 24-h observation and Silv-Ex was not regurgitated, the LD₅₀ for Silv-Ex was determined to be greater than 2,000 mg (active ingredient) per kg body mass (Table 1), which is the limit criteria for significant acute toxicity. The LD₅₀ was not quantifiable for Fire-Trol GTS-R, Phos-Chek D75-F, and Fire-Trol LCG-R and Phos-Chek WD-881 because the birds regurgitated the chemicals (Table 1).

Acute oral toxicity testing with white-footed mouse was conducted using Fire-Trol GTS-R, Fire-Trol LCG-R, and Phos-Chek WD-881. No dose-related mortalities were observed. The LD₅₀ for all three chemicals was greater than 2,000 mg (active ingredient) per kg body mass, which is the limit criteria for significant acute toxicity (Table 1).

Subacute toxicity testing with white-footed mice was also conducted using Phos-Chek D-75F and Silv-Ex. The single-dose 24-h median lethal dosage (LD₅₀) for both chemicals was above the pre-determined 2000 mg (active ingredient) per kg body mass limit criteria for significant acute toxicity. No mortalities were observed (Table 1).

Earthworm Testing. In tests with earthworms, the LD₅₀ for all five chemicals were above the pre-determined 1000 ppm (active ingredient) limit criteria for significant acute toxicity.

Embryotoxicity / Hatchability. Embryotoxicity and hatching success were evaluated for bobwhite quail and red-winged blackbird with Phos-Chek G75-F and Silv-Ex. Embryo survival and hatching success were determined for both species while survival of the chicks to 14 days was measured only in the quail. These studies were undertaken to simulated chemical precipitation onto eggs during application. The chemicals may be harmful to the embryo by entering through the egg shell and membranes or by coating the egg such that gas exchange in and out of the egg may be blocked. The two species were selected because they represent two different types of development strategies. The quail embryo undergoes rapid development in the egg and the hatching chicks are precocial. By contrast, the blackbird represents birds whose embryo development rate is slow, compared to the quail, and therefore the blackbird chicks are altricial. No effects on embryo survival or hatching success have been observed, however, additional work with the two species is continuing.

Literature Cited:

- Grue, C.E. 1982. Response of common grackles to dietary concentrations of four organophosphorus pesticides. Arch. Environ. Contam. Toxicol. 11:617-626.
- Rattner, B.A., and D.J. Hoffman. 1984. Comparative toxicity of acephate in laboratory mice, white-footed mice and meadow voles. Arch. Environ. Contam. Toxicol. 13:483-491.
- Smith, G. J. (1987). Pesticide Use and Toxicology in Relation to Wildlife: Organophosphorus and Carbamate Compounds (U.S.F.W.S. Resource Publication No. 170). Washington DC: U.S. Government Printing Office.
- Wiemeyer, S.N. and D.W. Sparling. 1991. Acute toxicity of four anticholinesterase insecticides to American kestrels, eastern screech-owls and northern bobwhites. Environ. Toxicol. Chem. 10:1139-1148.

Table 1. Toxicity¹ of selected fire retardants and foam suppressants to terrestrial wildlife. All chemicals are of a comparatively low order of acute toxicity.

SPECIES	CHEMICAL				
	Fire-Trol GTS-R	Phos-Chek D75-F	Fire-Trol LCG-R	Silv-Ex	Phos-Chek WD-881
American kestrel	NQ ²	NQ ²	NQ ²	>2,000	NQ ²
Red-winged blackbird	2,197	>2,000	>2,000	>2,000	>2,000
Northern bobwhite	>2000	>2000	>2000	>2000	>2000
White-footed mouse	>2,000	>2,000	>2,000	>2,000	>2,000
Earthworm	>1,000	>1,000	>1,000	>1,000	>1,000

¹ Toxicity reported as LD₅₀ in mg active ingredient per kg body mass.

² **Test not quantifiable** (NQ) because birds regurgitated the chemicals as presented in gelatin capsule.

III. PROJECT OBJECTIVE 3: ECOLOGICAL EFFECTS OF FIRE RETARDANT AND SUPPRESSANT CHEMICALS

A. EFFECTS OF FIRE RETARDANT CHEMICALS AND FIRE SUPPRESSANT FOAMS ON TERRESTRIAL, AQUATIC, AND VEGETATIVE COMMUNITIES IN PRAIRIE WETLAND HABITAT---A study of a North Dakota prairie wetland community

In May 1993, studies were implemented to evaluate the response of the aquatic, terrestrial, and vegetative communities associated with a prairie wetland habitat to several fire-fighting chemicals. The vegetative and terrestrial components were exposed to the retardant, Phos-Chek G75-F, and a foam suppressant, Ansul Silv-Ex. In the aquatic ecosystem, two foam suppressants, Silv-Ex and Phos-Chek WD-881, were compared. The purpose of this phase of the study was not only to provide information on aquatic, terrestrial, and vegetative responses to fire-fighting chemicals in a wetland environment, but also to develop field assessment methods that could be used to determine the effects of these chemicals in a more complex ecological system such as the Great Basin area of Nevada, a study planned and implemented during the summer of 1994. The following sections summarize results from the North Dakota study.

Description of Study Site:

The 1993 study was conducted at the Woodworth Station, a research facility of the Northern Prairie Science Center, Jamestown, North Dakota. The Station is located in Township 142N, Range 68W, on the Missouri Couteau physiographic region of central North Dakota. The region is characterized by thick deposits of glacial till with knob-and-kettle topography. The Station was established in 1963 as a field laboratory for the study of effects of land-use practices on wildlife. Records of land-use practices throughout the Station have been maintained since its establishment. Prior to 1960, the study area was sporadically grazed and hayed. The 65-ha field containing the study site has never been plowed. Biologists burned the field in 1969, 1970,

1971, 1972, 1976, 1979, 1981, and 1990; it has not been grazed since 1974. Currently, vegetation in the study area is dominated by *Poa pratensis*, an exotic cool-season grass. Other grass species found during previous studies on the site include *Stipa viridula*, *S. comata*, *Agropyron repens*, *Muhlenbergia cuspidata*, and *Bromus inermis*. *Rosa arkansana*, *Elaeagnus commutata*, and *Symphoricarpos occidentalis* are common woody plants.

The aquatic component of the study was conducted in Fish Lake, a permanent wetland located immediately north of the vegetation and terrestrial study plots. In the recorded history of Woodworth Station, including the drought of the 1930's, Fish Lake has never dried completely.

Task 1. vegetation and herbivorous insects

Task 2. vertebrate and invertebrate terrestrial species

Task 3. fish and aquatic invertebrate species

Task 1. Effects of fire retardant chemicals and fire suppressant foam on vegetation in a North Dakota prairie

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Chemicals tested: Phos-Chek G75-F
Silv-Ex

Introduction:

Fire suppressant foams and fire retardant chemicals are used in wildland fire suppression and in prescribed burns for habitat management. Despite their relatively widespread use, little is known about potential effects on terrestrial and aquatic ecosystems. The purpose of this study was to examine experimentally the effect of retardant and foam application on vegetation. We studied the effects alone and in combination with fire. In addition, we examined the effects of the chemicals and fire on insect herbivory, which provides a link to higher levels in the food chain. A simple ecosystem, represented by a mixed-grass prairie, was chosen for the first year's work, so that general patterns could be identified. Subsequent studies are being done in more complex habitat in Nevada, where fire suppressant foams are more often applied.

Objectives:

- (1) To estimate effects of fire suppressant foam and fire retardant chemical application on growth and species diversity of burned and unburned prairie vegetation.**
- (2) To assess the response of herbivorous insects, in terms of number of insects and their effects on plants, to burning and application of foam and retardant to their host plants.**

Methods:

We delineated a grid of 30 0.4-ha blocks in the study field (Figure 1). Each block was separated from adjacent blocks by a mowed, 5-m-wide fire break. Four treatments [burning (B), foam application (F), burning and foam application (BF), and no manipulation (C)] were each assigned at random to six blocks. We established a 10 m x 10 m plot in the center of each of the 24 blocks (Figure 1) for vegetation sampling. For the retardant study, we established five 10 m x 10 m vegetation plots in each of the remaining six 0.4 ha blocks. Four treatments [burning (B), retardant application (R), retardant application plus burning (RB), and no manipulation (C)] were assigned at random within 0.4 ha blocks; each 0.4 ha block had each of the four treatments. Vegetation plots for which the treatment included burning were surrounded by mowed 1.5 m wide fire breaks.

Inside each 10 m x 10 m vegetation plot we randomly selected five 1-m² permanent vegetation subplots and four 0.25-m² biomass subplots (Figure 1). Prior to treatment, we counted stems of the woody species, *Symphoricarpos occidentalis* and *Rosa arkansana*, counted the total number of plant species, and measured litter depth in each permanent vegetation subplot. We made all pretreatment measurements during 17 - 28 May 1993.

Retardant application and burning of retardant plots was accomplished on May 26. Representatives of Monsanto applied Phos-Chek G75-F at the rate of 1 gallon per 100 ft², resulting in approximately 12 pounds of retardant per plot, to the R and RB plots within each 0.4 ha block. This is the application rate recommended for grassland vegetation. The retardant was applied by hand using a hose from a slip on pumping unit. Because retardant reacts with cellulose when heated, it was necessary to burn the RB plots after retardant had been applied. The retardant was allowed to dry for 0.5 - 1 hour before an attempt was made to burn through the RB plot in each block. B plots were also burned at this time.

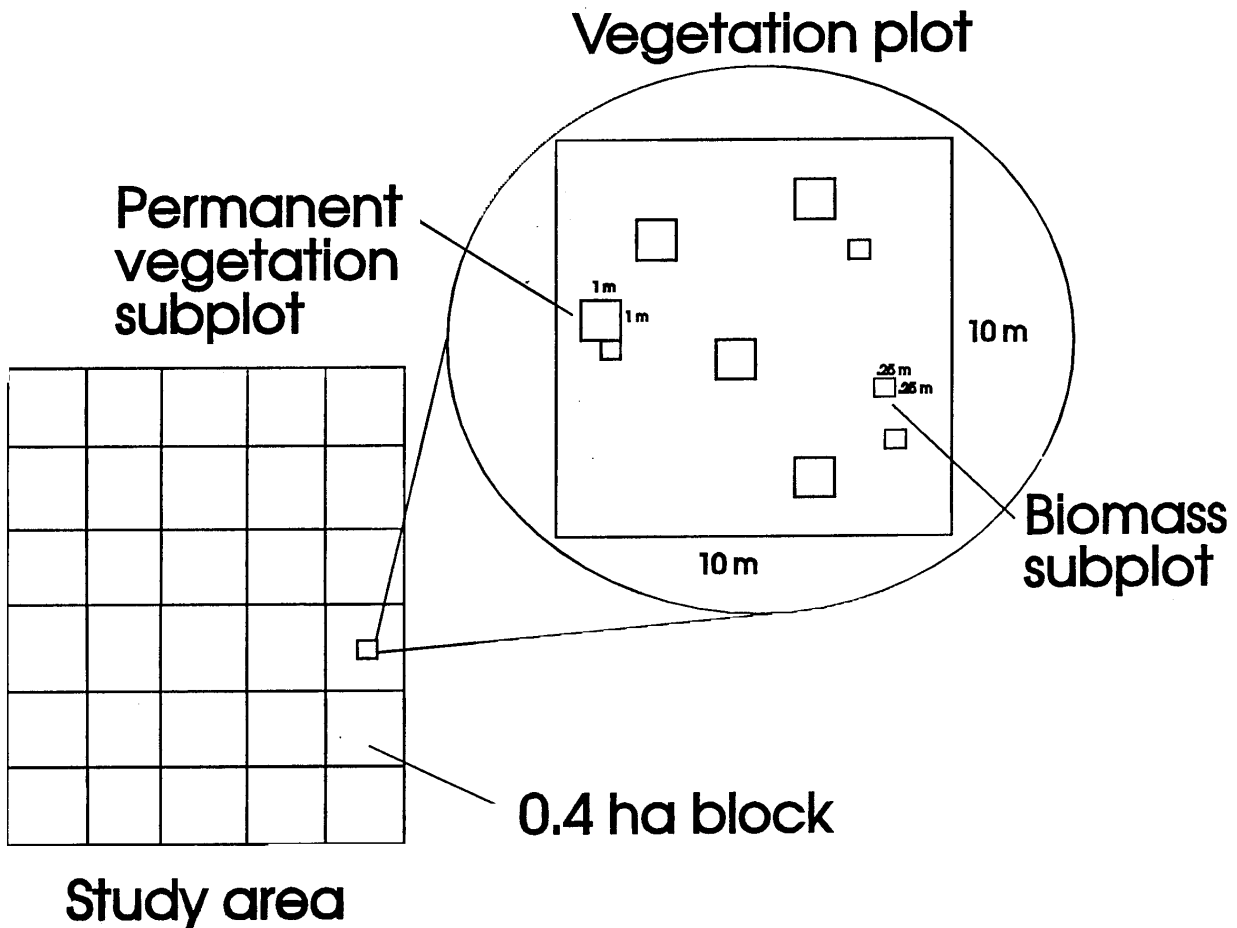


Figure 1. Study area showing 0.4-ha blocks, 10m x 10m vegetation plots, and randomly located 1-m² permanent vegetation subplots and 0.25m x 0.25m biomass plots. Treatments were assigned at random, six blocks per treatment for the Silv-Ex experiment. For the Phos-Chek experiment, each treatment was assigned at random to one of five 10m x 10m vegetation plots within each of 6 blocks.

On 1 June, B and BF blocks were burned. The entire 0.4 ha block was burned. All burn treatments were started with a drip torch to ignite the down-wind side. As the back fire progressed across the block, the flanks were ignited. As soon as these fires had blackened enough of the block to form a safe fire break, a head fire was started to complete the burn. All fires were allowed to burn to completion.

On 10 June, we applied Silv-Ex in 0.5%-solution maintained by a proportioner to F and BF blocks. The rate of application was approximately 189 liters per 10 m x 10 m plot, resulting

in approximately 1 L of Silv-Ex on each vegetation plot. Only the vegetation plots were treated on BF blocks. Although the entire 0.4-ha F blocks were treated, the smaller vegetation plots were treated first and at a higher rate than the remaining areas because a more even coverage was desired. The foam was applied with a 3.66-m boom mounted on bicycle tires. Nozzles mounted on the boom every 30 cm each produced approximately a 1:10 expansion. The boom was pushed by two people, while other personnel handled the hose between the boom and a 3785-liter pumper parked at the edge of the 0.4-ha block.

We conducted post-treatment vegetation sampling at 2-week intervals, beginning June 16 and ending August 27. Post-treatment vegetation sampling concentrated on four species: *Poa pratensis*, *Symphoricarpos occidentalis*, *Rosa arkansana*, and *Solidago rigida*. Height of *Poa pratensis* was measured at four locations on each subplot at each sampling period. For the other three species, we marked individual plants in each permanent vegetation subplot as follows: two *Symphoricarpos occidentalis*, two *Rosa arkansana*, and ten *Solidago rigida*. If fewer individuals were found in a subplot, we marked all individuals. Plants were marked near the base with either blue or red flagging (*Rosa arkansana* and *Symphoricarpos occidentalis*), or numbered metal tags (*Solidago rigida*). In addition, five shoots, defined as current year's growth, were marked and followed through the three sampling periods on each *Symphoricarpos occidentalis* and *Rosa arkansana* plant.

On each of the three non-grass plants, we measured the length of two fully expanded leaves. We measured the total length and counted the number of galls, leaf miners, aphids, chewed leaves, and flowers on each of the five shoots. Galls, leaf miners, aphids, and chewed leaves were recorded on a per-leaf basis. In each permanent subplot, we counted the total number of plant species and measured litter depth at four locations. Total stems of

Symphoricarpos occidentalis, *Rosa arkansana*, and *Solidago rigida* were also recorded in each plot at each sample period.

Two of the 0.25 m x 0.25 m biomass subplots were clipped to ground level on 23-29 June and 7-8 September, 1993 and 14-15 July, 1994 (retardant); and 7-8 July and 9-10 September, 1993 and 11-12 July, 1994 (foam). Dead and woody vegetation was removed and discarded. Live non-woody vegetation was oven dried to constant weight and weighed.

Statistical Methods:

We used analysis of variance (ANOVA) techniques in a repeated-measures type design with subsampling to assess the effects of the burn-foam treatments, time since treatment, and their interaction on all measured variables. Mean separations of significant effects in the ANOVAs were done with Fisher's protected least significant difference value (Milliken and Johnson 1984). Analyses were made in the original scale of measurement and with a $\log(y+1)$ transformation (Steel and Torrie 1980), but only results in the original scale of measurements are reported because only slight differences were observed in ANOVA results. ANOVAs were done using the General Linear Models procedure of SAS (SAS Inst. Inc. 1989). Significance was accepted at the 0.05 level.

Because vegetation plots differed significantly in number of plant species at pre-treatment in the foam experiment, this difference was taken into account in subsequent analysis by using the change in number of species between pre- and post-treatment as the response variable. Plots were similar in all other pre-treatment measurements for both experiments.

Results:

Silv-Ex

Of the 22 response variables for which we had enough data for analysis, only 5 showed significant ($p < 0.05$) effects after Silv-Ex application. Change in number of species, ratio of chewed to total leaves per shoot in *Symphoricarpos occidentalis* and *Rosa arkansana*, and mean shoot length and leaf length in *Symphoricarpos occidentalis* were affected by treatment.

The number of plant species increased between pre- and post- treatment in all plots, but the increase was smaller in plots treated with Silv-Ex than in untreated plots (Figure 2). Burning did not influence this difference.

Because the summer of 1993 was exceptionally cool and wet, insect abundances were uniformly low at our study site (D. Larson, personal observation). However, we found a significant 3-way interaction among foam, burning, and month on the change in ratio of chewed to total leaves on *Symphoricarpos occidentalis* (Figure 3A) and *Rosa arkansana* (Figure 3B). Silv-Ex treated plants of both species experienced greater herbivory late in the season. More untreated *Rosa arkansana* leaves were chewed early in the season; herbivory on burned *Rosa* was not affected by Silv-Ex.

Silv-Ex application had little effect on overall plant growth, as evidenced by the lack of difference in biomass accumulation between treated and untreated plots, irrespective of burning (Figure 4A, four weeks post-treatment; Figure 4B, end of growing season; Figure 4C, one year post-treatment). Nonetheless, growth characteristics between Silv-Ex treated and untreated *Symphoricarpos occidentalis* differed. Leaf length increased more rapidly on plants treated with Silv-Ex than on untreated plants (Figure 5A). Silv-Ex, fire, and month combined to produce a significant 3-way interaction on shoot length in *Symphoricarpos occidentalis* (Figure 5B);

burning significantly enhanced the rate of shoot growth compared with other treatments; Silv-Ex tended to depress shoot growth.

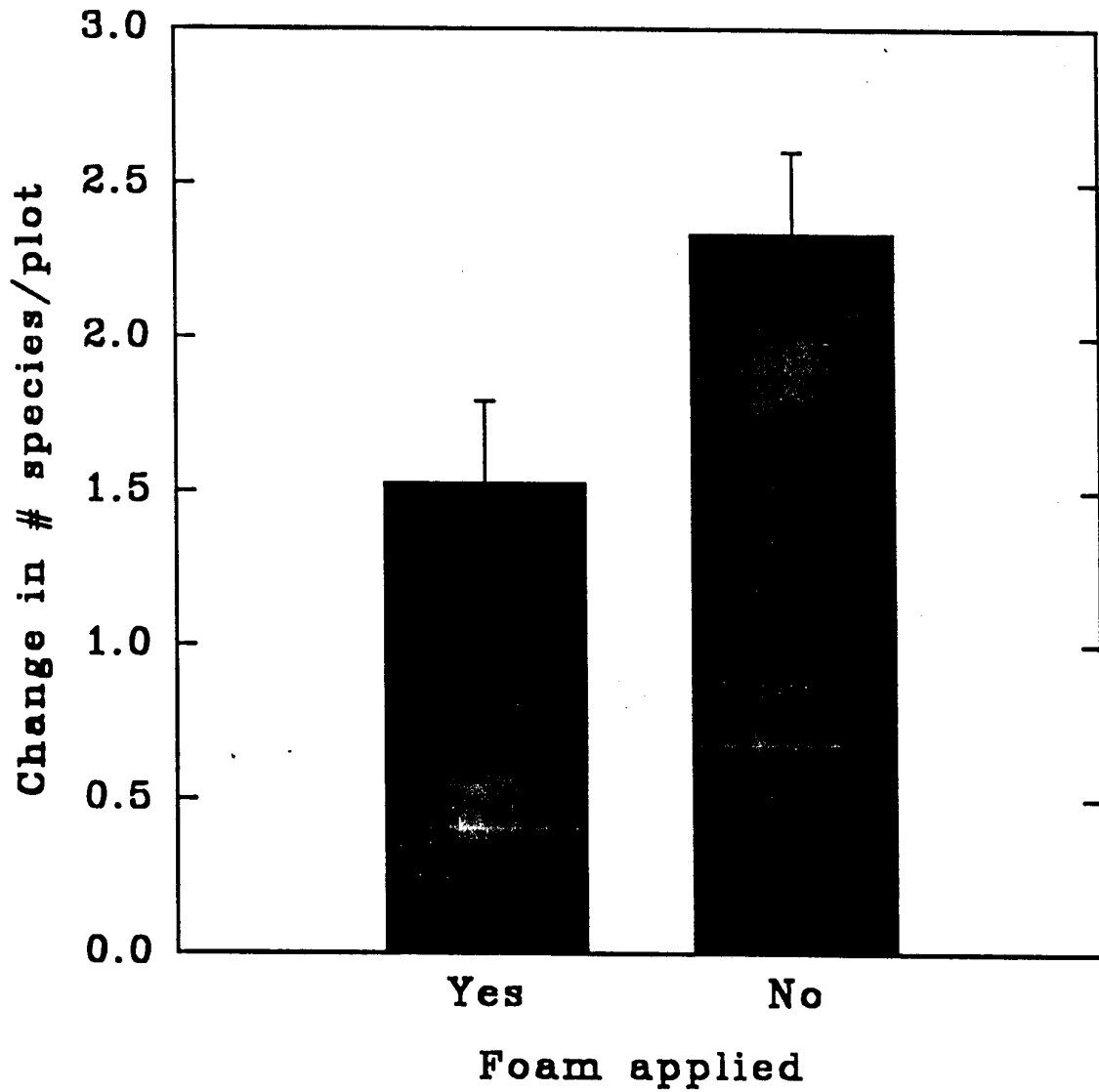


Figure 2. Change in mean number of species per plot between pre- and post-treatment, with and without Silv-Ex application. Error bar indicates one standard error of the mean.

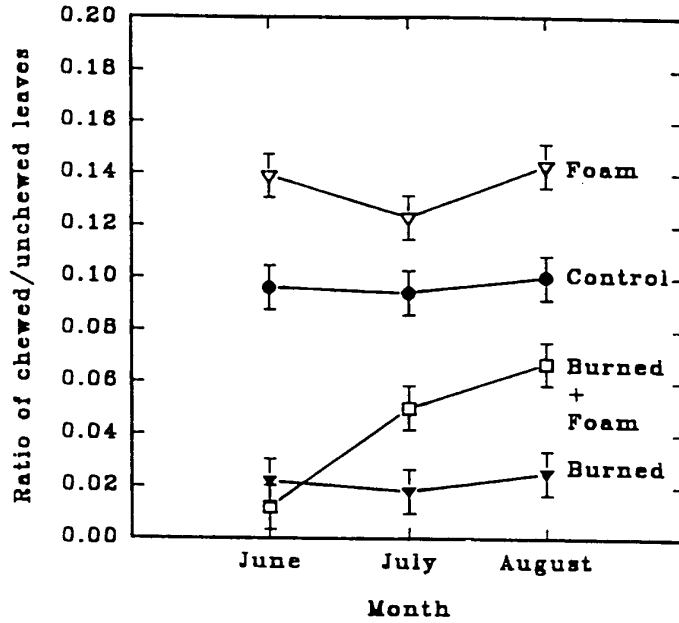


Figure 3A. Ratio of chewed to unchewed leaves on *Symphoricarpos occidentalis*. Shown is the mean \pm one standard error of the mean for the 3-way interaction foam x fire x month.

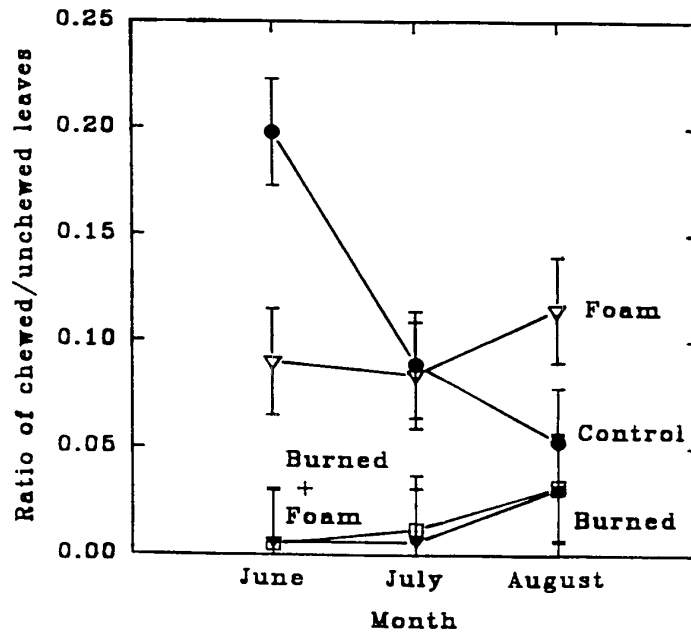


Figure 3B. Ratio of chewed to unchewed leaves on *Rosa arkansana*. Shown is the mean \pm one standard error of the mean for the 3-way interaction foam x fire x month.

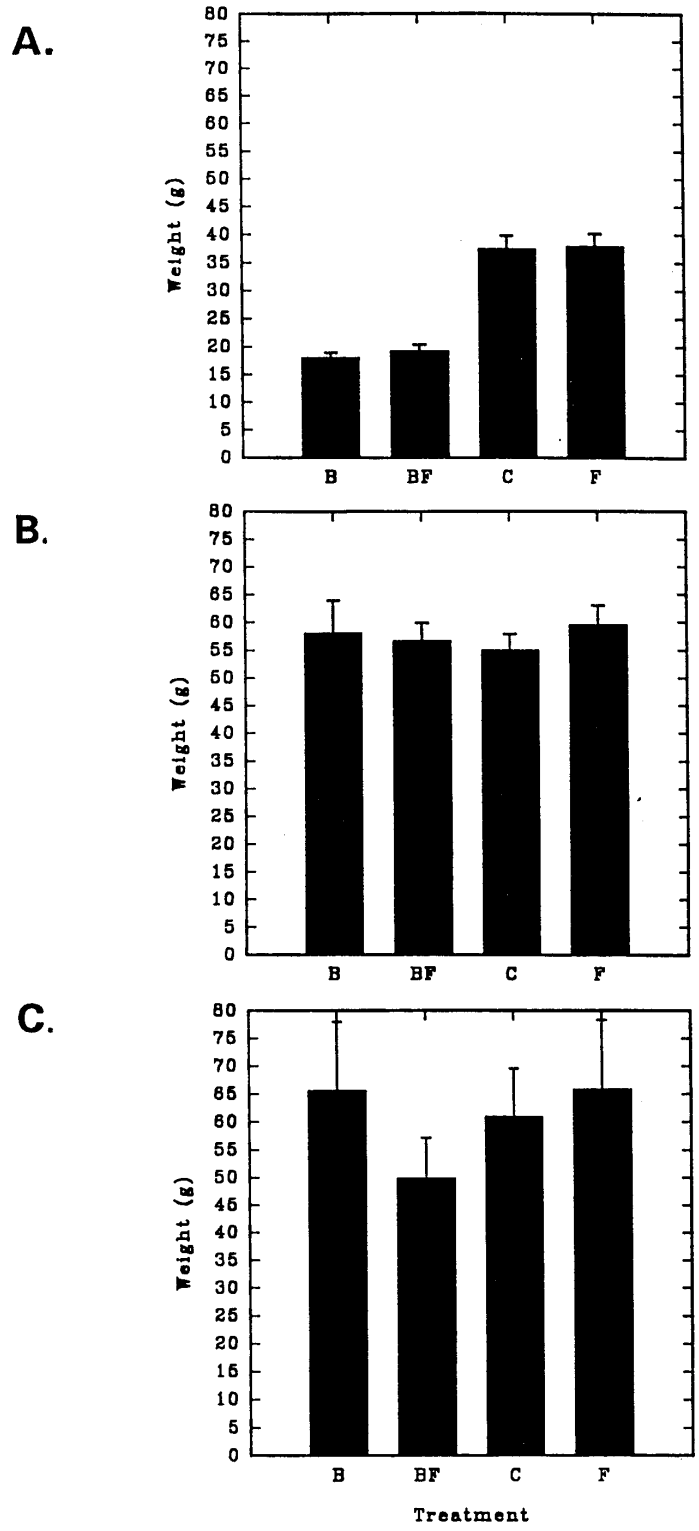


Figure 4. Mean (+ one standard error of the mean) herbaceous biomass accumulation for four weeks post-treatment (A), at the end of the growing season (B), and for one year post-treatment (C) with Silv-Ex.

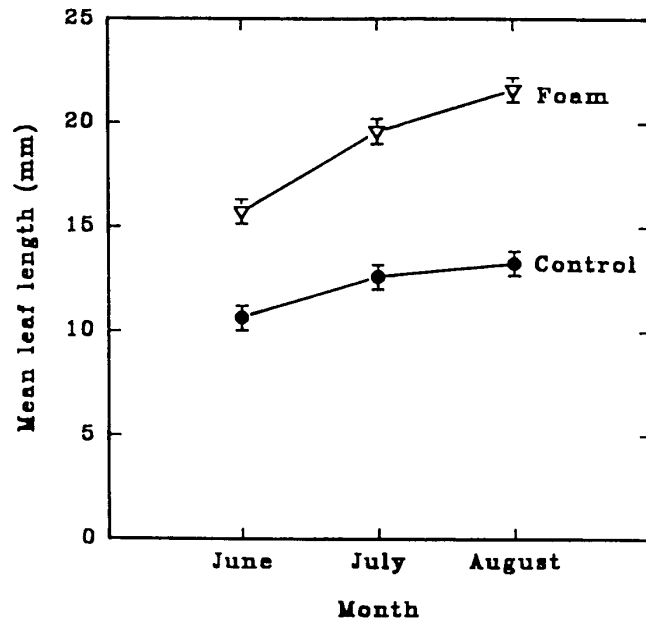


Figure 5A. Mean (\pm one standard error of the mean for the foam x month interaction) leaf length on *Symphoricarpos occidentalis* plants treated or not treated with Silv-Ex.

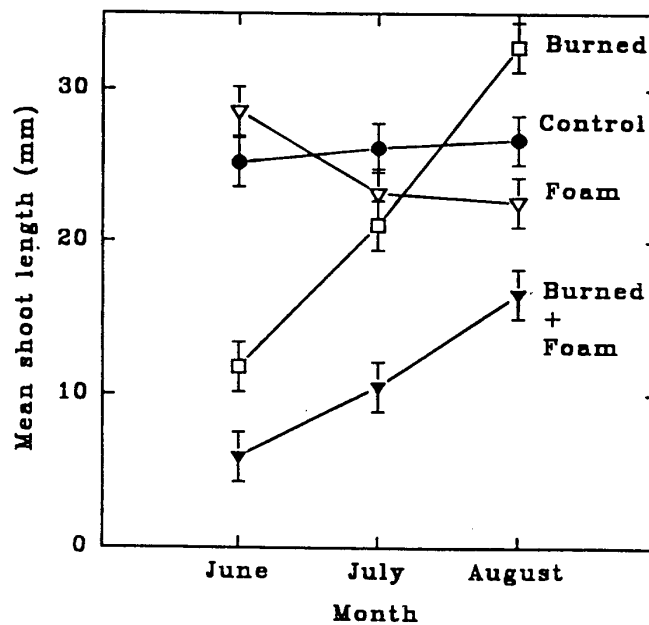


Figure 5B. Mean (\pm one standard error of the mean for the 3-way interaction) shoot length on *Symphoricarpos occidentalis* plants treated or not treated with Silv-Ex.

Results:

Phos-Chek D75-F

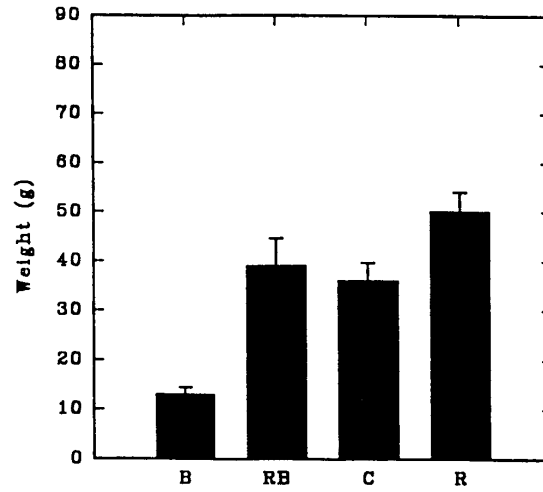
Of the 37 variables for which we had sufficient data for analysis, 5 showed a significant effect involving Phos-Chek treatment. Phos-Chek G75-F produced a pronounced fertilization effect, as measured by herbaceous biomass accumulation four weeks after treatment (Figure 6A) and at the end of the growing season (Figure 6B). Retardant application resulted in increased biomass, whether or not the plots were burned (two-way nested ANOVA; $F = 18.61$; $df = 1, 15$; $p = 0.0006$). No interaction between retardant and burning was evident ($F = 0.84$; $df = 1, 15$; $p = 0.3726$). Nonetheless, the effect was transitory; biomass did not differ among treatments the following year (Figure 6C).

Growth of *Poa pratensis* followed the pattern anticipated by the biomass results. Phos-Chek application combined with month to produce a significant interaction: not only was grass longer on plots treated with retardant, but the effect was enhanced over the course of the growing season (Figure 7A). The fertilization effect was not evident in stem growth or leaf size of any of the other plant species measured, however.

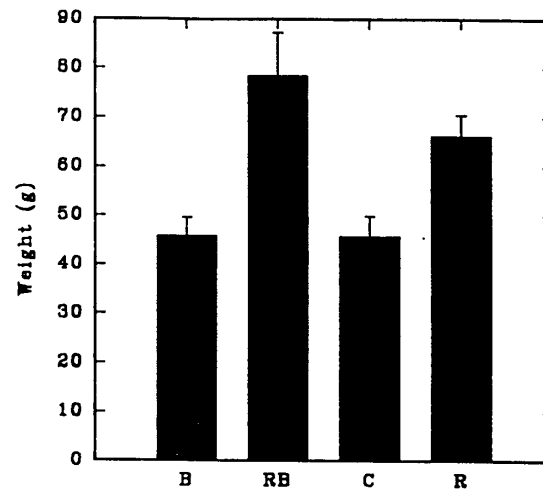
Phos-Chek interacted with both fire and month to produce a significant effect on the number of leaves per shoot in *Symphoricarpos occidentalis* (Figure 7B). Retardant seemed to enhance leaf production between June and July compared with burned or untreated plants. Leaf production continued to increase into August on burned plants, but not on plants in other treatments.

The change in number of species per plot from month to month was depressed on Phos-Chek treated plots (Figure 8). Number of species increased dramatically on burned plots through August; burned plots to which Phos-Chek was applied showed an increase between June and July, but little change late in the season.

A.



B.



C.

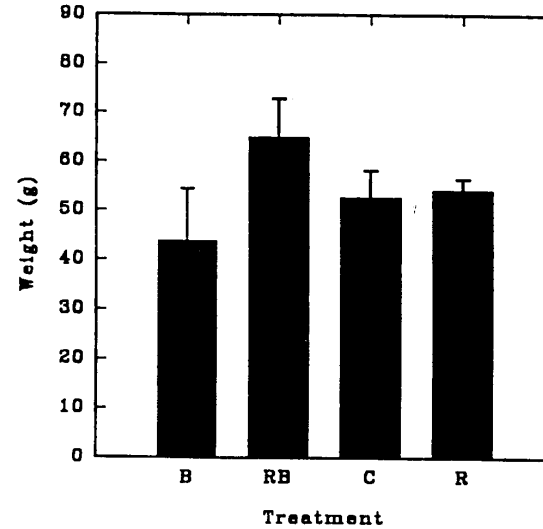


Figure 6. Mean (+ one standard error of the mean) herbaceous biomass accumulation for four weeks post-treatment (A), at the end of the growing season (B), and one year post-treatment (C) with Phos-Chek.

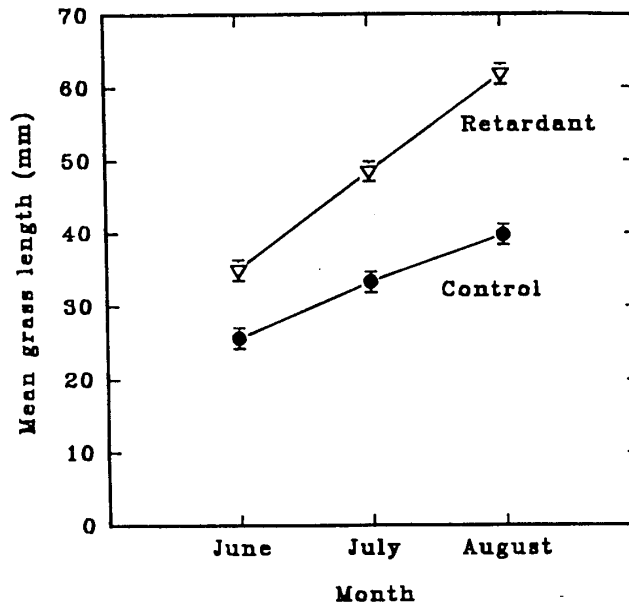


Figure 7A. Mean (\pm one standard error of the mean for the retardant x month interaction) length of *Poa pratensis* for plots treated or not treated with Phos-Chek.

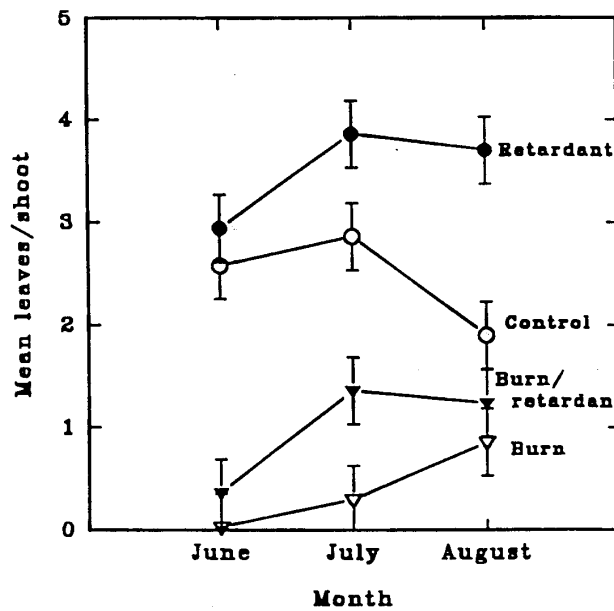


Figure 7B. Mean (\pm one standard error of the mean for the retardant x fire x month interaction) number of leaves per shoot on *Symphoricarpos occidentalis*, Phos-Chek experiment.

Discussion and Management Implications:

Overall, Silv-Ex application had little effect on the vegetation characteristics we measured. Effects we detected were subtle. Silv-Ex application influenced herbivory, as evidenced by the proportion of chewed leaves on *Symphoricarpos occidentalis* and *Rosa arkansana* (Figure 3). It also influenced growth of leaves and shoots of *Symphoricarpos occidentalis*, resulting in enhanced leaf growth, but depressing shoot growth (Figures 5). The decline of shoot length between June and July for Silv-Ex treated plants suggests either shoot damage and subsequent breakage or vertebrate herbivory. Nonetheless, herbaceous biomass accumulation was not affected by Silv-Ex (Figure 4), suggesting little effect on average plant vigor.

Although Phos-Chek produced a pronounced increase in herbaceous biomass (Figure 6), the fertilization effect seemed to be concentrated in the grass, *Poa pratensis*, which dominated our study site. Measures of shoots and leaves on woody species, and of stem length on *Solidago*, did not indicate any effect on growth of these species. The effect we saw in 1993 did not persist into 1994; herbaceous biomass collected at approximately the same date as in 1993 showed no significant differences among treatments (Figure 6C), with weights generally comparable to the control treatment in 1993.

Although Phos-Chek influenced the number of leaves per shoot in *Symphoricarpos occidentalis* (Figure 7A), the effect is not easily interpretable because it is embedded in a three-way interaction. Early in the season, retardant produced similar changes in leaf production on burned and unburned plants; between July and August, however, the relationship changed. Burned, untreated plants were the only group still producing leaves.

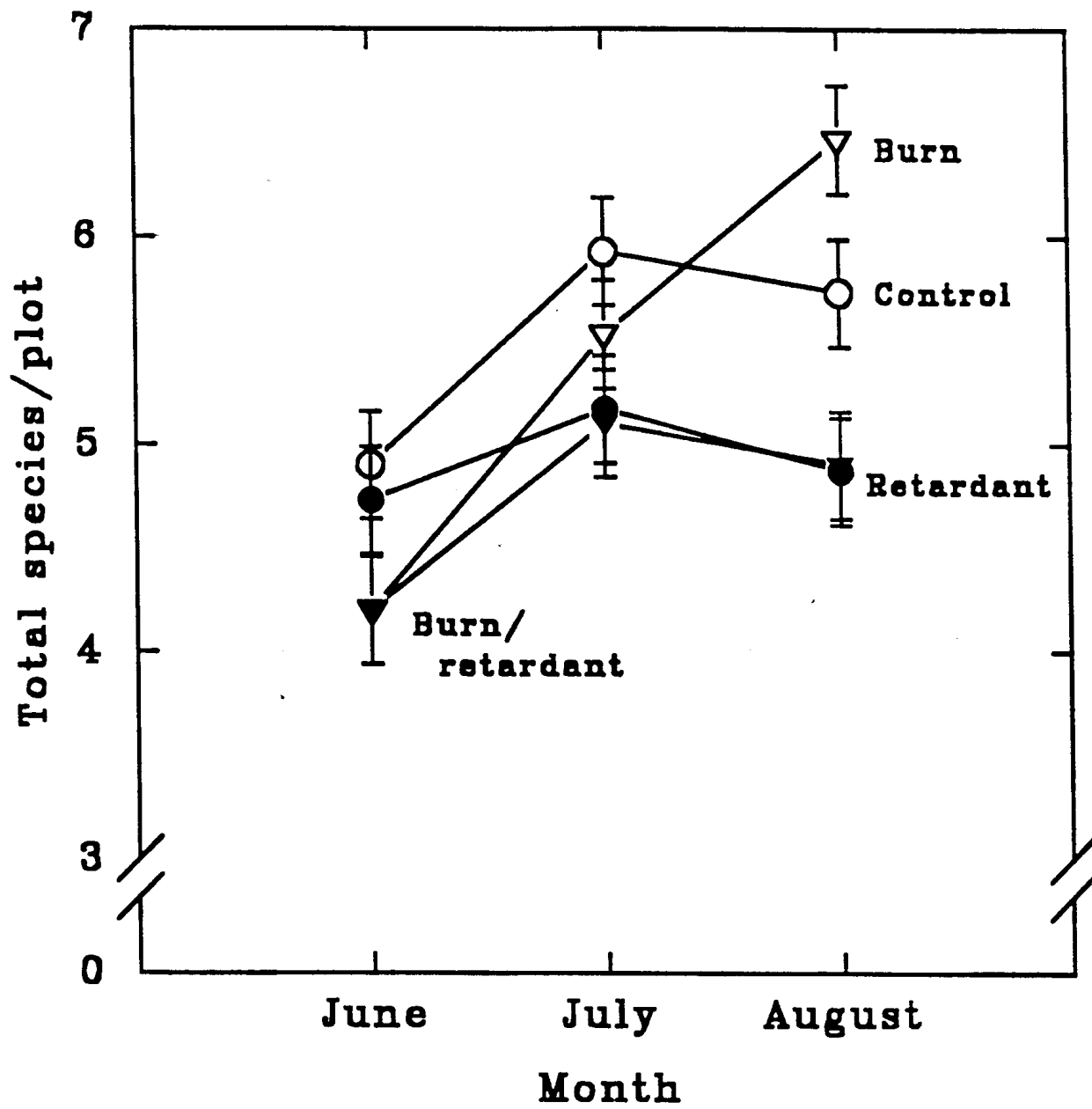


Figure 8. Mean (\pm one standard error of the mean for the retardant x fire x month interaction) total species per plot, Phos-Chek experiment.

Of concern to land managers is the potential depression in species diversity associated with both Silv-Ex (Figure 2) and Phos-Chek (Figure 8) application. The change in number of species per plot was significantly lower after Silv-Ex application, regardless of whether or not the plot was burned. Phos-Chek interacted with both month and fire, so again results are more difficult to interpret. The change in number of species per plot was depressed, especially between July and August, on Phos-Chek plots. All plots were dominated by *Poa pratensis*, which clearly benefitted from retardant fertilization, and may also have increased in response to the general disturbance, and crowded out other species. Further work in areas not dominated by *Poa pratensis* will help define this relation.

Implications of this research depend on the objectives of the manager. If the objective is to halt an uncontrolled fire, subtle changes caused by Silv-Ex and Phos-Chek may be of little importance. On the other hand, if the objective is to aid in the control of prescribed burns, the potential effect on species diversity should be considered. In particular, if the control of exotic, robust grasses such as *Poa pratensis* is important, these results suggest that use of these chemicals should be minimized or avoided.

Literature Cited:

- Milliken, G.A. and D.E. Johnson, 1984. Analysis of messy data, volume I: designed experiments. New York, NY: Van Nostrand Reinhold Co. 473 pp.
- SAS Institute Inc., 1989. SAS/STAT user's guide. Version 6, fourth edition, volume 2. Cary, NC: SAS Institute Inc. 846 pp.
- Steel, R.G.D. and J.H. Torrie, 1980. Principles and procedures of statistics, a biometrical approach, 2nd edition. New York, NY: McGraw-Hill Inc. 633 pp.

Task 2: Effects of fire retardant chemicals and fire suppressant foam on terrestrial species in a mixed-grass prairie ecosystem

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Wildlife Toxicology Group
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Chemical tested: Silv-Ex

Objectives:

- (1) To evaluate potential effects of fire fighting chemicals on small mammal and insect populations in a mixed-grass prairie ecosystem**
- (2) To develop and validate methods for the evaluation of ecological effects of fire fighting chemicals on terrestrial organisms in a more complex ecosystem during 1994 (Great Basin-Nevada)**

Methods:

This study was conducted during summer 1993 at the Woodworth Field Station of the Northern Prairie Science Center near Jamestown, North Dakota. Twelve 1-acre (0.4 ha) plots (6 controls and 6 treated with Silv-Ex) were sampled for approximately three months. (May-August 1993). Small mammal sampling was conducted using standard capture-recapture methodology (Pollock et al. 1990). A total of 1200 small mammal live traps were checked daily for 5 consecutive days at 2-week intervals. All animals were tagged, weighed, and their reproductive status recorded.

Insect populations were monitored by sampling ant mounds. Ants from control and treated mounds were collected using adhesive tape. Ants were sampled one week prior to treatment, and immediately post-treatment and then again 2 weeks later. Three samples per

mound were collected each time. Avian nestling survival data could not be collected because an unusually cool spring and early summer delayed nesting.

Results:

Small mammal population data was analyzed following procedures outlined by Pollock et al. (1990). Analysis revealed no exposure effect on small mammal populations. Although this is supported by laboratory information which suggests a relatively low level of acute toxicity for these chemicals, our field results are confounded by the extremely low population densities that likely resulted from the unusually cold and wet weather in North Dakota during summer 1993. Analysis of ant population data also revealed no dose-related effect.

Overall, interpretation of this information for management purposes must be guarded due to the unseasonal events of the 1993 summer. However, development of methods during this study greatly benefitted the experimental design and method selection for the 1994 field season in the Great Basin.

Literature Cited:

Pollock, K.H., Nichols, J.D., Brownie, C., and Hines, J.E. 1990. Statistical inference for capture-recapture experiments. Wildlife Monographs 107. 97 p.

Task 3: Effects of fire retardant foams on a prairie wetland aquatic ecosystem

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Chemicals tested: Phos-Chek WD-881
Silv-Ex

Objectives:

- (1) To determine the response of the aquatic invertebrate community to two foaming agents, Silv-Ex and Phos-Chek WD-881, in a wetland environment.**
- (2) To evaluate the survival of fish and invertebrates after exposure to Silv-Ex and Phos-Chek WD-881.**

Methods:

Two field exposures were performed in 1993 to examine the effects of two fire suppressant foams, Phos-Chek WD-881 and Silv-Ex, on aquatic life. These tests were conducted as 96-h limnocorral exposures in both North Dakota and Missouri during summer 1993. The first field exposure was performed June 5-9 in Fish Lake, a permanent wetland, at the Northern Prairie Science Center's Woodworth Field Station near Jamestown, North Dakota. The second field exposure was performed August 2-6 in an experimental pond (0.4 ha) at the Environmental and Contaminants Research Center (ECRC, formerly the Midwest Science Center) in Columbia, Missouri.

Limnocorral exposures:

Portable limnocorrals were used for both field exposures. Octagonal limnocorrals were designed to enclose approximately 2500 L of water so that chemical dosage could be accomplished without leakage into the surrounding open water area. They were constructed from 1.5" schedule 40 polyvinyl chloride (PVC) pipe and fittings with a bottom edging of polyethylene to create a seal during placement into soft sediments. The limnocorrals were 2.5 m wide x 1.0 m high, and were encircled with 10-mil clear polyethylene plastic. The limnocorrals were built with a 4-way PVC center cross to strengthen the frame and divide the limnocorral into 4 quadrants. A 5-cm hole was drilled in the center to aid in positioning each limnocorral onto 1" galvanized conduit pipe. Limnocorrals were consecutively numbered.

For the North Dakota field exposure, 24 locations with depths ranging from 55-60 cm were identified by placing 1" galvanized conduit pipe at each location along a single transect in Fish Lake. This pipe was used for anchoring and positioning the limnocorrals and for attachment of the artificial substrates. The 24 limnocorrals in this field exposure (8 Silv-Ex, 8 Phos-Chek WD-881, 8 control) were positioned to enclose previously colonized artificial substrates 24 hours before chemical addition.

For preparation of the field exposure at ECRC, conduit pipe was placed at 9 locations in a 3 x 3 block at 30-55 cm depths in the experimental pond. Nine limnocorrals (3 Silv-Ex at 6 mg/L, 3 Silv-Ex at 24 mg/L, 3 control) were anchored and positioned in the same manner as in the Fish Lake exposure, 24 hours before chemical addition.

Chemicals and Dosage:

Based on laboratory tests conducted by the Yankton Field Research Station (Hamilton and Buhl, Objective 1, this report) using fire retardant liquids and suppressant foams, we determined that the foams Silv-Ex and Phos-Chek WD-881 were more toxic to aquatic organisms than the liquid retardants tested. For use in field exposures, concentrations

representing the lowest observable effect level (LOEL) for these chemicals were calculated based on this laboratory test data for *Daphnia magna*. The LOEL was calculated as 6 mg/L for Silv-Ex, and 4.7 mg/L for Phos-Chek WD-881. These concentrations were used during the Fish Lake exposure.

To further delineate the effects of foams on aquatic communities, we conducted the second field exposure at ECRC. Two exposure concentrations of Silv-Ex were used during this field exposure, one representing the LOEL (6 mg/L) and one representing 4 times the LOEL (24 mg/L). The highest concentration also approximated the laboratory-derived LC₅₀ (concentration expected to induce 50% mortality in a population) for daphnids.

Chemicals were pre-weighed in the laboratory using a Mettler PE360 top-loading balance. Chemical doses were added as a liquid to a 23 L polyethylene container of lake water, then mixed and poured slowly into each limnocorral to avoid agitation. Chemical dosage was added to numbered limnocorrals in a randomized fashion in both field exposures.

Water Chemistry:

Continuous Water Quality Monitoring. The variables of pH, dissolved oxygen, conductivity, and water temperature were measured at hourly intervals throughout the study period with DataSonde II units (Hydrolab Inc., Austin, Texas), self-contained submersible dataloggers. One unit was placed outside the limnocorrals in Fish Lake, and 6 units were deployed within randomly selected limnocorrals (2 Silv-Ex, 2 Phos-Chek WD-881, 2 control). Hydrolab units were suspended in the limnocorrals at mid-depth using stainless steel cable clamps. During the second field exposure at ECRC, units were deployed in the same manner in each of the three treatments and in open water areas.

Dissolved Oxygen. A YSI dissolved oxygen meter was also used to measure dissolved oxygen between 0700-0900 daily in each limnocorral and in the open water (and the ECRC experimental pond) throughout the test period, during both field exposures.

Nutrients. One-liter water samples were also collected from each limnocorral and the open water areas between 0700-0900 daily during both field exposures. Ammonia (APHA 1985) and orthophosphorus were measured daily throughout the test period. EPA method 365.1 (Colorimatic Automated Ascorbic Acid Method) was used for orthophosphorus determination. Nitrate and nitrite were measured once before and once after each 96-h exposure (APHA 1985).

Other Parameters. The pH was also measured daily from 1-L water samples. Chlorides, sulfates, alkalinity, and hardness were measured once before and once after each 96-h field exposure (APHA 1985). Depth-integrated chlorophyll *a* samples were collected daily throughout both field exposures with a 45.7 cm acrylic tube placed vertically in each limnocorral and plugged on the top with a rubber stopper. The fluorimetric technique with acetone extraction (APHA 1985) was used for chlorophyll *a* determination in both exposures. Filters used in this procedure were frozen for further laboratory analysis at ECRC.

Single Species Exposures:

Cenocorixa. The water boatman *Cenocorixa* sp. (Hemiptera: Corixidae) was collected from Fish Lake in sufficient numbers for use in the first in-situ field exposure. Winged adults were collected on June 5 prior to chemical addition. Exposure chambers were constructed of 15-L polyethylene containers. For each chamber, 10 x 20 cm windows were cut and fitted with 1-mm mesh polypropylene netting. One chamber with 10 *Cenocorixa* sp. was placed in each of the 24 limnocorrals 1 h after chemical addition. Chambers were suspended from each limnocorral frame. Number of organisms remaining alive was recorded daily throughout the 96-h exposure, with the exception of June 7; inclement weather with extremely high winds made access to the exposure chambers impossible on this day.

Fathead Minnows. An in-situ field exposure with 48-h old fathead minnows (*Pimephales promelas*) was also conducted in an experimental pond at the Midwest Science Center to better define the distribution of mortality for Silv-Ex exposure. Logistical constraints had prohibited

use of these organisms in the North Dakota study. Environmental exposure chambers to contain the larval fathead minnows were constructed of 16 x 18 cm cylindrical polyethylene containers fitted with stainless steel 0.5-mm mesh screening. The chambers were covered and placed on square polystyrene floats so that the screened portion of each chamber was below the water. Three chambers were placed in each of the 9 limnocorrals, and 3 additional chambers were placed outside the limnocorrals in the test pond. Twenty fathead minnow larvae were placed in each chamber and acclimated to pond water temperature for 2 hours. Chambers were placed inside each limnocorral 1 h after chemical addition. Number remaining alive was recorded daily throughout the 96-h exposure.

Community Effects:

Aquatic Macroinvertebrates. The response of the macroinvertebrate community was evaluated in both the North Dakota and the Midwest Science Center studies using artificial substrate trays that were constructed of 1/2" mesh aquaculture netting (Memphis Net & Twine, Memphis, TN) with a base of PVC pipe and fittings. The tray base was designed specifically for placement into soft sediments. A 30-cm circle of knotless nylon netting was positioned underneath the trays to eliminate organism loss during sampling. The trays contained 5 g of pre-weighed, air-dried Cottonwood (*Populus deltoides*) leaves. Substrate trays were deployed with a PVC pole by pushing the base into the sediment, with tray and netting attached. A nylon pull cord attached to each substrate tray was fastened to the center conduit pipe that anchored and positioned the limnocorral. Substrate trays were sampled by pulling the nylon cord vertically, and then placing the tray into a white pan. Substrate trays and nylon netting were placed in zip-lock bags and preserved with 90% ethanol.

For the field exposure in North Dakota, a total of 120 substrate trays were deployed during May 10-11 and allowed to colonize with organisms for 4 weeks before the 96 h dose on June 5. Five substrate trays were deployed at each of the 24 limnocorral locations. Single trays

were placed in each limnocorral quadrant (Figure 9), and an additional tray was located immediately outside the corral area. The 24 trays outside the limnocorrals were sampled on June 4 before corral placement. One tray from each limnocorral was also randomly sampled on June 5 after limnocorral placement and before chemical addition to determine disturbance effects associated with limnocorral placement. The remaining 3 trays in each limnocorral were sampled at the end of the 96-h exposure.

For the field exposure at ECRC, a total of 116 substrate trays were deployed in the experimental pond on June 20-21, and allowed to colonize with organisms for 6 weeks before the 96-h dose on August 2. Twelve substrate trays were deployed at each of the 9 limnocorral locations (3 for each quadrant), and 8 additional trays were deployed at locations around the outside of the exposure area. The 8 external trays were removed on August 1 before limnocorral placement. One tray from each limnocorral quadrant was removed on August 2 after limnocorral placement and before chemical addition to determine disturbance effects. One tray from each quadrant was removed 24 h after chemical addition, and again after the end of the 96-h exposure. Substrate trays that were sampled after chemical addition were immediately washed, processed, and sorted with a #40 brass sieve and white pan to separate dead organisms from the sample before preservation. For a period of 10 min., dead organisms were removed and placed in a separate sample bottle, then preserved in 80% ethanol.

Zooplankton. An 18 x 25 cm aquarium net mounted on a PVC handle at a 100° angle was used to sample zooplankton from each limnocorral and the main lake during the North Dakota field exposure. The net was positioned at a 45.7 cm depth and drawn to the surface. Samples were washed into a bottle and preserved with 80% ethanol. One sample was taken from each limnocorral and 3 samples were taken from the main lake on June 5 before chemical addition. An additional sample was taken from each limnocorral 24 h and 96 h after chemical addition.

Top View - Portable Limnocorral

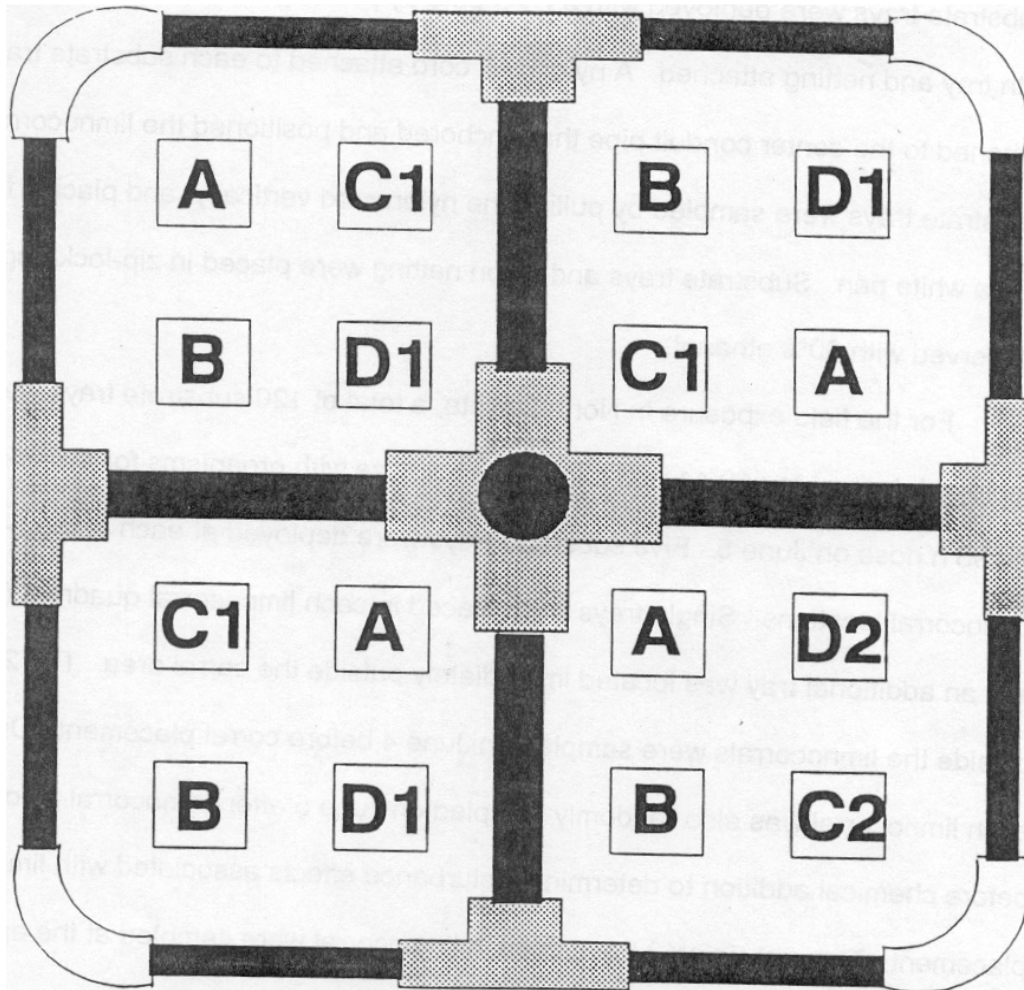


Figure 9. Schematic diagram of limnocorral illustrating placement of artificial substrate trays within each corral. Additional trays were also deployed in open water areas.

Results:

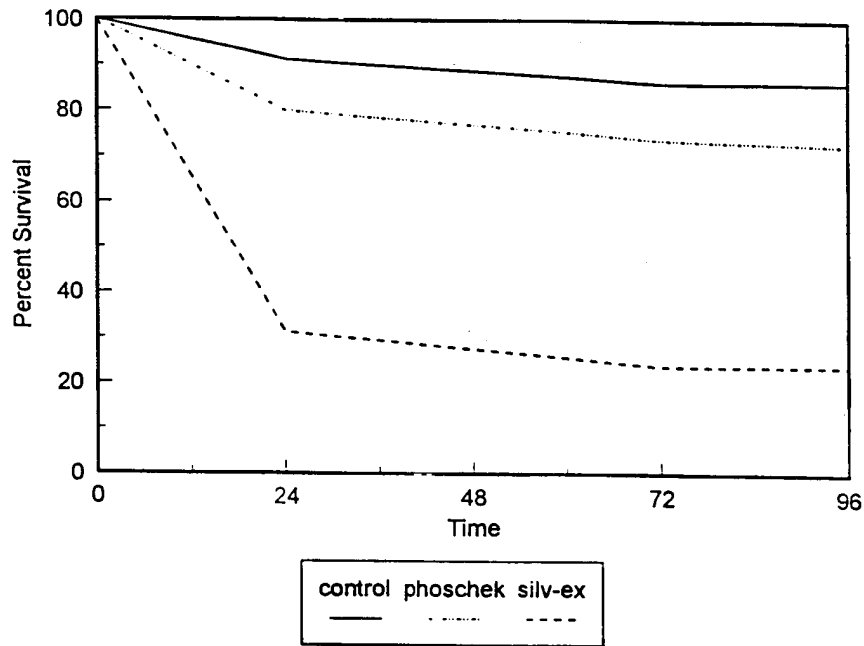
A. In-Situ Exposures

Single Species Exposures

Cenocorixa. After 24 h, exposure to Silv-Ex at 6 mg/L resulted in significantly higher mortality of water boatmen (*Cenocorixa* sp.) than in controls ($p = 0.003$) (Figure 10A). The most dramatic decrease in survival (69%) occurred during the first 24 h, but survival continued to decrease throughout the 96-h exposure to Silv-Ex until only 11% of the organisms remained. Contrastingly, the 4.7 mg/L Phos-Chek WD-881 treatment did not cause mortality significantly different from that of controls during the 96-h experiment. However, organisms showed impaired movement that suggested a sublethal response related to chemical exposure.

Fathead Minnows. After 24 hours, the highest Silv-Ex treatment, 24 mg/L, resulted in significantly higher mortality than in the controls ($p = 0.02$) (Figure 10B). As was the case in studies with *Cenocorixa*, survival decreased markedly (64%) during the first 24 hours. After 96 h of exposure, 70 % of the fish had died. The 6 mg/L Silv-Ex treatment did not significantly reduce survival as compared with controls during the 96-h experiment. In addition, survival of fish in the control limnocorrals was similar to survival of fathead minnows in the open pond, indicating no effects directly attributable to the limnocorral enclosures.

A. Cenocorixa



B. Fathead Minnows

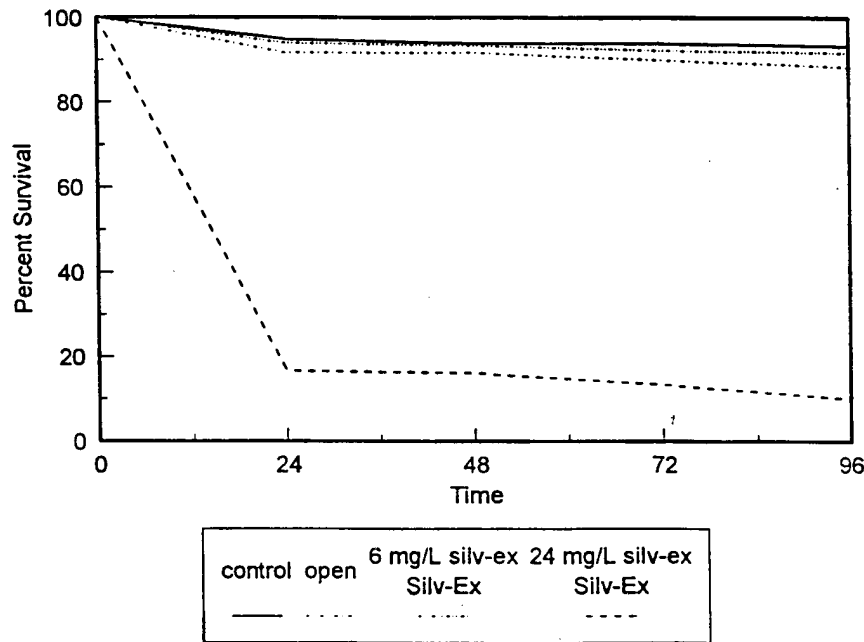


Figure 10A. Survival of *Cenocorixa* sp. (water boatmen) after 96-h exposure to Silv-Ex and Phos-Chek WD-881.

Figure 10B. Survival of fathead minnow larvae after 96-h exposure to treatments of 6 mg/L and 24 mg/L Silv-Ex.

Water Chemistry:

Fish Lake is an alkaline, extremely hard, well-buffered aquatic ecosystem. The pH ranged from 8.3 to 8.7 with mean hardness and alkalinity of 1345 and 766 mg/L as CaCO₃, respectively. In the North Dakota study, water quality conditions among limnocorrals were similar. Hourly data from Hydrolab units indicated that daily patterns in temperature, pH, dissolved oxygen, and conductivity did not differ among limnocorrals or between the corrals and the open water of Fish Lake during the exposure (Figures 11 and 12). Water temperatures dropped from 19 to 13°C during the study; this decrease in temperature was caused by a strong cold front on 7 June and resulted in below normal conditions for June in North Dakota. However, this temperature depression did not occur until day 3 of the study and thus, did not coincide with the high mortality observed after a 24-h exposure to Silv-Ex. Dissolved oxygen remained above saturation throughout the exposures and never represented a hazard to aquatic life. No dose-related fluctuations in phosphates, sulfates, chlorides, chlorophyll a, conductivity, or pH occurred during the exposure.

Water quality patterns were also similar among limnocorrals as well as between the corrals and the open water during the study conducted in the Midwest Science Center's experimental pond. This pond is a hard, well-buffered aquatic system with pH ranging from 7.5 to 8.3, a mean hardness of 138 mg/l as CaCO₃, and a mean alkalinity of 133 mg/L as CaCO₃. Temperature during this exposure ranged from 21 to 26 °C. Ammonia was the only water quality variable measured that was affected by treatment. Ammonia in limnocorrals containing the high dose of Silv-Ex (24 mg/L) increased with time; this trend was not evident in controls or other treatments. However, unionized ammonia never exceeded concentrations known to be acutely toxic to fish or invertebrates. No dose-related fluctuations in phosphates, sulfates, chlorides, chlorophyll a, conductivity, or pH occurred during the exposure.

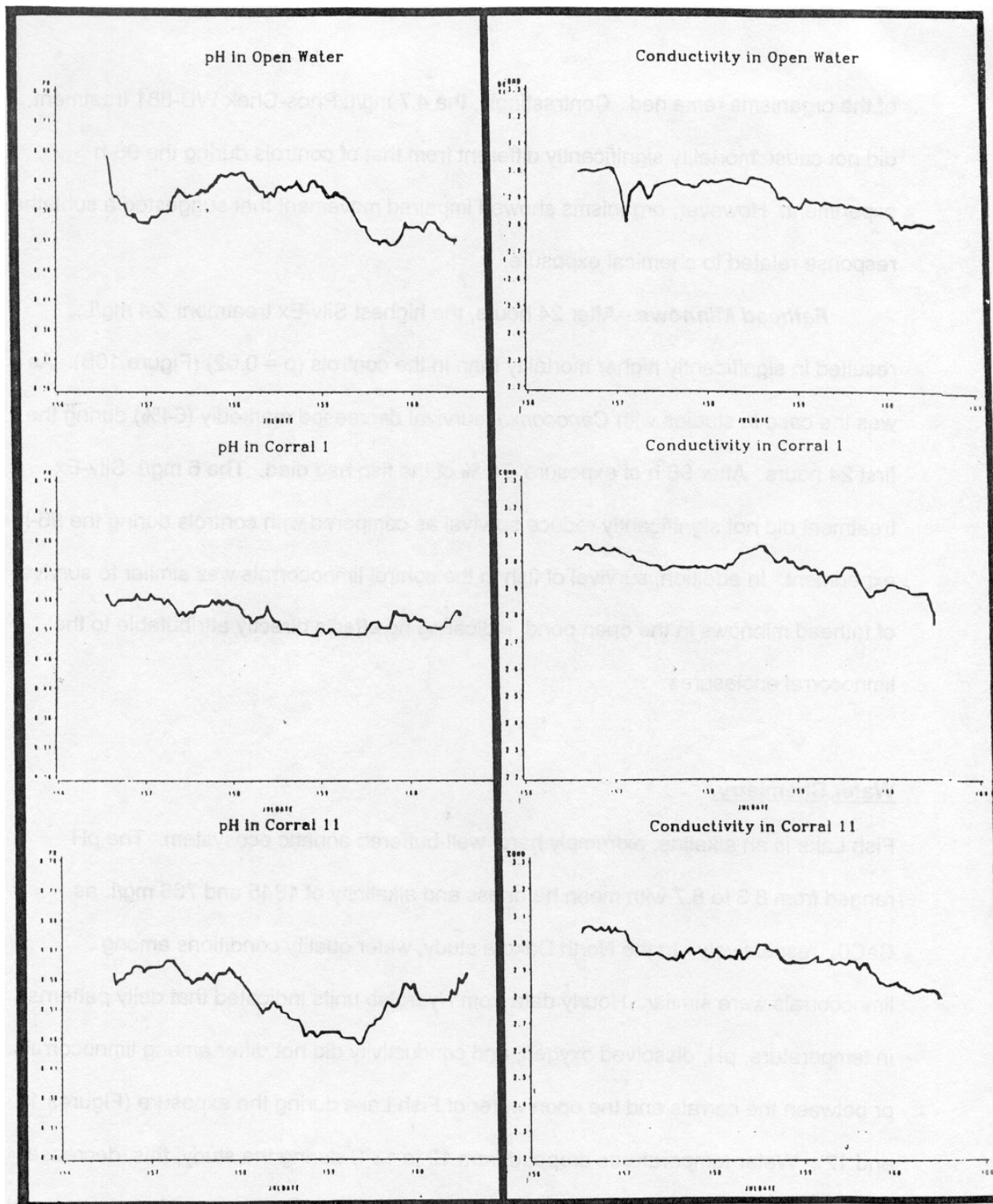


Figure 11. Similarity of measurements of pH and conductivity in open water and two limnocorrals recorded hourly by DataSonde Hydrolab units during the 96-h in-situ exposure with Silv-Ex and Phos-Chek WD-881.

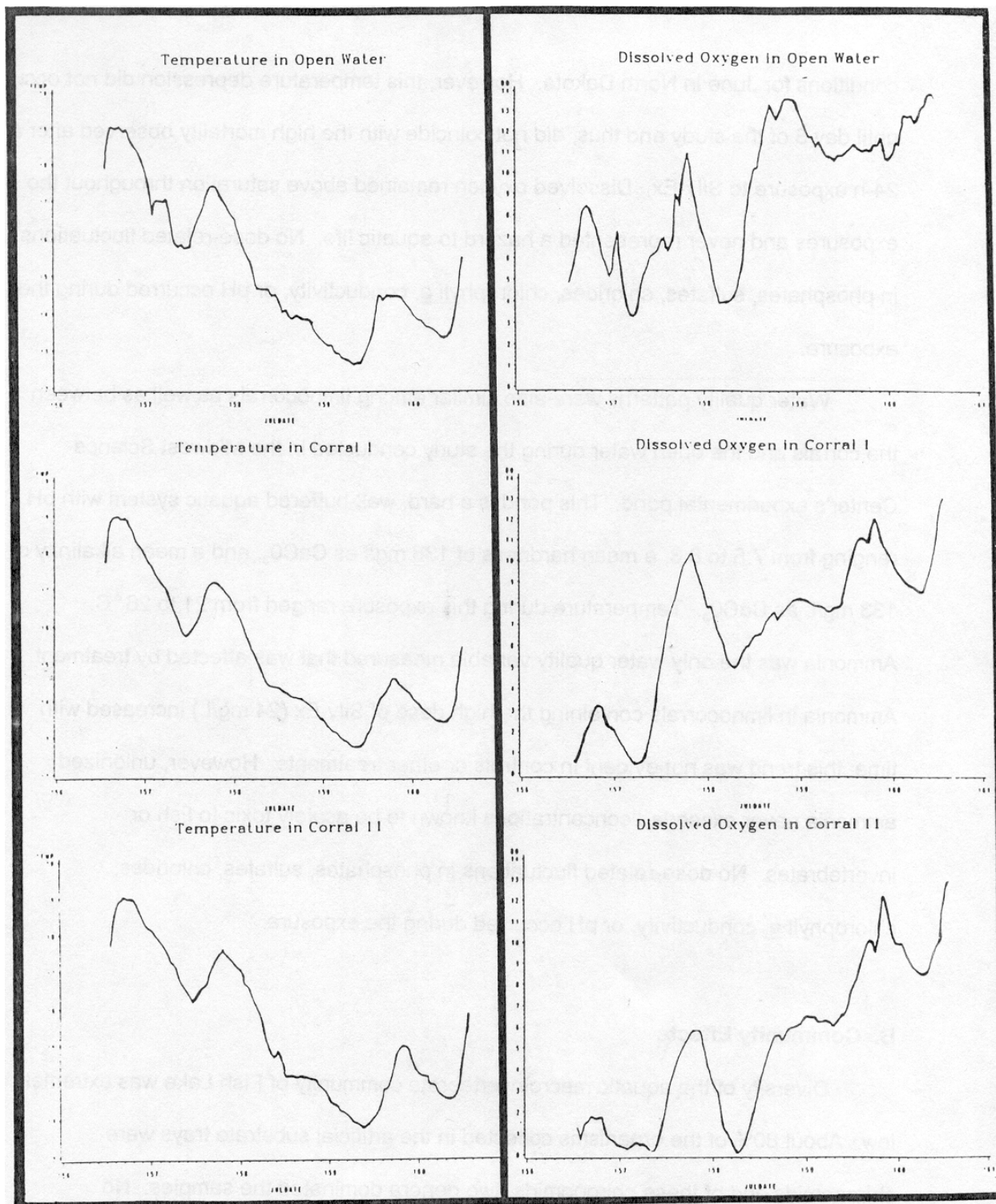


Figure 12. Similarity of measurements of temperature and dissolved oxygen in open water and two limnocorrals recorded hourly by DataSonde Hydrolab units during the 96-h in-situ exposure with Silv-Ex and Phos-Chek WD-881.

B. Community Effects

Diversity of the aquatic macroinvertebrate community of Fish Lake was extremely low. About 80% of the organisms collected in the artificial substrate trays were chironomids and of these chironomids, two genera dominated the samples. No community-level effects resulting from either Silv-Ex or Phos-Chek WD-881 were evident after the 96-h exposure. Total number of organisms and relative abundance of organisms did not differ among treatments. The Pinkham and Pearson Similarity Index, a similarity index highly sensitive to community disturbance, indicated that for both total number of organisms and relative abundance, treatments did not differ from controls (Figure 13).

Discussion and Management Implications:

Under field conditions, the toxicity of Silv-Ex to fathead minnows was similar to that observed in laboratory exposures. For fathead minnows, the Silv-Ex exposure (24 mg/L) that caused significant mortality under field conditions was within the confidence interval of the calculated laboratory LC₅₀ for hard water (22 mg/L; 95% CI=17-28) (Hamilton 1993 Progress Report). In both instances, the highest mortality occurred during the first 24 hours (Figure 10). In the event of an actual spill or accidental overspray, an organism's response during the first 24 h is an ecologically relevant measure of the severity of a chemical effect. Chemical concentration would be highest at this time because degradation begins immediately; under laboratory conditions, Silv-Ex degrades by 42% in about 20 days (Norecol 1989). Degradation studies (this report) demonstrated similar rates of degradation with highest degradation occurring in organic-rich sediments. Under natural conditions with elevated temperatures commonly associated with fires, that degradation may be further accelerated.

To put these results in perspective using an actual field application example, the acutely toxic concentration of 24 mg/L Silv-Ex identified in our study is equivalent to a spill of 12 L (2.6 gallons) of an 0.5% Silv-Ex (500 mg/L) mixture directly into one 2500 L limnocorral (550

Pinkham & Pearsons Similarity - Fish Lake, ND Limnocorral Exposure

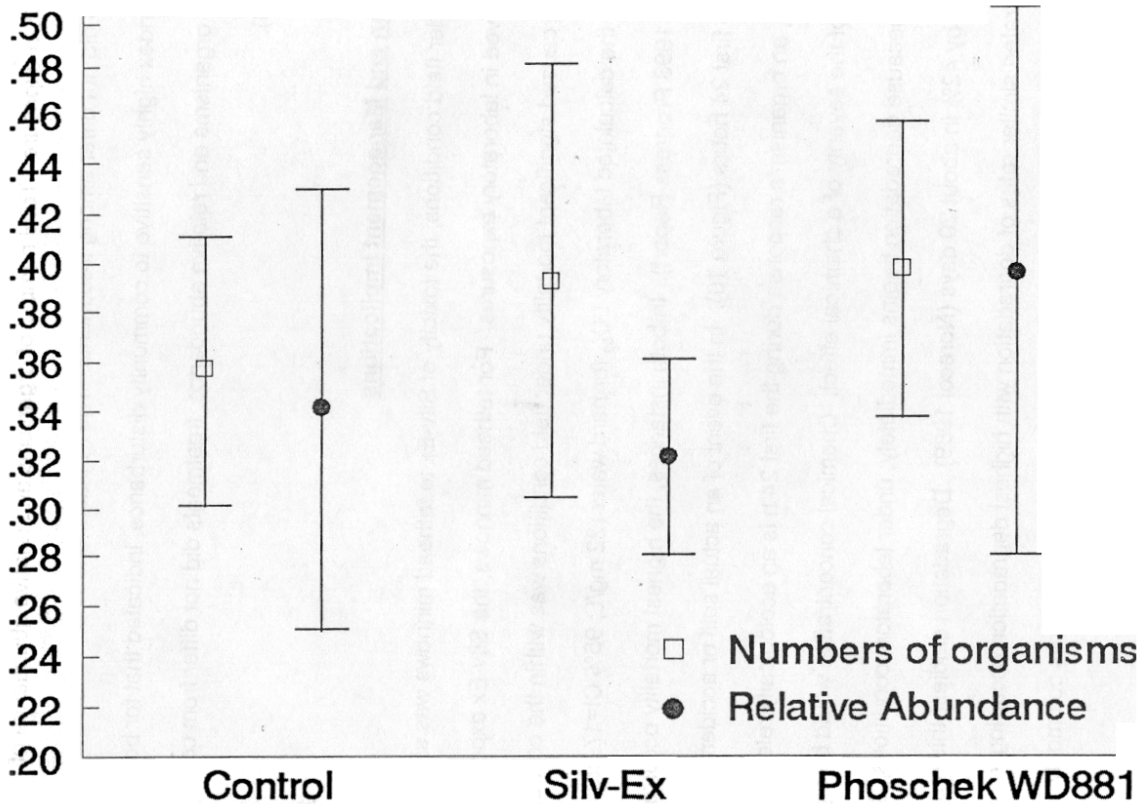


Figure 13. Pinkham and Pearsons Similarity Index calculated for total number of macroinvertebrates and for relative abundance in the North Dakota limnocorral study with Silv-Ex and Phos-Chek WD-881.

gallons). This equates to a dilution factor of 208 in a natural aquatic system. Therefore, in a pond or wetland environment one would expect mortality of larval fish to occur if a 0.5% Silv-Ex spill was not diluted more than 208 fold. Accordingly, if the Silv-Ex was applied at 1% (10,000 mg/L), the amount of Silv-Ex required to reach the acutely toxic concentration demonstrated in this study would be 6 L in the 2500 L limnocorral; the dilution factor would double to 416. Similar dilution factors are also suggested by Hamilton's data (1993 Progress report) for aquatic invertebrates such as amphipods.

A safety factor is often applied to toxicity data to estimate a "safe" or maximum acceptable toxicant concentration (MATC) for the protection of aquatic organisms. A safety

factor of 100 is commonly used (Rand and Petrocelli 1984). Thus, a safety factor of 100 applied to toxicity information for a 1% Silv-Ex mixture from our study would require a 41,600-fold dilution. For example, in a one acre pond with an average depth of 10 feet, use of a safety factor of 100 would estimate that about 78 gallons of 1% Silv-Ex spilled directly into the pond would represent minimal hazard to aquatic organisms. Use of this safety factor provides a conservative value for the protection of larval fish based on results from our study. In the limnocorral exposures, no mortality of fish occurred at a concentration that would be equivalent to a spill of about 1600 gallons of 1% Silv-Ex in a one-acre pond. However, this same concentration resulted in significant mortality of water boatmen (*Cenocorixa*). It is likely that the mortality of Silv-Ex is related to the surfactants present in the formulation. In rainbow trout, Muller (1980) demonstrated that the toxicity of surfactants was related to their effect on surface tension. As surfactant concentration increased, surface tension decreased and toxicity to trout increased. Because the mobility of water boatmen is dependent on the surface tension of the water, it is likely that their mortality was directly related to the reduction of surface tension caused by Silv-Ex.

Another scenario through which fire fighting chemicals affect the aquatic environment is by incidental overspray. If this were to occur with Silv-Ex and one were to assume the coverage across the water surface was even, the application of a 0.5% Silv-Ex mixture would result in a 23.6 mg/L chemical concentration in a 2500-L limnocorral. This exposure is similar to the concentration that resulted in significant mortality of larval fathead minnows in both field and laboratory exposures. However, it is highly unlikely that the entire surface of an aquatic system would be covered by foam, such as Silv-Ex. Thus, dilution of the area affected by overspray would occur rapidly. It is unlikely that a concentration as high as that predicted (23.6 mg/L) would actually occur. Nonetheless, organisms such as water boatmen and other invertebrates that utilize the water surface would suffer adverse effects from direct exposure resulting from the chemical application. These organisms along with other important invertebrates such as

daphnids and amphipods must be considered as important components contributing to the integrity of an ecosystem. Although aquatic invertebrates are neither economically or recreationally important aquatic resources, they are an integral part of the food chain essential to the support of higher trophic levels such as fish.

Literature Cited:

American Public Health Association (APHA) 1985. Standard Methods for the Examination of Water and Wastewater. 16th ed. Washington, D.C.

Muller, R. 1980. Fish toxicity and surface tension of non-ionic surfactants: Investigations of antifoam agents. *J.Fish.Biol.* 16:585-589.

Norecol Environmental Consultants, LTD. 1989. Toxicological review of fire fighting foams. (Report prepared for Manager, Fire Management Forest Protection Branch, Ministry of Forests and Lands, Victoria, BC, Canada.

Rand, G.M. and S.R.Petrocelli (eds.). 1984. Fundamentals of Aquatic Toxicology. Hemisphere Publishing, Washington, D.C.

IV. PROJECT OBJECTIVE 4: ECOLOGICAL EFFECTS OF FIRE RETARDANT CHEMICAL AND FIRE SUPPRESSANT FOAMS

A. *EFFECTS OF FIRE RETARDANT CHEMICALS AND FIRE SUPPRESSANT FOAMS ON TERRESTRIAL, AQUATIC, AND VEGETATIVE COMMUNITIES*
--A study of a Great Basin ecosystem in northern Nevada

Description of study site:

This 1994 study was conducted in Humboldt County, Nevada along the North Fork of the Little Humboldt River and along Cabin Creek in northern Nevada. The study site was located at an elevation of about 6000 ft. within the Santa Rosa Mountains north of Winnemucca, approximately 30 km from Paradise, Nevada. The land is under the management of Nevada First Corporation and is used primarily for grazing; written permission for the National Biological Survey to conduct this research was obtained from Gary Benochea, Vice President of Nevada First Corporation.

Suitable in-stream exposure sites for the aquatic studies were selected at 3 locations on the North Fork of the Little Humboldt River. The stream is a small, permanent stream with healthy invertebrate communities and adequate size to support trout year round. No Lahontan cutthroat are currently reported in this stream, but the area has been identified as potential habitat for Lahontan reintroduction. Each of the three sites had pool and riffle sequences of comparable physical, chemical, and biological characteristics, thus allowing for in-stream treatment of several sections of the stream.

Vegetation at the site is predominately sagebrush (*Artemisia* sp.) and rabbitbrush (*Chrysothamnus* sp.), grading into low, riparian vegetation (mainly *Salix* sp.) near the river. Reeds (*Juncus* sp.) And sedges (*Carex* sp.), along with the exotic grass *Poa pratensis*, dominated the riparian zones; dominant upland grasses included *Poa secunda* and *Agropyron trachycaulum*.

The relatively low sagebrush overstory allowed maximum exposure of wildlife species after the chemicals were applied.

General Introduction:

The following summaries address the research initiated during year 3 of the study on the toxicity of fire retardant chemicals funded through the Interagency Fire Coordination Committee.

During summer 1994, the toxicity of Ansul Silv-Ex, and either Phos-Chek D75-F or Phos-Chek G75-F were evaluated in three biological compartments:

Task 1. vegetation and herbivorous insects

Task 2. vertebrate and invertebrate terrestrial species

Task 3. fish and aquatic invertebrate species

Task 1: Response of vegetation and herbivorous insects to burning and fire retardant chemical application

Principal Investigator: Diane L. Larson
Northern Prairie Science Center
Jamestown, North Dakota

Objectives:

- (1) To determine effects of fire retardant chemical and fire suppressant foam application on growth and flowering phenology of burned and unburned vegetation.**
- (2) To determine the population-level response of herbivorous insects to burning and application of retardants and foams to their host plants.**

Introduction:

Fire retardant chemicals and fire suppressant foams are frequently used in wildland fire fighting and fire suppression activities. These chemicals are often applied in environmentally sensitive areas of the United States which may contain endangered, threatened, or economically significant plant and animal species. Relatively little information is available on the toxicity of these chemicals to aquatic and terrestrial life; even less information is available concerning effects at the community and ecosystem levels.

Based on results from the previous year's study on a mixed-grass prairie site in North Dakota, main effects to be expected include changes in growth, including biomass accumulation, and changes in species diversity. Fire retardants such as Phos-Chek are primarily fertilizers, and stimulate growth. Species diversity was depressed at the prairie site, however, possibly as a result of the strong positive response of the exotic grass, *Poa pratensis*, to fertilization. Foams such as Silv-Ex did not affect growth, but did depress species diversity.

Several aspects of the Great Basin environment suggest that results may vary from those we obtained in the more mesic midwest. The growing season tends to be divided into two peaks: an early response to melting snow, and a second response to late summer storm precipitation. Many species are dormant during the hottest summer months, when natural fires are most likely to occur. Like the mixed-grass prairie, vegetation cover can be nearly 100% in riparian areas of the Great Basin; in contrast, upland areas tend to be dominated by sparse bunch grasses and shrubs, with large areas of exposed soil.

Objectives of this study were (1) to determine effects of fire retardant chemical and fire suppressant foam application on growth, species diversity, resprouting, and flowering of burned and unburned vegetation and on galling insect activity on that vegetation; and (2) to quantify the amount of chemical reaching the soil, and persistence of the chemical through the growing season.

Description of study site:

The study was conducted along the North Fork of the Humboldt River and along Cabin Creek in northern Nevada, within the Santa Rosa Mountains north of Winnemucca. Elevation was approximately 6000 ft. at each site. Woody vegetation at the site is predominately sagebrush (*Artemisia* sp.) and rabbitbrush (*Chrysothamnus* sp.), grading into low, riparian vegetation (mainly *Salix* sp.) near the river. Reeds (*Juncus* sp.) and sedges (*Carex* sp.), along with the exotic grass *Poa pratensis*, dominated the riparian zones; dominant upland grasses included *Poa secunda* and *Agropyron trachycaulum* (Table 1).

Procedures:

Treatments

We treated 24-1m² plots per treatment, divided equally between the North Fork and Cabin Creek drainages. Within drainages, plots were located randomly and treatments assigned

randomly to plots. Treatments included: (1) Phos-Chek; (2) Phos-Chek/burned; (3) 0.5% Silv-Ex; (4) 0.5% Silv-Ex/burned; (5) 1% Silv-Ex; (6) 1% Silv-Ex/burned; (7) water, equivalent in volume to chemical application; (8) water/burned. We applied one set of treatments 28 June - 1 July and one set 19 - 20 July. Two-thirds of the plots were used for measuring growth, resprouting, flowering, and species diversity; one-third were used for biomass.

Chemicals were mixed as appropriate for operational use on sagebrush communities. Phos-Chek was applied at coverage level 3 (3 gallons/100 ft²). Silv-Ex (Table 1.) was mixed at two concentrations, 0.5% and 1.0%, and applied at the rate of 0.33 gallons/m². We used motorized 6-gallon backpack pumps to apply the chemicals. We did not quantify expansion of the foam. When treatment included burning, plots were ignited with a propane torch; all vegetation within the plot was burned, although all was not reduced to ash. Chemicals or water were applied to extinguish the fire, depending on treatment.

Cattle exclosures measuring 1 m² x 1 m high were placed around each plot. Exclosures were made of 6 cm hardware cloth, and were anchored with rebar.

Vegetation response - plots

We marked five individual shoots on each woody plant within the vegetation sampling plots. We measured current year's growth of these shoots, and counted number of stems of each woody species occurring on each plot, the number of post-burn resprouts of *Chrysothamnus*, and the number of flowers and galls per shoot on *Artemesia* and *Chrysothamnus*. We measured abundance, height, number of species, and species density for all herbaceous species on each plot. The sampling schedule is listed in Table 2.

Plots designated as biomass plots were clipped at the end of the study; timing was determined by noting when the majority of plants had senesced. Samples were dried to constant weight and weighed.

Table 1. Plant species encountered on study plots at North Fork and Cabin Creek study sites, June – October, 1994.

FAMILY	GENUS	SPECIES	ZONE	N*
Lilliaceae	Allium	spp.	upland	1
Cruciferae	Arabis	glabra	riparian	1
Poaceae	Bromus	inermis	riparian	1
Asteraceae	Crepis	acuminata	upland	1
Portulacaceae	Lewisia	redivia	upland	1
Polemoniaceae	Phlox	longifolia	upland	1
Lameaceae	Prunella	vulgaris	riparian	1
Polygonaceae	Rumex	cCrispus	riparian	1
Labiatae	Scutellaria	angustifolia	riparian	1
Brassicaceae	Thlaspi	arvense	riparian	1
Scrophulariaceae	Verbascum	thapsus	riparian	1
Polemoneaceae	Ipomopsis	aggregata	upland	2
Poacea	Koeleria	cristata	riparian	2
Scrophulareaceae	Penstemom	rydbergii	upland	2
Iridaceae	Iris	missouriensis	riparian	3
Gentianaceae	Gentiana	affinis	riparian	4
Scrophulareaceae	Mimulus	guttatus	riparian	4
Rosaceae	Rosa	woodsii	riparian	4
Lilliaceae	Smilacina	stellata	riparian	4
Poaceae	Stipa	thurberiana	upland	4
Poaceae	Bromus	tectorum	riparian	5
Brassicaceae	Descurainia	richardsonii	upland	5
Rubiaceae	Galium	aparine	riparian	5
Juncaceae	Juncus	ensifolius	riparian	6
Poaceae	Koeleria	cristata	upland	6
Saxifragaceae	Ribes	cereum	upland	6

Polygonaceae	Eriogonum	ovalifolium	upland	7
Poaceae	Deschampsia	elongata	riparian	8
Lameaceae	Mentha	arvensis	riparian	8
Asteraceae	Senecio	integerrimus	upland	9
Poaceae	Poa	secunda	riparian	10
Polemoniaceae	Leptodactylon	pungens	upland	10
Asteraceae	Taraxacum	officinale	upland	11
Asteraceae	Agroseris	glauca	riparian	12
Asteraceae	Chrysothamnus	nauseosus	upland	12
Rosaceae	Potentilla	glandulosa	riparian	12
Fabaceae	Lupinus	caudatus	riparian	18
Ranunculaceae	Ranunculus	cymbalaria	riparian	19
Asteraceae	Agroseris	glauca	upland	20
Asteraceae	Achillea	millefolium	upland	22
Asteraceae	Cirsium	vulgare	riparian	24
Polemoniaceae	Phlox	hoodi	upland	24
Malvaceae	Sidalcea	neomexicana	riparian	25
Caryophyllaceae	Stellaria	lingipes	riparian	29
Poaceae	Elymus	cinereus	upland	30
Asteraceae	Cirsium	foliosum	upland	31
Fabaceae	Astragalus	curvicaupus	upland	32
Cyperaceae	Carex	microptera	riparian	36
Scrophulareaceae	Penstemon	rydbergii	riparian	37
Equisetaceae	Equisetum	arvense	riparian	41
Rosaceae	Ponentilla	gracilis	upland	41
Cyperaceae	Carex	douglasii	upland	45
Asteraceae	Arnica	chamissonis	riparian	46
Asteraceae	Artemisia	ludoviciana	riparian	47
Fabaceae	Lupinus	caudatus	upland	47

Rosaceae	Geum	macrophyllum	riparian	50
Poaceae	Hordeum	pusillum	riparian	52
Poaceae	Sitanion	hystrix	upland	62
Onagraceae	Epilobium	glaberrimum	riparian	63
Juncaceae	Juncus	balticus	upland	69
Asteraceae	Erigeron	spp.	upland	69
Cyperaceae	Carex	nebraskensis	riparian	78
Poaceae	Agropyron	trachycaulum	riparian	102
Asteraceae	Cirsium	foliosum	riparian	107
Linaceae	Linum	perenne	upland	113
Linaceae	Linum	perenne	riparian	132
Poaceae	Agropyron	trachycaulum	upland	142
Poaceae	Poa	secunda	upland	159
Asteraceae	Artemisia	tridentate	upland	193
Fabaceae	Thermopsis	Montana	riparian	213
Rosaceae	Potentilla	gracilis	riparian	247
Asteraceae	Chrysothamnus	vicidiflores	upland	284
Cyperaceae	Carex	praegracilis	riparian	287
Asteraceae	Achillea	millefolium	riparian	288
Juncaceae	Juncus	balticus	riparian	298
Asteraceae	Taraxacum	officinale	riparian	337
Poaceae	Poa	pratensis	riparian	352

* Number of plots in which the species was encountered.

Table 2. Sampling schedule for vegetation.

Herbaceous plant measurements (variables: abundance, height, number of species present)		
Treatment	Variables Measured	Sampling Interval
June burned post	all	pre + 4, 6, 8, 13 weeks
June unburned	all	pre + 8, 13 weeks post
post	number of species	pre + 4, 6, 8, 13 weeks
July burned post	all	pre + 2, 4, 6, 11 weeks
July unburned	all	pre + 6, 11 weeks post
post	number of species	pre +2, 4, 6, 11 weeks

Woody plant measurements (variables for unburned treatments: leader length, flowers per number of leaders, gall; variables for burned treatments: number of <i>Chrysothamnus viciciflours</i> (CHVI) resprouted, number of stems resprouted per plant, average stem length.)		
Treatment	Variables Measured	Sampling Interval
June burned	no CHVI resprouted	4, 6, 8, 13 weeks post
	no. stems resprouted, stem length	13 weeks post
June unburned	all unburned variables	pre + 13 weeks post
July burned	no. CHVI resprouted	2, 4, 6, 11 weeks post
	no. stems resprouted, stem length	11 weeks post
July unburned	all unburned variables	pre + 11 weeks post

Herbaceous biomass	
Treatment	Vegetation Clipped
June unburned	9 weeks post
June burned	13 weeks post
July unburned	8 weeks post
July burned	11 weeks post

Vegetation response - *Artemisia tridentata*

Non-burn treatments (i.e., 0.5% Silv-Ex, 1.0% Silv-Ex, Phos-Chek, and water) were applied to randomly selected big sage plants, 10 per treatment. The volume of chemical applied to each plant was scaled according to the volume of the plant, using 0.33 gal/m³. We applied the chemical using the same motorized backpack pumps as were used for the vegetation plots.

We randomly selected and tagged four branches on each plant. Current annual growth (terminal leader and four subsequent leaders), and number of galls per branch were recorded prior to treatment and at the end of the growing season, 5-6 October. When flowers were present, we measured inflorescence length at the end of the season.

Statistical Methods:

We used analysis of variance (ANOVA) techniques to assess the effects of burning, Silv-Ex and Phos-Chek on plant abundances, plant growth, plant species diversity, biomass, number of galls, and number and size of flowers. The exact ANOVA model varied with the response variable. All response variables were log-transformed to increase normality. For plant species abundances, heights, and diversity, the model was a repeated measures design with treatment as main effect and sampling date as the repeated measures factor. For the other response variables, the ANOVA designs were simple one- or two-way factorials. Analyses were done separately for riparian and upland habitat zones because species varied between them; separate analyses were also performed for the June and July treatment applications because of phenological differences among species. We used Fisher's protected LSD test to isolate mean differences for significant effects or interactions in ANOVAs (Milliken and Johnson, 1984). We used the general linear models procedure (PROC GLM) of SAS (SAS 1989) to conduct the analyses. Significance was set at $p = 0.05$.

Results:

Vegetation response, herbaceous species. Of the 67 species found on riparian and upland plots, only 19 occurred on ≥ 50 plot-sample combinations, and thus were sufficiently abundant to likely exhibit treatment effects. Four of the 19 species (21%) were clearly affected by one or more treatments: *Achillea millefolium*, *Carex praegracilis*, *Linum perenne*, and *Poa pratensis* (Table 3). No species showed a significant treatment effect, other than pre-treatment differences among plots, on burned plots.

Table 3. Plant species affected by Silv-Ex or Phos-Chek application.

					Effect		
Species	Family	Lifespan	Origin	Growth Form	Burned	Month Applied	Zone
<i>Achillea millefolium</i>	Asteraceae	perennial	introduced	forb	no	July	riparian
<i>Carex praegracilis</i>	Cyperaceae	perennial	native	sedge	no	June, July	riparian
<i>Linum perenne</i>	Lineaceae	perennial	native	forb	no	June	riparian
					no	June	upland
<i>Poa pratensis</i>	Poaceae	Perennial	introduced	grass	no	June, July	riparian

A. millefolium, yarrow, was more abundant and of greater stature on retardant and 1% Silv-Ex plots before July treatment (Figure 1). Plants in all treatments had senesced by 6 weeks post-treatment. By the eleventh week, however, plants in unburned riparian 1% Silv-Ex treatment plots had returned to their pre-treatment abundance; none of the plants in the other treatments had increased significantly in either abundance or height beyond their mid-season senescence. *A. millefolium* did not respond significantly to treatments that included burning, nor did it respond to June applications, regardless of burn status.

The sedge, *C. praegracilis*, responded dramatically to Phos-Chek application on unburned riparian plots (Figure 2) after both June and July applications. Abundance did not differ significantly from control prior to treatment; by the 6th week after June treatment both Phos-Chek and 1% Silv-Ex plots had fewer plants of lower stature than did either control plots or plots with 0.5% Silv-Ex. By the thirteenth week, sedges on Phos-Chek plots nearly had returned to pre-treatment abundances, and were significantly more abundant than on control plots. In contrast, plots with 1% Silv-Ex had fewer sedges than any other treatment by the thirteenth week.

July applications to unburned riparian plots produced the same dramatic response to Phos-Chek in both height and abundance (Figure 2), but Silv-Ex treatments were not statistically different from the control at any sampling period. Although common on upland plots (Table 1), *C. praegracilis* showed no significant treatment effects in the upland zone.

L. perenne, flax, was the only plant to exhibit significant treatment effects in the upland zone; abundance varied significantly after June treatments in both riparian and upland zones, although riparian effects were primarily due to pre-treatment differences among plots in different treatments (Figure 3). Although there were pre-treatment differences among upland plots, treatment effects persisted throughout the study in both abundance and height; in each case,

Achillea millefolium

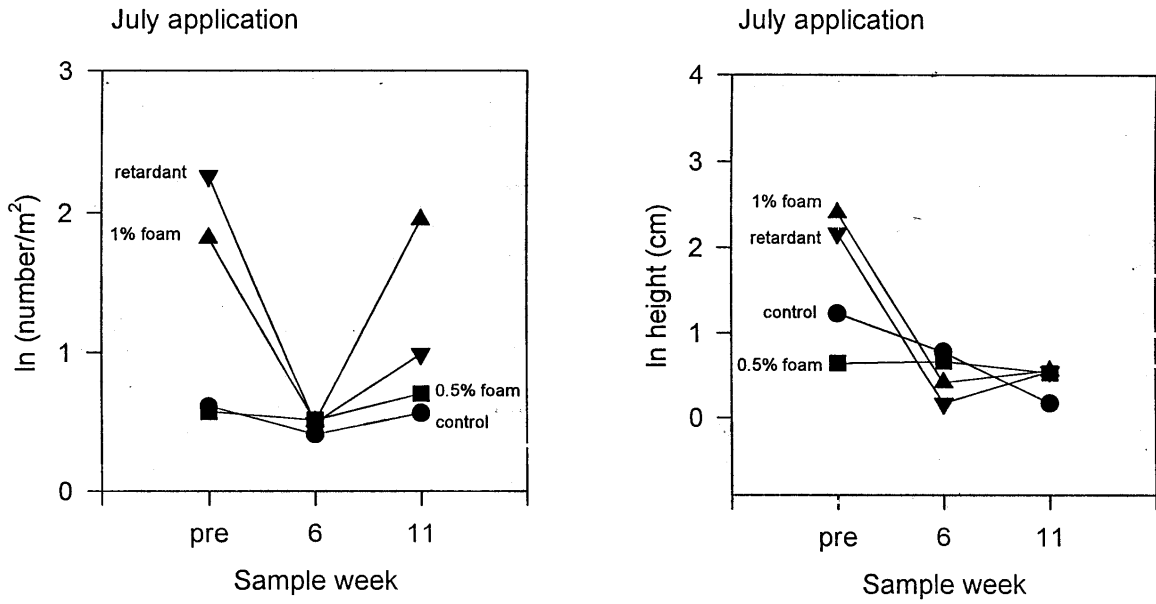


Figure 1. Changes in abundance and height of *Achillea millefolium* from pre-treatment through 11 weeks after the July application on unburned riparian plots.

Carex praegracilis

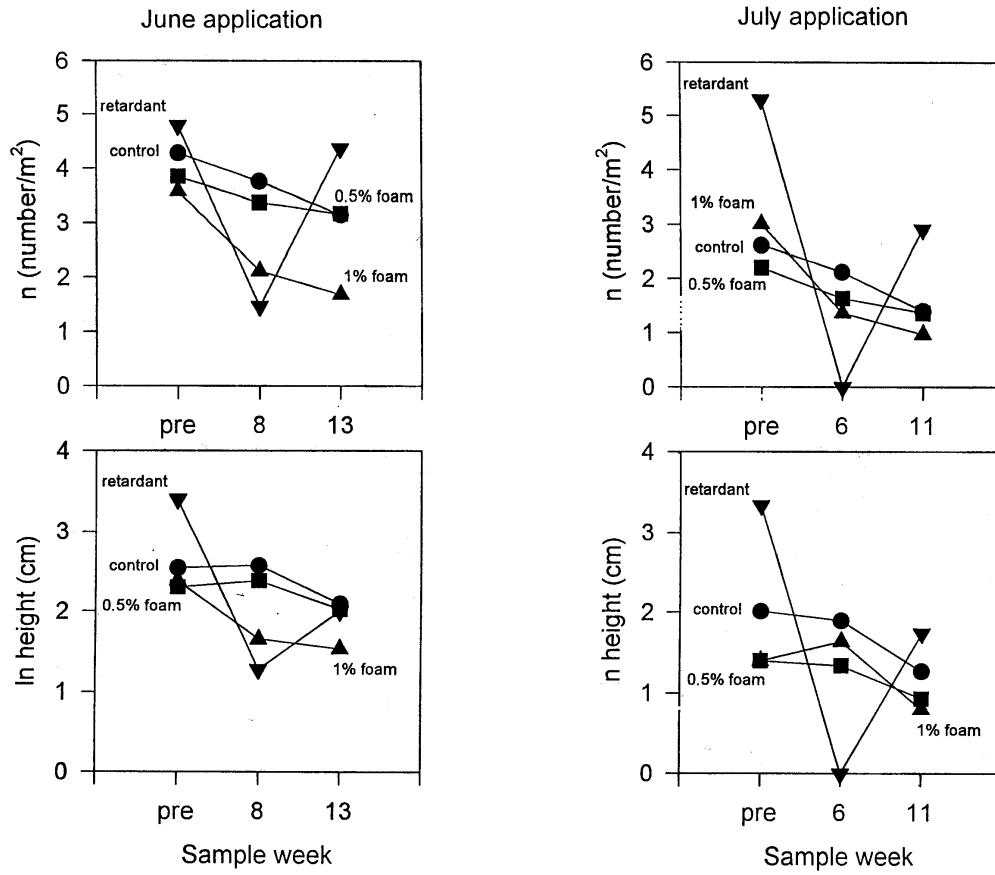


Figure 2. Changes in abundance and height of *Carex praegracilis* from pre-treatment through 11 or 13 weeks after June and July applications on unburned riparian plots.

Linum perenne

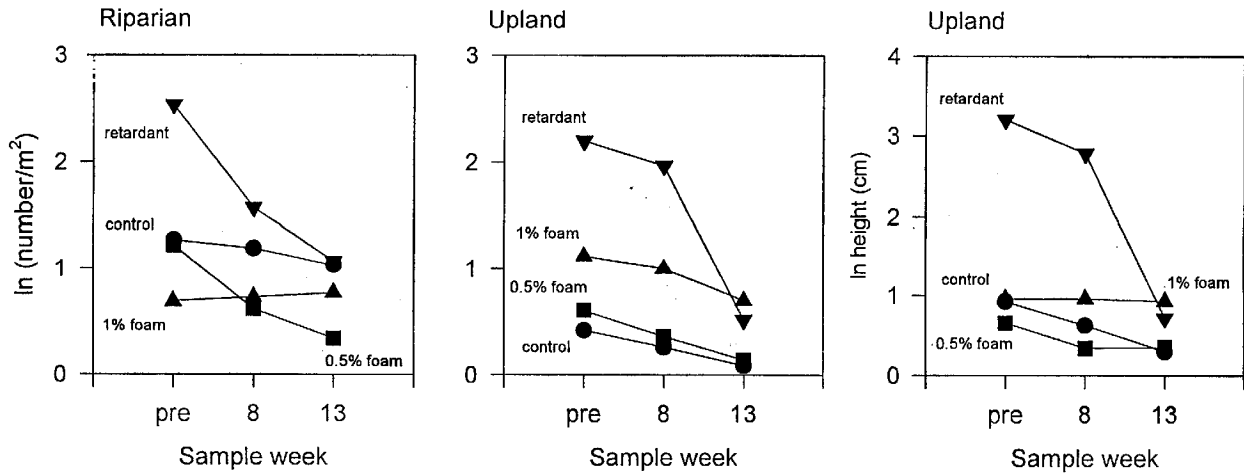


Figure 3. Changes in abundance of *Linum perenne* from pre-treatment through 13 weeks after June applications on unburned riparian and upland plots, and change in height after June applications on upland plots.

control and 0.5% Silv-Ex plots had fewer and smaller flax plants than did 1% Silv-Ex and Phos-Chek plots.

P. pratensis, bluegrass, responded to Phos-Chek application in unburned riparian plots by nearly complete senescence by the first sample period after treatment (Figure 4). Although indistinguishable from control plots before treatment, abundance of bluegrass treated with 0.5% Silv-Ex in June remained below that of 1% Silv-Ex or control through the remainder of the growing season; heights of grasses treated with 0.5% Silv-Ex or Phos-Chek in June ended the season significantly shorter than grasses receiving the other treatments. Plants receiving July applications did not differ among treatments by the end of the growing season.

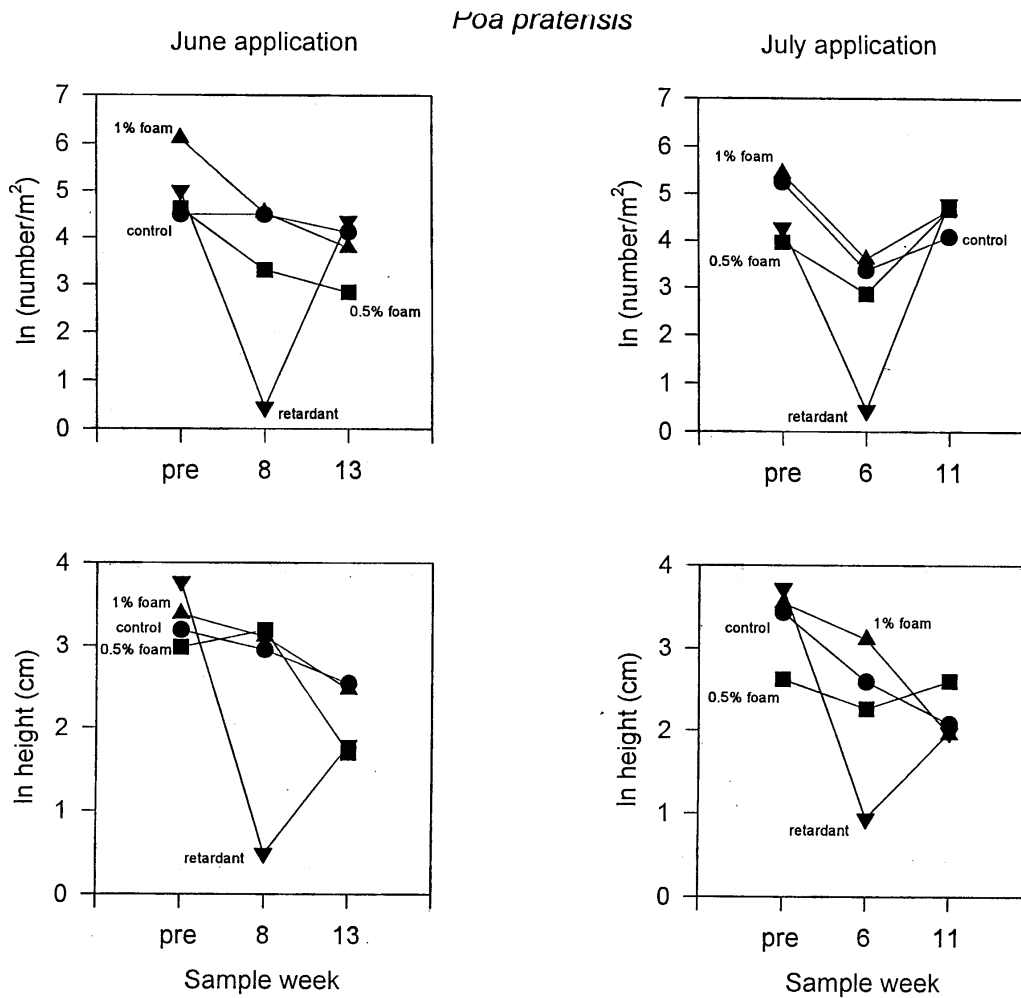


Figure 4. Changes in abundance and height of *Poa pratensis* from pre-treatment through 11 or 13 weeks after June and July applications on unburned riparian plots.

Vegetation response - woody species.

We found no difference in any variable we measured on woody species occupying vegetation plots after treatment. Growth, resprouting, number of flowers, and number of galls were unaffected by any chemical application.

Vegetation response - community characteristics.

Riparian zones were more responsive to June treatments than to July treatments; upland zones responded only to July treatments. In the riparian zone, burning obscured responses to

chemicals that were seen in unburned sites. In the upland zone, different variables responded to chemical treatment in burned and unburned plots.

Before treatment, the total number of stems/m² in the upland zone was higher on 1% and 0.5% Silv-Ex plots than on the other two treatments (Figure 5). After burning and chemical application, all plots remained essentially bare until late summer rains stimulated growth after the eighth week post-treatment. By the thirteenth week, Phos-Chek plots had significantly more stems/m² than did the control or the two Silv-Ex treatments. The 0.5% Silv-Ex treatment had significantly fewer stems/m² than did all other treatments.

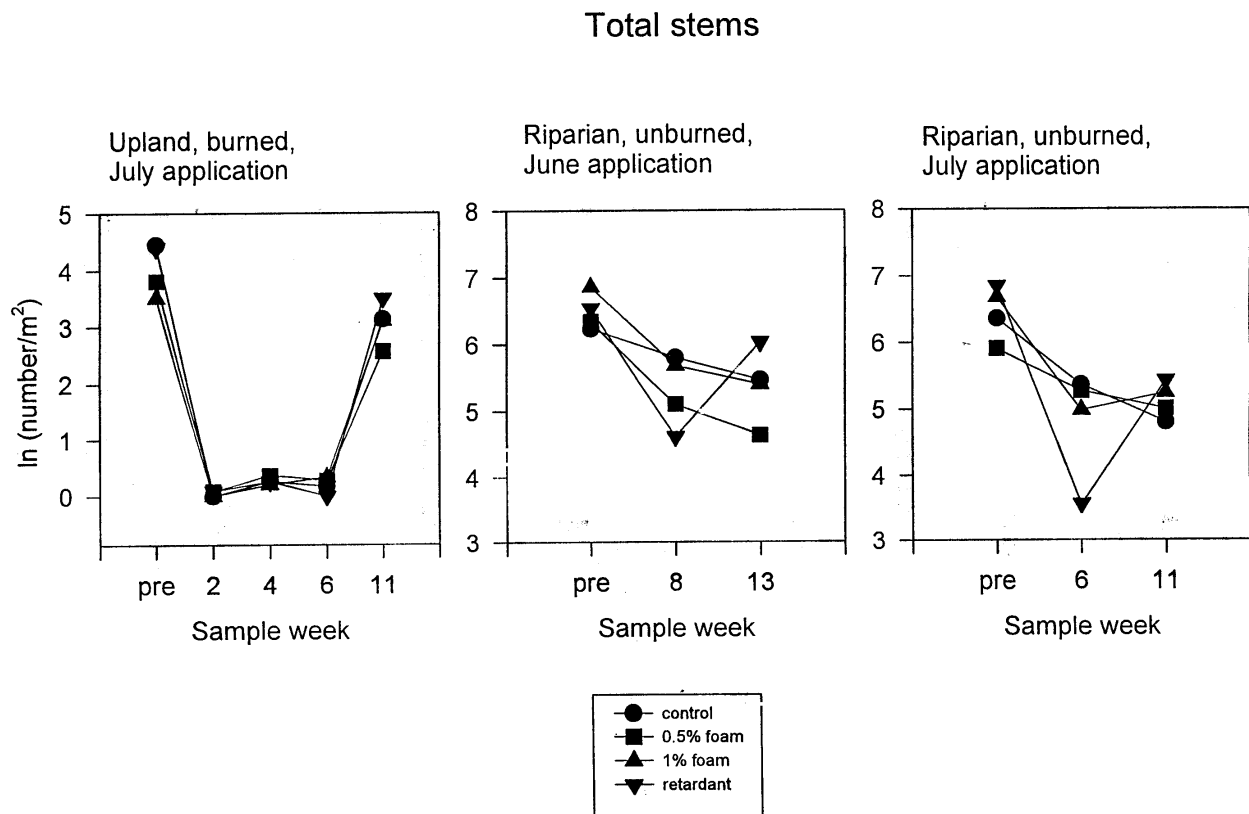


Figure 5. Change in density of stems on upland burned plots after July chemical application and on riparian unburned plots after June and July chemical application.

On unburned riparian plots, significant treatment effects on number of stems/m² were seen after both June and July applications (Figure 5). Number of stems/m² showed the greatest response to Phos-Chek after both June and July applications, although by the eleventh week after the July applications all treatments were statistically indistinguishable. At 13 weeks after the June application, 0.5% Silv-ex plots had significantly fewer stems/m² than any other treatment; Phos-Chek plots had significantly more.

Total number of species/m² did not differ from control on unburned riparian plots prior to June or July applications; after treatment, the number of species declined on all plots, with Phos-Chek plots having significantly fewer species than the other treatments up to 6 or 8 weeks after July and June applications, respectively (Figure 6). By the thirteenth week after June applications, control plots still had higher numbers of species than did the three chemical treatments. In contrast, by the eleventh week after July applications, the number of species in chemically treated plots was statistically indistinguishable from the number in control plots.

Shannon's index of species diversity (H') revealed significant treatment effects in both riparian and upland unburned plots. In the riparian zone, H' declined markedly after Phos-Chek application in June (Figure 7). By the thirteenth week after treatment, only 1% Silv-Ex treated plots had significantly lower H' than the control plots.

In the upland zone, all three chemical treatments resulted in significantly lowered H' six weeks after July applications (Figure 7). By the eleventh week after treatment, however, only Phos-Chek plots had significantly lower H' than the control plots.

Vegetation response - *herbaceous biomass*.

We found no significant effect on biomass of any chemical treatment.

Vegetation response - *Artemisia study*.

We found no significant effect on growth, flower production, or galling insect activity of any chemical treatment.

Total species

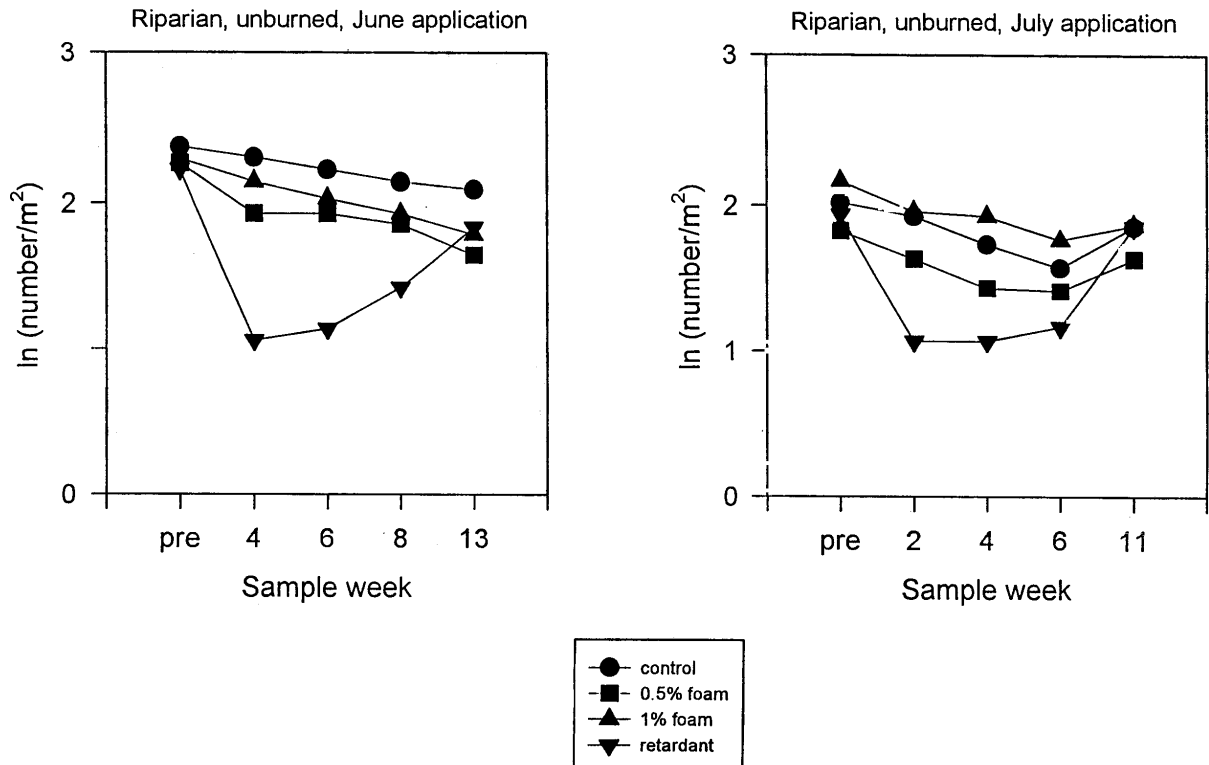


Figure 6. Change in number of species on riparian unburned plots after June and July chemical application

Species diversity

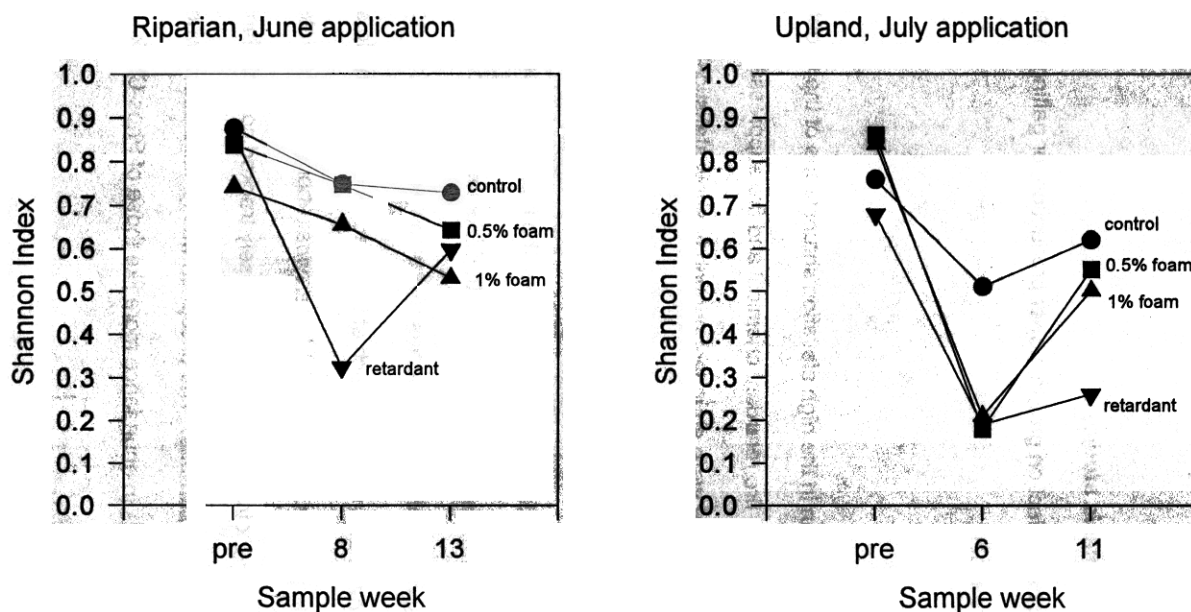


Figure 7. Change in species diversity (H') on riparian unburned plots after June chemical application and on upland unburned plots after July chemical application.

Discussion:

Herbaceous species. Vegetation in the high elevation shrubsteppe of the Great Basin responded in measurable ways to fire retardant chemical and fire suppressant foam application. Of the 19 species that were abundant enough to show statistically significant treatment effects, four (21%) responded to one or more chemical treatment (Table 3). All four are perennial; all but *L. perenne* are known to spread by rhizomes or creeping rootstock (Munz and Keck, 1968). Nonetheless, the majority of species that failed to exhibit a treatment effect also are perennial with rhizomes or creeping rootstock.

Examination of responses of the four species reveal few similarities. Phos-Chek produced a marked decline in abundance of each of the species on riparian plots when the first

counts were made post-treatment; all but *L. perenne* rebounded after late summer rains. Trends in Silv-Ex treated plants more closely tracked control plants in *P. pratensis*, *C. praegracilis*, and *L. perenne*. The exception was *A. millefolium*, where 1% Silv-Ex treatment produced trends in abundance more like those of Phos-Chek treatment. Neither abundance nor height of plants subjected to any chemical treatment was consistent, with respect to controls, among species.

The lack of significant effects in any individual species after burning likely reflects the short duration of the study rather than an actual lack of effect. Responses to burning in the sagebrush steppe are more appropriately measured over the course of several years (Young and Evans, 1978), or even decades (Harniss and Murray, 1973). Annuals, in particular, could not be expected to regrow on plots until the following season. Most upland species, after early spring growth that largely occurred before roads were passable to the study site, were dormant through most of the study. It should be kept in mind, however, that most natural fires will also occur during this dormant season; if chemicals do not persist in the soils until the next growing season, there may, in fact, be little long-term effect of their use, at least in terms of individual species. Results of soil analyses may shed light on this question.

Community effects. As with individual species abundances, the total number of species (Figure 6) and their total abundance (Figure 5) declined after Phos-Chek application far more than for any other treatment. Because H' reflects both species richness and relative abundance, H' also declined after Phos-Chek application, while other treatments followed trends more similar to the control (Figure 7). Interestingly, although H' returned to levels similar to the control in riparian areas after late summer rains, no such response was observed in the uplands. In addition, all chemical treatment in the uplands produced a marked decline in H' within 6 weeks of application, which was not evident in riparian sites.

The dramatic response of H' to Phos-Chek application may reflect the addition of nitrogen to the soils. Wilson and Shay (1990), working in mixed prairie, found that H' declined

after nitrogen fertilization, but not in response to burning. If this is the case on our study sites, then the difference between H' in riparian and upland sites at the end of the study may be the result of differences in nitrogen holding capacities of riparian and upland soils. We presently have no data to test this hypothesis.

Lack of response. The majority of species demonstrated no response to chemical application over the course of the growing season in which the chemicals were applied. Flowering progressed normally in *Artemesia* and *Chrysothamnus*. The well-known post-fire sprouting of *Chrysothamnus* (Young and Evans, 1974) was not disrupted. Activity of galling insects was not influenced, suggesting that structural components of leaves and stems were unaffected by Silv-Ex and Phos-Chek.

Unlike in our prairie site, biomass in the Great Basin sites was not increased by Phos-Chek application. Because rains did not immediately carry the chemicals into the soil in Nevada, as they did in North Dakota, the fertilization effect may have been attenuated. Differences in soils alluded to in the discussion of species diversity may also play a role in translation of nitrogen into biomass production. It's also interesting to note that biomass measurements on all treatments had returned to control levels at the prairie site during the next growing season.

Management Implications:

None of the effects we detected on vegetation suggest that either Phos-Chek or Silv-Ex, applied as directed, should not be used to control wildland fire. One caveat, however, is the short duration of this study. We cannot say with certainty what changes may occur in species that were dormant until the next growing season. The fact that most species that showed immediate response returned to control levels by the end of the study does suggest that effects are likely transitory.

Managers intending to use these chemicals to control prescribed burns may wish to consider effects on species diversity, or on individual species of interest. Most significant treatment effects occurred in the riparian zone (Table 4); care should be exercised in riparian areas, especially if there are particular species of concern.

Finally, this study did not adequately address the interaction between burning and chemical application. Studies of longer duration, in which plots can be followed for several seasons after treatment, are essential in assessing these interactions. Plots in North Dakota were burned and treated this year with the hope of following them for several years to come, so that this concern can be addressed.

Table 4. Effects of Phos-Chek and Silv-Ex application on abundance and diversity measures.

Significant variable	<i>Month Applied</i>	Burned	Zone Affected
Total Stems	June	no	riparian
	July	no	riparian
	July	yes	upland
Total Species	June	no	riparian
	July	no	riparian
Species Diversity	June	no	riparian
	July	no	upland

Literature Cited:

- Harniss, R. O. and R. B. Murray. 1973. 30 years of vegetal change following burning of sagebrush-grass range. *Journal of Range Management* 26:322-325.
- Sas, Inc. 1988. SAS/STAT User's Guide, Release 6.03 Edition. SAS Institute Inc., Cary, North Carolina.
- Milliken, G. A. and D. E. Johnson. 1984. *Analysis of Messy Data: Designed Experiments*. Van Nostrand Reinhold, New York, New York.
- Munz, P. A. and D. D. Keck. 1968. *A California Flora*. University of California Press, Berkeley, California.
- Wilson, S. D. and J. M. Shay. 1990. Competition, fire, and nutrients in a mixed-grass prairie. *Ecology* 71:1959-1967.
- Young, J. A. and R. A. Evans. 1974. Population dynamics of green rabbitbrush in disturbed big sagebrush communities. *Journal of Range Management* 27:127-132.
- Young, J. A. and R. A. Evans. 1978. Population dynamics after wildfires in sagebrush grasslands. *Journal of Range Management* 31:283-289.

Task 2: Toxicity of Fire Retardant Chemicals to Vertebrate and Invertebrate Wildlife Species

Principal Investigators: Nimish B. Vyas and Elwood F. Hill
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Laurel, Maryland

Chemicals tested: *Silv-Ex*
10:1 ratio of a 1.0% batch
7.5 gallons of Silv-Ex in 750 gallons of water/acre

Phos-Chek G75-F
3 gallons of mixture/100 sq. ft.
Each gallon contains 1.12 lbs chemical/ gallon of water.

Objectives:

In general, the objectives involved conducting an experiment to identify the effects of the most toxic fire retardant and most toxic foam suppressant (based on toxicity to aquatic organisms) on wildlife populations.

- (1) To determine the population-level effects of operational applications of Phos-Chek G75-F and Silv-Ex on small mammals.**
- (2) To determine residual fate of Phos-Chek G75-F and Silv-Ex on vegetation and in soil.**
- (3) To determine changes in insect populations in response to applications of Phos-Chek G75-F and Silv-Ex.**
- (4) To monitor changes in the occurrence of reptiles at the study sites.**
- (5) To evaluate liver and skeletal muscle tissue of wild mammals for biochemical marker analysis.**

Procedures:

This experiment was conducted in the Santa Rosa Mountains of Nevada and consisted of two studies. Study 1 examined the effects of Silv-Ex and study 2 examined the effects of Phos-Chek G75-F on terrestrial wildlife. Phos-Chek G75-F and Silv-Ex were selected as test chemicals in this field study based on laboratory results on aquatic organisms. Chemical applications were conducted according to standard procedures used in wildfire management with the U.S. Bureau of Land Management administering the chemical treatment. Staff members from the National Interagency Fire Center, Boise, Idaho were present at and coordinated all chemical applications.

The two studies were conducted along the North Fork of the Little Humboldt River and included riparian and sagebrush habitats. Each of the studies consisted of eight 0.4-ha plots (4 control plots and 4 treatment plots). Treatments were randomly assigned to the plots. The experiment was initiated on June 17 and lasted until September 16, 1994. Two additional plots were treated identically to the above for procurement of specimens for the biochemical marker and vegetation and soil residue analysis.

Small Mammal Population Assessment

Data on small mammal abundance, survival, recruitment and movement were collected using live capture-recapture methodology. Experimental design followed the combined closed and open population models (Pollock et al., 1990). One hundred Sherman live traps were arranged in a 10 X 10 matrix on the control and treatment plots. Small mammals were individually marked with Monel metal fingerling tags and released immediately at the capture site. Data on body weight and reproductive condition were recorded at the initial capture and at all subsequent recaptures. Each plot was sampled for five consecutive days (sampling period) with four sampling periods per study. A 9-day interval lapsed between each of the four sampling periods. Two of the sampling periods per study were conducted prior to chemical application and two were conducted post-application. Mortalities were recorded and the carcasses were

frozen for biochemical markers and residue level analysis. Ten animals of the most abundant species were euthanized by decapitation from each of two supplemental plots for biomarker analysis. Carcasses were analyzed using procedures analyzed following procedures presented by Van-Meter et al. (1985). All samples were frozen and shipped frozen to Patuxent Environmental Science Center for analysis.

Vegetation and soil analysis

Vegetation and soil samples were collected from the study plots at 24, 48 and 72 h and 2 weeks post application. Samples were frozen and shipped frozen to Patuxent Environmental Science Center for analysis (Van-Meter et al. 1985).

Insect population assessment

Insects were collected from the study plots to determine changes in insect populations in response to applications of Phos-Chek G75-F and Silv-Ex. Insects were collected using sweep nets and pit traps. In addition to the above sampling, ant mounds were monitored for activity. Collection and monitoring schedules consisted of one pre-application and two post application periods for each plot. Thirty pit traps were placed per plot. Pit traps were opened for a period of 24-h. Insect collection using sweep nets and ant mound activity monitoring was also conducted during this period.

Reptile and amphibian monitoring

Plywood boards measuring 2'X4' were placed on the plots to attract reptiles and monitor changes in their occurrence at the study plots. Fifteen boards were placed per plot. Boards were checked once during the pre-application period and twice during the post-application period for each plot. This method did not provide a meaningful sample.

Results:

Evaluation of small mammal and insect population data indicated no detectable response related to chemical application. Biochemical analysis of tissues and blood collected from the field failed to confirm an adverse response to either chemical. Degradation of both Phos-Chek and Silv-ex as indicated by sample analysis of soils and vegetation indicated that chemical application rates used in this study resulted in relatively short-term exposures. Based on the combined results from laboratory and field studies, no direct toxicity to terrestrial organisms should result from application of these chemicals at the currently recommended rates.

Literature Cited:

Pollock, K.H., Nichols, J.D., Brownie, C., and Hines, J.E. 1990. Statistical inference for capture-recapture experiments. Wildlife Monographs 107. 97 p.

Van-Meter, W.P., George, C.W., and Johnson, C.W. 1985. Chemical analysis procedures for forest fire retardant constituents. USDA Forest Service Gen.Tech. Rep.181. 25 p.

Task 3: Effects of fire retardant chemicals on fish and aquatic macroinvertebrates in aquatic ecosystems of the Great Basin

Principal Investigator: Barry C. Poulton
Environmental and Contaminants Research Center
4200 New Haven Road
Columbia, MO, 65201

Chemicals tested: Silv-Ex
Phos-Chek D75-F

Objectives:

- 1) **To compare results from laboratory 96-h exposures in reconstituted waters to results from similar exposures using water from the North Fork of the Little Humboldt River for Silv-Ex and Phos-Chek D75-F.**
- 2) **To compare results from these toxicity tests with laboratory-cultured tests organisms to results from toxicity tests conducted with indigenous organisms.**
- 3) **To evaluate the toxicity of these chemicals to rainbow trout, Lahontan cutthroat, and fathead minnows and to evaluate the potential for recovery of these species after chemical exposure in streamside recirculating channels.**
- 4) **To determine the toxicity of Silv-ex and Phos-Chek D75-F to fish and aquatic invertebrates in actual stream exposures in the North Fork of the Little Humboldt River.**
- 5) **To evaluate the degradation of fire retardants and foam suppressants applied to different soil types**

Procedures:

A total of 15 on-site field exposures were performed in 1994 (13 + 2 range-finding tests) to examine the effects of fire retardant and suppressant chemicals on aquatic life. All exposures were conducted between June 23-July 15 at the North Fork of the Little Humboldt River, Humboldt County, located in the Santa Rosa Mountains about 30 km from Paradise, Nevada.

Tests included acute toxicity tests conducted in a mobile laboratory using water from the Little Humboldt instead of standard reconstituted water, on-site exposures in artificial stream channels, and actual in-stream exposures. Data were analyzed using probit (2 partial kills), Spearman-Kärber (1 partial kill), and binomial (no partial kills) methods for each time period where at least 50% mortality resulted.

Acute toxicity tests with stream water

Acute toxicity tests (USEPA 1991) were conducted for 96 h with fathead minnow larvae and *Daphnia magna* using Silv-Ex and Phos-Chek D75-F. These tests duplicated chemical exposure concentrations used by the Yankton Field Research Station (Objective 1- ECRC Protocols P93-04-01 and P93-04-04). This standard technique included 8 treatments and a control with readings routinely taken at 24-h intervals. During tests in the mobile laboratory, observations were also taken at 30 min, 1 h, 2 h, 4 h, 8 h, 24 h, 48 h, and 96 h. In contrast to the reconstituted water used in the original Yankton exposures, stream water collected from the North Fork of the Little Humboldt was used for dilution water and controls. Organisms were shipped from the ECRC to the study site and held for observation for 24 h prior to testing. Test results from organisms exposed to the chemicals mixed in stream water were compared to organism responses in identical concentrations of chemical mixed with reconstituted water (similar in hardness and alkalinity to the North Fork of the Little Humboldt).

Artificial stream channels

Twelve portable 12" x 12" x 48" fiberglass tanks with polypropylene covers and PVC fittings were set up streamside to simulate stream channels for continuous exposures of fish and invertebrates. These artificial stream channels were fitted with a 3MD-SC Little Giant Centrifuge pump (Little Giant Pump Co., Tulsa, OK). Each channel recirculated 75 L of stream water at a rate of 40 L/min, and provided a current velocity and water movement similar to

stream flow conditions. Pumps were operated with gas-powered generators during the tests. In these channels, indigenous invertebrates were exposed in 15 cm x 25-cm cylindrical baskets constructed with 1-mm mesh stainless steel wire. Similar chambers constructed of polypropylene and 1/8" plastic mesh were used for fish exposures. Both types of chambers were suspended with aluminum brackets inside each recirculating tank. These 12 artificial stream channels were designed to determine the effect levels for indigenous macroinvertebrates and to provide critical information to determine appropriate chemical concentrations for the in-stream exposures.

Chemicals and Dosage

Based on laboratory tests using fire retardant liquids and suppressant foams, concentrations of Silv-ex and Phos-Chek D75-F were chosen to provide a range of responses to aquatic organisms, including sublethal effects and mortality. To adequately address effects of short-term exposure due to accidental chemical spillage or entry into aquatic systems from aerial spray, observations were recorded at 30 min intervals for 4 h following treatment in the artificial stream channel tests. Concentrations used in each test are given in Table 1.

Water Chemistry

Portable meters were used to measure dissolved oxygen, pH, conductivity, and water temperature during tests in the recirculating channels. To measure additional variables, a 1-L grab sample was collected from each tank 5 min after addition of the chemical. Water chemistry variables included alkalinity, hardness, turbidity, ammonia, nitrates, sulfates, chlorides, and orthophosphate.

Two Hydrolab Units (Hydrolab Inc., Austin, TX) were deployed in the North Fork of the Little Humboldt River during the in-stream chemical doses, one at a reference site upstream of the dose addition and one immediately downstream. Each unit was calibrated and set to monitor pH, dissolved oxygen, conductivity, and water temperature each minute for the duration of each test.

Artificial Channel Exposures with Indigenous Aquatic Invertebrates

Two streamside exposures with nymphs of the predacious stonefly *Hesperoperla pacifica* (Plecoptera: Perlidae) and the mayfly *Epeorus (Iron) albertae*, both collected from the Little Humboldt River, were conducted in the artificial stream channels. Nymphs were collected with a D-frame kicknet and pre-counted the day before each test. Nymphs were held overnight in the stream using cylindrical stainless steel chambers suspended in rectangular styrofoam floats. Before each test, nymphs were transferred to the test site in coolers and acclimated to within 1°C of the test water in the tanks. Ten stonefly nymphs were placed in each of 3 cylindrical chambers within each tank (Table 1) and were exposed to a range of concentrations of each chemical. Identical numbers of mayfly nymphs were exposed concurrently in the same manner. Sublethal behavioral effects and mortality were recorded at 30 min, 1 h, 2 h, 3 h, and 4 h during each test.

Initial examination of mortality and sublethal effects indicated that the mayfly was more sensitive than the stonefly during tests with both chemicals. To further define effects of Silv-Ex on *Epeorus (Iron) albertae*, and to more accurately define an appropriate concentration to be used during later in-stream chemical doses, an additional test with 5 concentrations and 12 tanks was conducted with mayfly nymphs. Test concentrations of Silv-Ex ranged from 20.63 - 330.0 mg/L for this test.

Table 1. Summary of 1994 on-site exposures performed on the North Fork of the Little Humboldt River, Humboldt County, Nevada, during an evaluation of the effects of Phos-Chek D75-F and Silv-Ex on aquatic organisms.

TEST #	DATE(S)	CHEMICAL	DURATION	ORGANISM USED	# CHAMBERS	CONCENTRATIONS TESTED(mg/L)
*1	6/23-25	Silv-Ex	48 hr	<i>Daphnia magna</i>	18	0, 3.6, 6, 10, 17, 28, 47, 78, 130
*2	6/23-25	Silv-Ex	96 hr	Fathead Minnow	18	0, 2.16, 3.6, 6, 10, 17, 28, 47, 78
3	6/24	Phoschek D75-F	4 hr	Mayfly / Stonefly	6	0, 14.65, 46.88, 150, 480, 1536
4	6/26	Silv-Ex	4 hr	Mayfly / Stonefly	6	0, 14.65, 46.88, 150, 480, 1536
5	6/28	Silv-Ex	4 hr	Mayfly	12	0, 20.63, 41.25, 82.5, 165, 330
6	7/2	Silv-Ex	4 hr	Rainbow Trout / LC Trout	12	0, 20.63, 41.25, 82.5, 165, 330
7	7/3	Phoschek D75-F	4 hr	Rainbow Trout / LC Trout	12	0, 82.5, 165, 330, 660, 1320
*8	7/7-11	Phoschek D75-F	96 hr	Fathead Minnow	18	0, 78, 130, 216, 360, 600, 1000, 1700, 2800
*9	7/7-9	Phoschek D75-F	48 hr	<i>Daphnia magna</i>	18	0, 13, 21.6, 36, 60, 100, 170, 280, 470
**10	7/13	Silv-Ex	2 hr	Rainbow Trout / LC Trout	5	0, 82.5, 104, 131, 165
11	7/14	Phoschek D75-F	30 min	In-Stream		72.4 (Macroinvertebrate Drift), 121.8 (Fathead Minnow)
12	7/14	Silv-Ex	30 min	In-Stream		82.5
13	7/15	Phoschek D75-F	30 min	In-Stream		228.2

* Test performed in mobile laboratory

** Test performed with recovery after 30 min. of exposure time

Artificial Channel Exposures with Trout

Hatchery-raised, sixty-day old Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) and 120-day old rainbow trout (*Oncorhynchus mykiss*) were tested concurrently during 3 streamside exposures in the artificial channels. Fish were transported from the Lahontan Fish Hatchery and the Mason Valley Hatchery, respectively, and then acclimated to stream water 24 h prior to testing. During acclimation, holding chambers were tethered on square styrofoam floats in the North Fork of the Little Humboldt River. For each species, 4 chambers containing 10 fish each were placed in the artificial channels. Sublethal behavioral effects and mortality were recorded at 30 min, 1 h, 2 h, 3 h, and 4 h for tests on 2 July and 3 July. An additional test set up with the same configuration was performed on 13 July to document recovery after Silv-Ex exposure. In this study, after a 30-min exposure to Silv-ex, fish were moved to 5 adjacent channels containing fresh stream water. Mortality, sublethal effects and recovery were documented after chemical exposure every 15 min for 90 min.

In-Stream Chemical Dose and Community Effects

A total of three in-stream chemical exposures were conducted in the North Fork of the Little Humboldt River to document ecological effects of Silv-ex and Phos-Chek D75-F. Concentrations were selected based on effects observed after 30 min. of exposure in the artificial channels for rainbow and Lahontan cutthroat trout, and the mayfly *Epeorus (Iron) albertae*. A stream reach including 2-3 riffle-run-pool sequences was designated for each test. Stream sections were dosed using a 1050-L (230 gal) polyethylene tank fitted with a 3MD-SC pump, water meter, and gate valve connected to 15 m of hose. The chemical was mixed with stream water to the desired concentrations in the tank, and the gate valve was adjusted so the tank would empty in 30 minutes. Two drift nets constructed of 15.5 X 17 cm steel cylinders and 363-um mesh Nitex netting were placed in a run below the first riffle exposed by the chemical. Drift samples were taken at 30 min. intervals before, during, and after chemical addition for each test. A Surber sampler was used to take quantitative macrobenthos samples from the center of the

affected riffle before and after chemical dose to document any decrease in density and community loss due to chemical exposure. Macroinvertebrates collected by the drift nets and Surber sampler were placed in jars, labeled, and preserved with 80% ethanol. All samples were sorted and enumerated under a dissecting microscope at 10X magnification, and macroinvertebrates were identified to the genus level or lowest possible taxon.

Prediction of Chemical Concentration and Exposure Time

To determine how in-stream exposures would relate to actual situations where spillage or aerial spray reaches a stream, and to determine worst-case concentrations and exposure times, physical stream parameters were measured along the 3 stream segments used for the in-stream exposures in the North Fork of the Little Humboldt River. Stream width (wettable perimeter area) was measured along 10 transects in each riffle and pool segment with a 100 m tape. Current velocity and depth were determined along the same transects with a Swoffer model 2100 current velocity meter and measuring rod. Discharge was determined for each of the stream segments by summation of current velocity and depth measurements for every 1 ft. of cross-sectional width across the stream. Measurements of velocity, width and depth were adjusted for riffle and pool length, and averaged for the entire set of 3 stream segments. Discharge, and mean values for width, depth, and current velocity were used to determine: 1) stream distance the chemical would travel in 30 minutes, 2) stream area of chemical coverage that would result in a maximum of 30 minutes of organism exposure, and 3) theoretical worst-case concentrations that might result from the use of different chemical coverage rates.

Determination of rates of degradation

Studies were conducted to determine the rates of degradation in soil for all five chemicals that had been originally tested under laboratory conditions (Fire-Trol GTS-R, Phos-Chek D75-F, Fire-Trol LCG-R, Silv-Ex, and Phos-Chek WD-881). Chemicals were applied to 500 g samples of five soil textures (sand, sandy loam, silty clay loam, clay loam, and clay) and to 5 samples of silty clay loam with different organic matter content. Chemical concentrations of foams were

evaluated at the time of application and at 24, 48, 96, 120, and 168 hours after application. Chemical concentrations of non-foams were evaluated at the time of application and at 24, 48, 96, 120, and 168 hours and 15 and 30 days after application to determine the potential of each chemical to be mobilized by a rainfall event. Soil was added to a 500 ml flask containing 300 ml of reconstituted soft water with a pH of 6.0, and vigorously shaken. A sample of the overlying water was then analyzed to determine the amount of chemical released from the soil.

Results:

Water Chemistry

A summary of each on-site exposure and corresponding 12 water quality variables measured during the tests are given in Tables 1-2. The North Fork of the Little Humboldt River is a relatively soft water system with pH ranging from 7.4-8.6, alkalinity of 44-51 (mg/L as CaCO₃), hardness of 28-34 mg/L, and conductivity of 100-120 μ mhos/cm. In general, these water quality conditions were similar to the reconstituted soft water used for tests with aquatic organisms in the laboratory (Hamilton and Buhl, this report). Dissolved oxygen remained above saturation and water temperatures were maintained at appropriate levels for each test organism exposed in the mobile laboratory. Similar to tests by Hamilton and Buhl, addition of Phos-Chek D75-F into test chambers resulted in lower pH values and substantial increases in ammonia, sulfates, orthophosphate, and conductivity. During Phos-Chek D75-F tests, levels of total ammonia were as high as 579 mg/L at the highest test concentration of 1536 mg/L. Addition of Silv-Ex into test chambers resulted in slight increases in conductivity, ammonia, sulfate, chlorides, and orthophosphate (Table 2).

In the North Fork of the Little Humboldt River, natural variability was observed in some water quality variables due to the dynamic nature of streams in the Great Basin; diel fluctuations of 8-15 C° in stream water temperature were common. Temperatures of stream water used in the recirculating channels were 13-15 C° in early morning at the beginning of tests, and gradually

Table 2. Range of values for 12 water quality parameters measured during 13 exposure tests with Phos-Chek D75-F and Silv-Ex on the North Fork of the Little Humboldt River, Humboldt County, Nevada. Test numbers correspond with Table 1.

Test #	Temp. (C°)	D.O. (mg/L)	pH	Conductivity (µmhos/cm)	Alkalinity (mg/L)	Hardness (mg/L)	Turbidity (NTU)	Ammonia (mg/L)	Nitrate (mg/L)	Sulfate (mg/L)	Chlorides (mg/L)	Ortho-P (mg/L)
1	20-21	4.8-7.6	7.4-7.6	110-150	44-48	30-32	N/A	0.1-1.84	0.07-0.16	6-19.3	0.3-2.3	0.08-0.26
2	20-21	5.1-8.0	7.4-7.5	105-170	48**	28-30	N/A	0.1-0.36	0.7-0.8	12-18	0.32-2.3	N/A
3	17-21	8.6-9.0	6.5-7.7	100-2000	44-87	34-39	N/A	0.18-579	N/A	9.8-1050	0.20-0.36	0.65-9.2
4	14-19	6.8-7.4	7.5-7.6	105-200	N/A	N/A	N/A	0.032-11.9	0.032-0.081	3.2-11.7	0.004-0.1	N/A
5	24-30	7.3-8.5	7.3-7.8	100-145	46-52	34-35	2.1-14	0.07-3.2	0.003-0.089	2.4-11.6	N/A	0.18-0.33
6	25-26	6.7-8.2	7.3-7.5	105-140	44-52	32-34	0.08-15	0.22-6.2	0.02-0.11	0.25-9.8	N/A	0.18-5.2
7	25-26	6.2-7.7	6.6-7.4	120-1880	51-80	34-37	2.7-44	0.13-261	0.02-0.1	9.6-639	1.9-4.4	0.05-10.2
8	18-20	6.3-7.0	6.5-7.8	115-2150	45**	29**	2.5**	0.02-35.9	0.05-0.12	11.14**	5.0**	0.33-10.6
9	19-20	6.4-7.3	6.6-7.6	110-700	36-81	29-32	2.5-99	0.03-310	0.08-0.14	11.1-693	3.5-10.5	0.33-12.4
10	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
11	14-18*	8.3-9.0*	7.5-7.8*	112-183*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
12	25-26*	6.4-7.6*	8.5-8.6*	107-110*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
13	17-21*	8.2-8.9*	6.7-7.9*	110-388*	N/A	N/A	1.9-14*	N/A	N/A	N/A	N/A	N/A

* = Values are from Hydrolab Unit
 ** = Values are listed for control only
 N/A = Analysis not performed

increased to as high as 30 C° at mid-day. Water temperatures in the streamside recirculating channels generally followed that of the stream temperatures during the 4-hour exposure periods. In-stream exposures were conducted during morning hours when stream temperatures were similar to those experienced in artificial channel exposures. All artificial channel and in-stream tests were conducted at the same time of day to minimize effects of temperature (Table 2). Chemical dosages used for in-stream exposures were selected based on the lowest level of effect observed in laboratory and artificial channel tests to insure that minimal ecological perturbation would occur in the stream. During in-stream exposures with Phos-Chek D75-F, measurements of conductivity were used to confirm concentrations dosed in the stream (Figures 1-3). Dose-conductivity relationships from tests #3 and #7 (Table 1) yielded a regression coefficient of $r=0.99$. Regression (Figure 1) determined that Phos-Chek concentrations of 72.4 mg/L and 228.2 mg/L were present during these exposures. Nominal dosages of 82.5 mg/L were used for in-stream tests with Silv-Ex.

Acute Test Results

Acute toxicity tests with Phos-Chek D75-F and Silv-Ex combined with stream water indicated that, similar to results from laboratory studies, Silv-Ex was more toxic to both fathead minnows and daphnids than was Phos-Chek D75-F (Table 3). The 96 h LC₅₀ for fathead minnows exposed to Phos-Chek in stream water was comparable to the LC₅₀ determined in the laboratory with reconstituted hard water (168 mg/L, Hamilton and Buhl, this report). Fathead minnows were slightly more sensitive to Silv-Ex when exposed in stream water than in reconstituted soft water, but the distribution of mortality was similar to results from standard laboratory testing. Response of daphnids exposed to Phos-Chek D75-F in stream water (Table 3), was within the confidence intervals reported by Hamilton and Buhl (this report). The sensitivity of *D. magna* to Silv-Ex (Table 3) was also similar to laboratory tests with reconstituted soft water, and 95% confidence intervals overlapped with Hamilton's data.

Phos-Chek D75F Dose vs. Conductivity

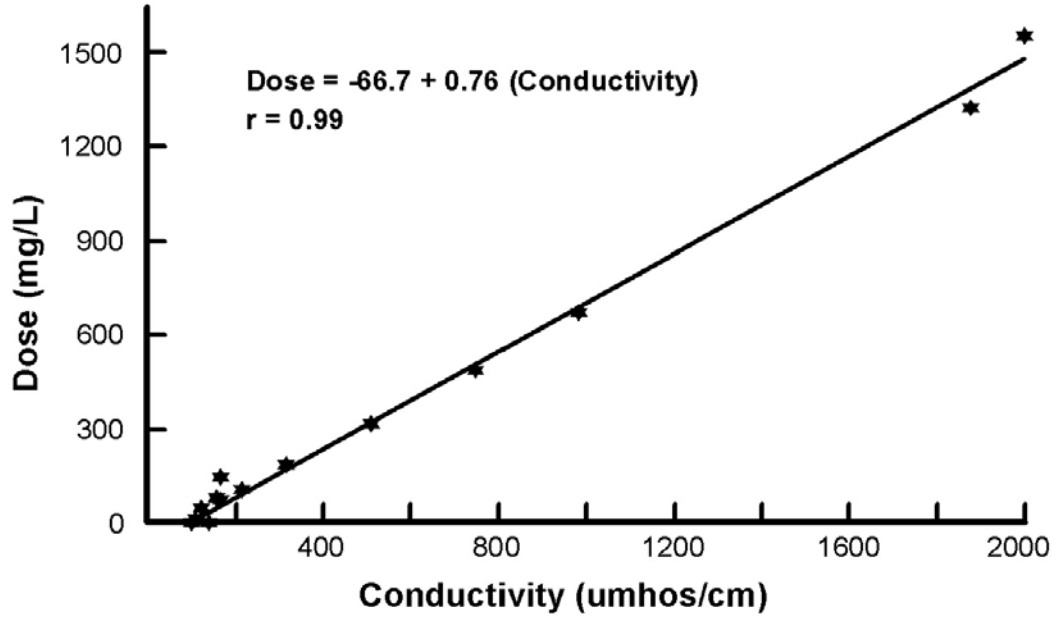


Figure 1. Regression, prediction equation, and line of best fit for Phos-Chek D75-F dose vs. conductivity based on data from on-site exposure tests #3 and #7 (from Table 1) on the North Fork of the Little Humboldt River, Humboldt County, Nevada.

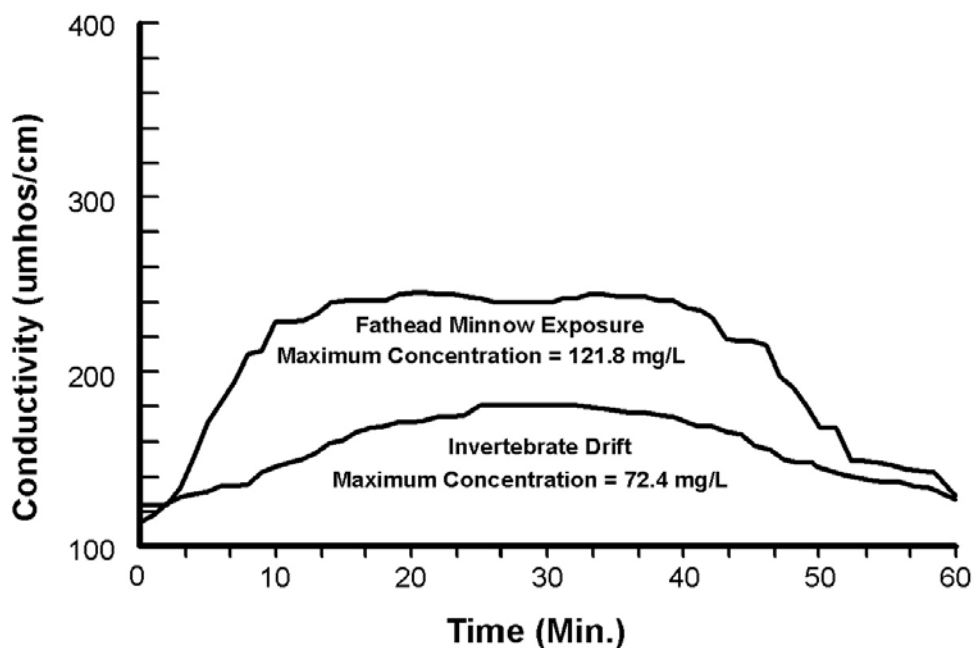


Figure 2. Conductivity curves for the first in-stream Phos-Chek D75-F exposure on the North Fork of the Little Humboldt River, Humboldt County, Nevada. Conductivity was measured every minute with a Hydrolab unit, and maximum chemical concentrations were determined with regression (Figure 1).

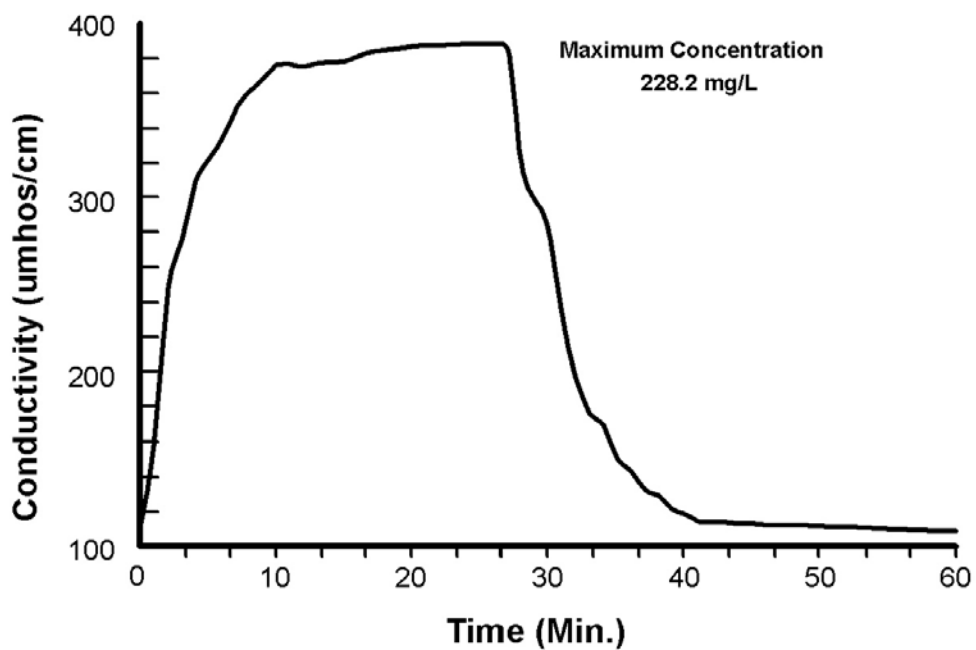


Figure 3. Conductivity curve for the second in-stream Phos-Chek D75-F exposure on the North Fork of the Little Humboldt River, Humboldt County, Nevada. Conductivity was measured every minute with a Hydrolab unit, and maximum chemical concentration was determined with regression (Figure 1).

Table 3. Summary table of on-site toxicity results for aquatic organisms exposed to Silv-Ex and Phos-Chek D75-F. LC₅₀ and EC₅₀ values were calculated with Probit (2 partial kills), Spearman-Kärber (1 partial kill), and binomial (no partial kills) methods.

ORGANISM	TIME (hr)	Phos-Chek D75-F		Silv-Ex	
		LC50 (C.I. RANGE)	EC50 (C.I. RANGE)	LC50 (C.I. RANGE)	EC50 (C.I. RANGE)
Mayfly <i>Epeorus (Iron) albertae</i>	0.5	no mortality	no effect	< 50% mortality	117 (83-165)
	1	< 50% mortality	< 50% effect	< 50% mortality	80 (73-88)
	2	< 50% mortality	< 50% effect	42 (33-50)	27 (21-41)
	3	1033 (857-12456)	807 (698-934)	35 (30-41)	(0-21)
	4	1051 (627-1300)	798 (670-949)	25 (20-29)	(0-21)
Stonefly <i>Hesperoperla pacifica</i>	0.5	< 50% mortality	< 50% effect	No mortality	no effect
	4	1545 (1193-2448)	767 (672-876)	< 50% mortality	689 (536-888)
Rainbow Trout <i>Oncorhynchus mykiss</i>	0.5	1262 (660-1320)	459 (443-475)	233 (165-330)	141 (128-156)
	1	853 (765-954)	366 (330-407)	154 (138-171)	117 (83-165)
	2	354 (317-396)	233 (165-330)	117 (83-165)	29 (21-41)
	3	282 (256-311)	233 (165-330)	78 (70-87)	29 (21-41)
	4	237 (229-246)	233 (165-330)	68 (59-77)	29 (21-41)
Lahontan Cutthroat Trout <i>Oncorhynchus clarki henshawi</i>	0.5	no mortality	467 (330-660)	225 (215-236)	141 (128-156)
	1	< 50% mortality	392 (357-432)	117 (83-165)	117 (83-165)
	2	616 (553-686)	233 (165-330)	58 (41-83)	29 (21-41)
	3	459 (443-475)	233 (165-330)	29 (21-41)	(0-21)
	4	435 (408-465)	233 (165-330)	29 (21-41)	(0-21)
Daphnia magna	0.5	< 50% mortality	*	< 50% mortality	*
	1	< 50% mortality	*	66 (56-79)	*
	2	< 50% mortality	*	36 (25-51)	*
	4	< 50% mortality	*	27 (18-42)	*
	48	76 (45-159)	*	17 (12-24)	*
			133		

Table 3 cont. Summary table of on-site toxicity results for aquatic organisms exposed to Silv-Ex and Phos-Chek D75-F. LC₅₀ and EC₅₀ values were calculated with Probit (2 partial kills), Spearman-Kärber (1 partial kill), and binomial (no partial kills) methods.

ORGANISM	TIME (hr)	Phos-Chek D75-F		Silv-Ex	
		LC50 (C.I. RANGE)	EC50 (C.I. RANGE)	LC50 (C.I. RANGE)	EC50 (C.I. RANGE)
Fathead Minnow <i>Pimephales promelas</i>	0.5	< 50% mortality	*	< 50% mortality	*
	1	< 50% mortality	*	41 (36-47)	*
	2	1562 (1330-1871)	*	28 (17-47)	*
	4	1047 (895-1232)	*	22 (20-24)	*
	48	312 (260-379)	*	17 (13-22)	*
	96	312 (260-379)	*	8 (5-12)	*

* not determined (sub-lethal effects were not recorded)

Invertebrate Responses

Silv-Ex was more toxic to invertebrates than was Phos-Chek D75-F in exposures conducted in the mobile laboratory with stream water and in the artificial channels with recirculating stream water (Table 3). Mayflies were consistently more sensitive to both Silv-Ex and Phos-Chek than were stoneflies; after two hours of exposure to a concentration of 480 mg/L Silv-Ex, total mortality had occurred in mayflies while no stoneflies had died. The 4-h LC₅₀ and 95% confidence intervals for the mayfly *Epeorus (Iron) albertae* exposed to Silv-Ex were very similar to that for both daphnids and fathead minnows, as well as Lahontan Trout (Table 3). However, after 4 h of Silv-Ex exposure at concentrations up to 1536 mg/L, less than 50% mortality occurred in the stonefly *Hesperoperla pacifica*. Results of the Phos-Chek D75-F exposures indicate that, in general, indigenous organisms were much less sensitive to Phos-Chek when compared to either of the trout species (Fig. 4). The mayfly LC₅₀'s for Phos-Chek were

similar to that for fathead minnows, but could not be compared to daphnids because less than 50% mortality had occurred by the end of 4 hours.

Fish Responses

Both Lahontan and rainbow trout were less sensitive to Phos-Chek D75-F and Silv-Ex than were daphnids and fathead minnows, yet were more sensitive than mayflies and stoneflies (Fig. 4, Table 3). Hamilton and Buhl (this report) 96-h LC₅₀ for rainbow trout exposed to Phos-Chek in laboratory reconstituted water was similar to the 4-h LC₅₀ determined in stream-side recirculating channels.

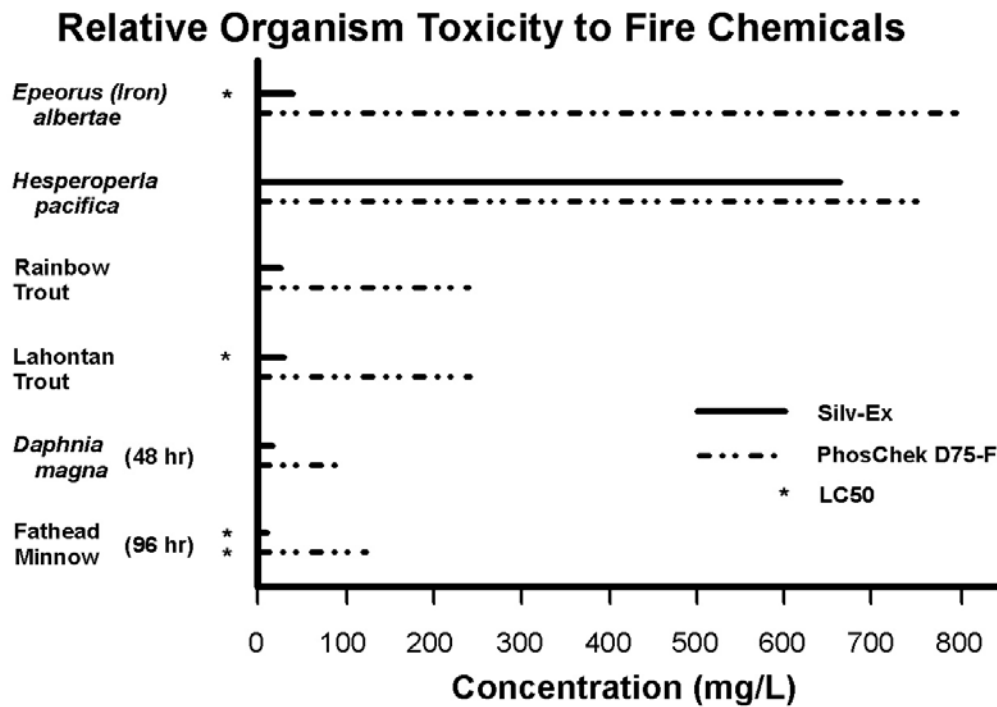


Figure 4. Relative sensitivity of 5 aquatic organisms exposed to Silv-Ex and Phos-Chek D75-F on the North Fork of the Little Humboldt River, Humboldt County, Nevada. Concentrations represent EC₅₀ values based on 4 hr exposures, except as otherwise indicated.

Results of the 4 h tests in recirculating channels indicate that Lahontan trout were more sensitive to Silv-Ex and less sensitive to Phos-Chek than were rainbow trout. In part, this may be due to the difference in age of the fish species tested. Differences in dose-response curves were also evident between the two trout species exposed to Phos-Chek D75-F (Figures 5-6).

Differences also exist in the relative sensitivity (Figure 4) and dose-response (Figures 7-8) of the 2 trout species when exposed to Silv-Ex in stream-side recirculating channels. This presumption is further supported by the 4 h Silv-Ex exposure with 60-d old Lahontan trout in the recirculating channels; LC₅₀'s and confidence intervals were similar as compared to the 96-h laboratory-determined LC₅₀ for 60-d old rainbow trout tested in reconstituted soft water (Hamilton and Buhl, this report). When trout were returned to fresh stream water after 30 min of Silv-Ex exposure (Test #10, Table 1), mortality of both species continued to occur (Figures 9-10). This indicates that short-term exposure to Silv-Ex may continue to cause trout mortality, even after the chemical has been eliminated by dilution or flushing.

In-Stream Effects on Aquatic Macroinvertebrates

Data for aquatic macroinvertebrate drift and density during the 3 in-stream exposure tests are summarized in Table 4. In general, taxa richness (Figures 11-13) and total number of organisms in the drift (Figures 14-16) was low during the 30 minutes prior to each of the exposures, and increased during the 30 minute dose period. For some taxa, drift continued to be elevated during the 30 minutes after chemical addition. For the first Phos-Chek D75-F exposure, drift of *Baetis* sp., *Zapada cinctipes*, and *Brachycentrus* sp. returned to zero during the post-dose period (Fig. 14). However, drift response of these taxa during the second Phos-Chek exposure did not show the same trend, even though the chemical concentration was higher (Fig. 15).

Macroinvertebrate drift during the in-stream Phos-Chek D75-F exposures was low as compared to the response observed during the Silv-Ex exposure of 82.5 mg/L. During the Silv-Ex dose, substantial increases in drift were observed in 7 of the 10 dominant macroinvertebrate taxa present in the North Fork of the Little Humboldt River (Table 4). As compared to the 30

Table 4. Table of drift and mean benthic density for the 10 most dominant macroinvertebrate taxa from the North Fork of the Little Humboldt River, Humboldt County, Nevada, during in-stream exposure tests performed on July 14-15, 1994. Benthic samples were taken before (0-30 min), during (30-60), and after (60-90) addition of chemicals into the stream.

TAXA	SAMPLE	Phos-Chek D75-F Test #1			Phos-Chek D75-F Test #2			Silv-Ex Test		
		Before	During	After	Before	During	After	Before	During	After
<i>Baetis</i> sp.	Drift	0	18	0	12	54	61	12	8482	278
	Surber	106	-	211	256	-	218	15	-	37
<i>Leucrocuta</i> sp.	Drift	0	0	0	0	0	0	0	248	42
	Surber	228	-	589	1351	-	562	96	-	107
<i>Skwalla</i> sp.	Drift	0	0	0	0	0	0	0	290	24
		211	-	311	181	-	174	70	-	85
<i>Zapada inctipes</i>	Drift	0	6	0	12	18	12	0	115	18
	Surber	222	-	56	462	-	489	18	-	111
<i>Brachycentrus</i> sp.	Drift	0	6	0	0	0	0	0	0	0
	Surber	0	-	22	59	-	33	851	-	381
<i>Hydropsyche</i> sp.	Drift	0	0	0	0	0	0	0	0	0
	Surber	106	-	39	429	-	822	30	-	96
<i>Optioservus</i> sp.	Drift	0	0	0	0	0	0	0	91	6
	Surber	122	-	167	473	-	329	84	-	84
<i>Zaitzevia</i> sp.	Drift	0	0	0	0	0	0	0	121	0
	Surber	194	-	117	156	-	181	292	-	96
<i>Tanytarsus</i> sp.	Drift	0	54	18	12	12	24	0	6	0
	Surber	1083	-	589	1603	-	2081	89	-	33
<i>Acarina</i>	Drift	0	42	24	79	79	115	6	1634	24
	Surber	67	-	228	333	-	296	522	-	492

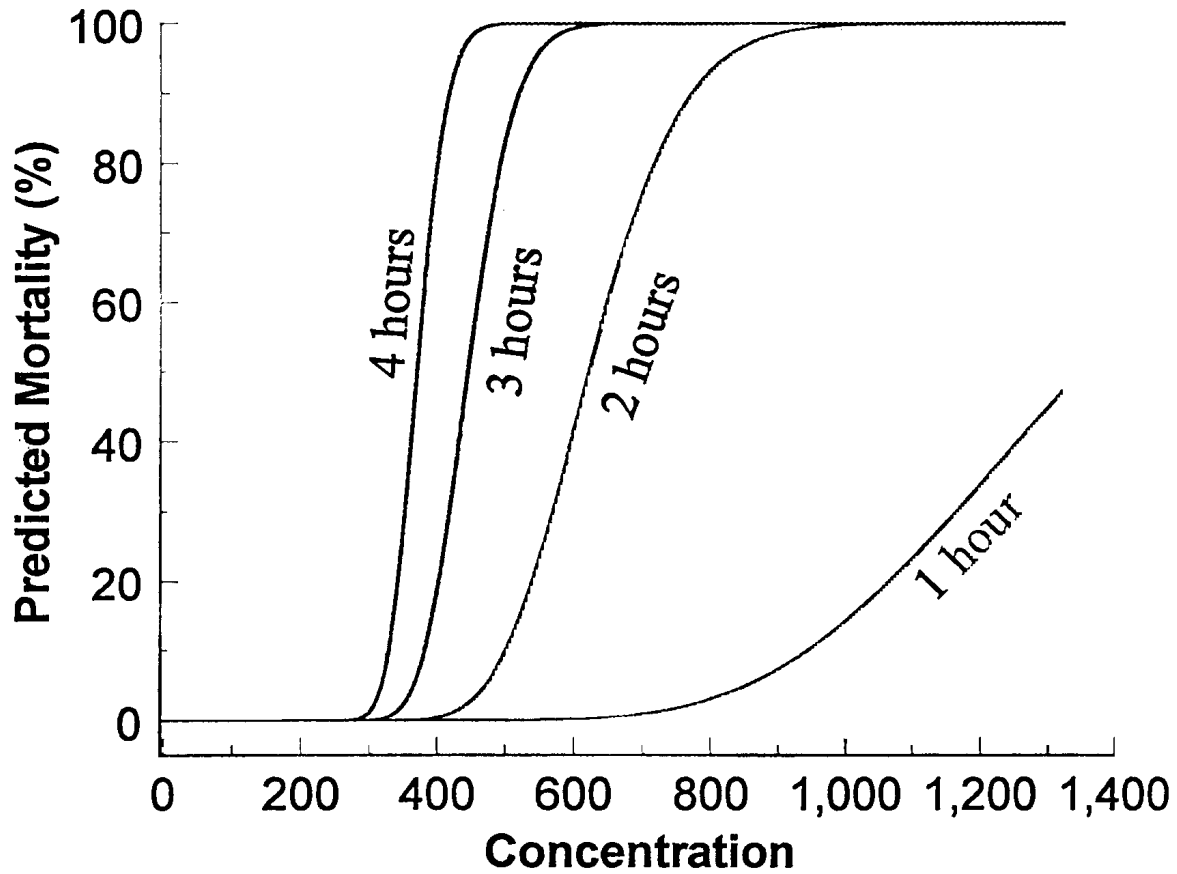


Figure 5. Dose-response curves for Lahontan Cutthroat Trout (*Oncorhynchus clarki henshawi*) exposed to Phos-Chek D75-F in stream-side recirculating channels on the North Fork of the Little Humboldt River, Humboldt County, Nevada.

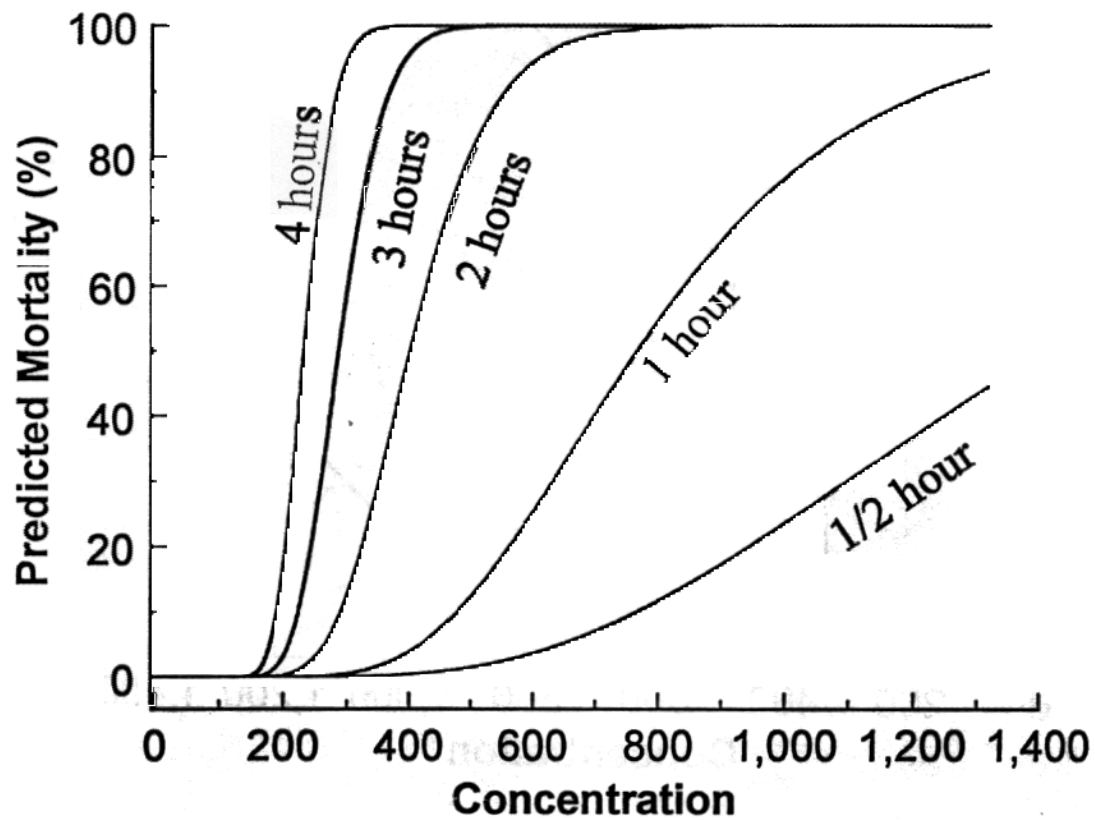


Figure 6. Dose-response curves for Rainbow Trout (*Oncorhynchus mykiss*) exposed to Phos-Chek D75-F in stream-side recirculating channels on the North Fork of the Little Humboldt River, Humboldt County, Nevada.

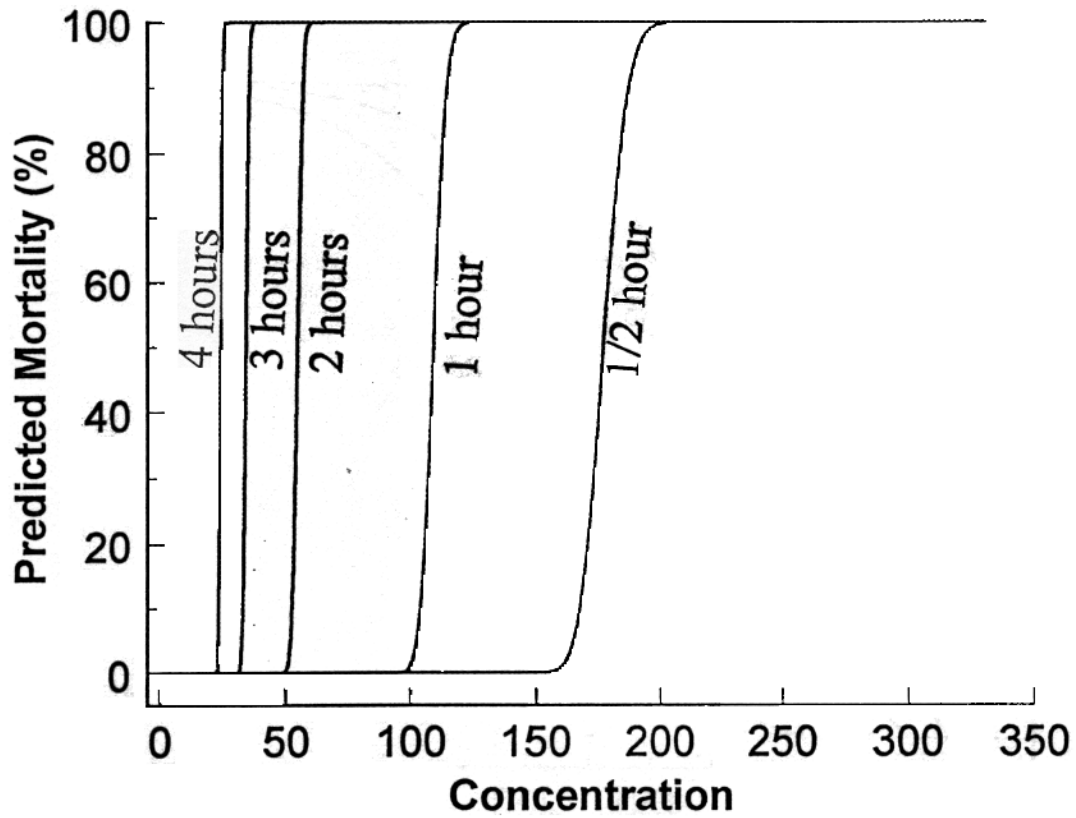


Figure 7. Dose-response curves for Lahontan Cutthroat Trout (*Oncorhynchus clarki henshawi*) exposed to Silv-Ex in stream-side recirculating channels on the North Fork of the Little Humboldt River, Humboldt County, Nevada.

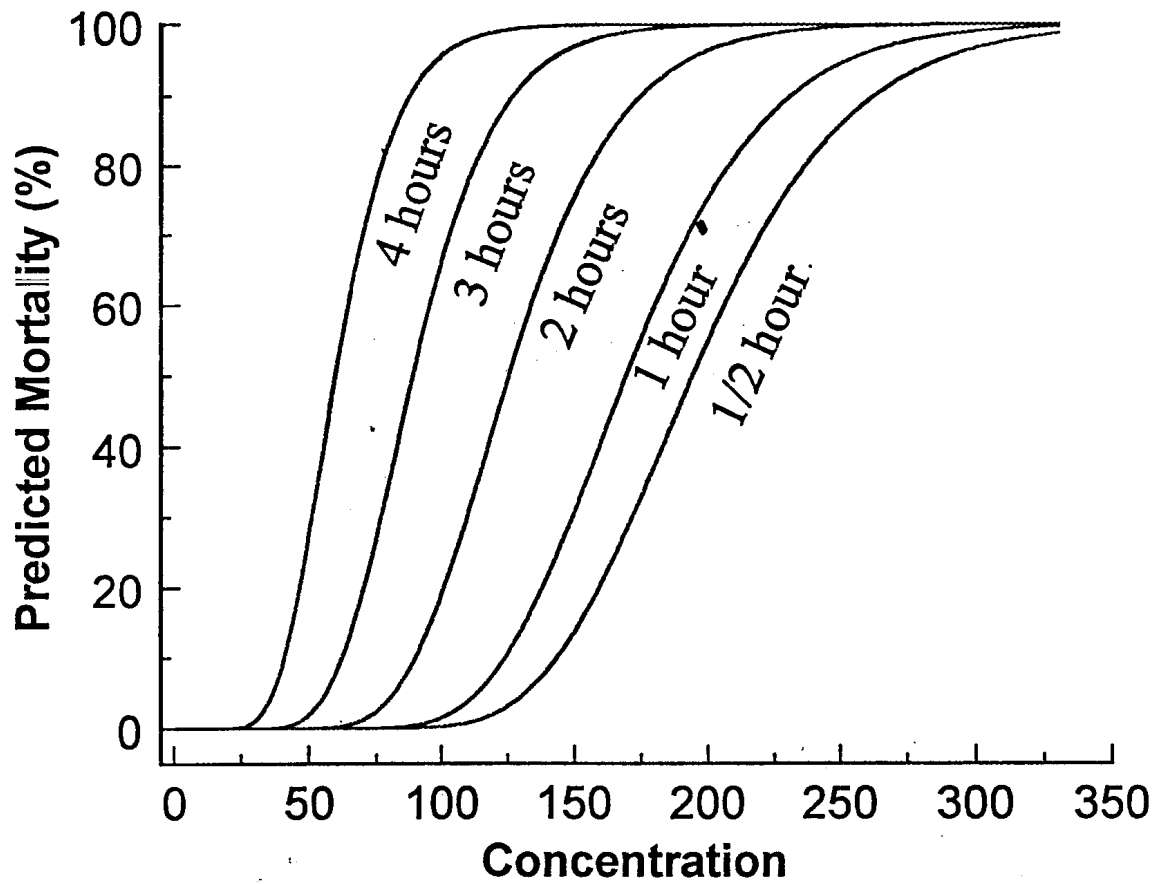


Figure 8. Dose-response curves for Rainbow Trout (*Oncorhynchus mykiss*) exposed to Silv-Ex in stream-side recirculating channels on the North Fork of the Little Humboldt River, Humboldt County, Nevada.

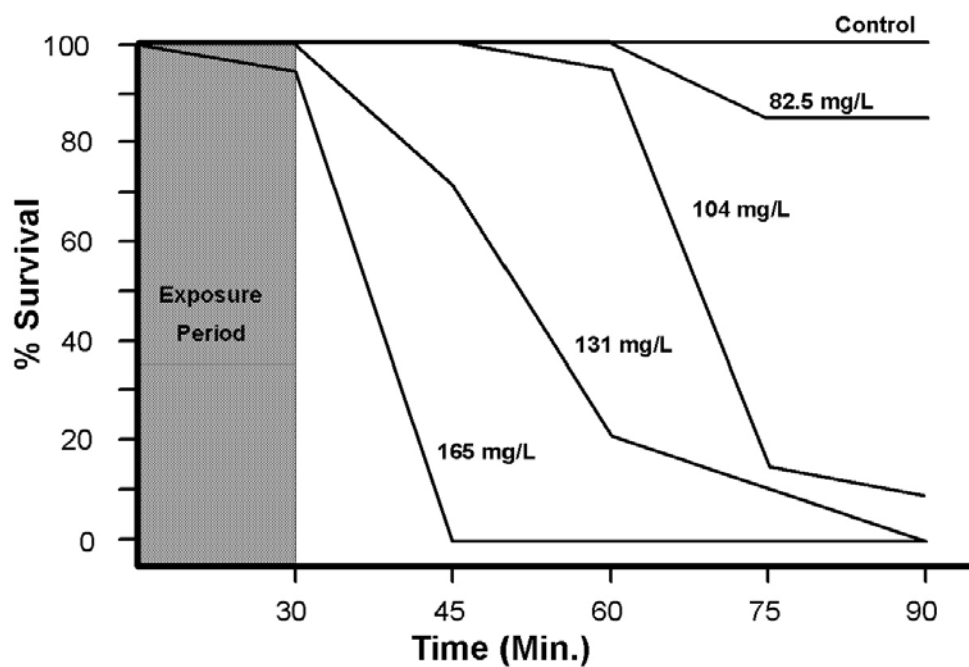


Figure 9. Percent survival of Lahontan cutthroat trout (*Oncorynchus clarki henshawi*) after exposure to 5 concentrations of Silv-Ex (Test #10, Table 1) on the North Fork of the Little Humboldt River, Humboldt County, Nevada, on 7/13/94. Fish were removed from the chemical after 30 min. and allowed to recover in stream water.

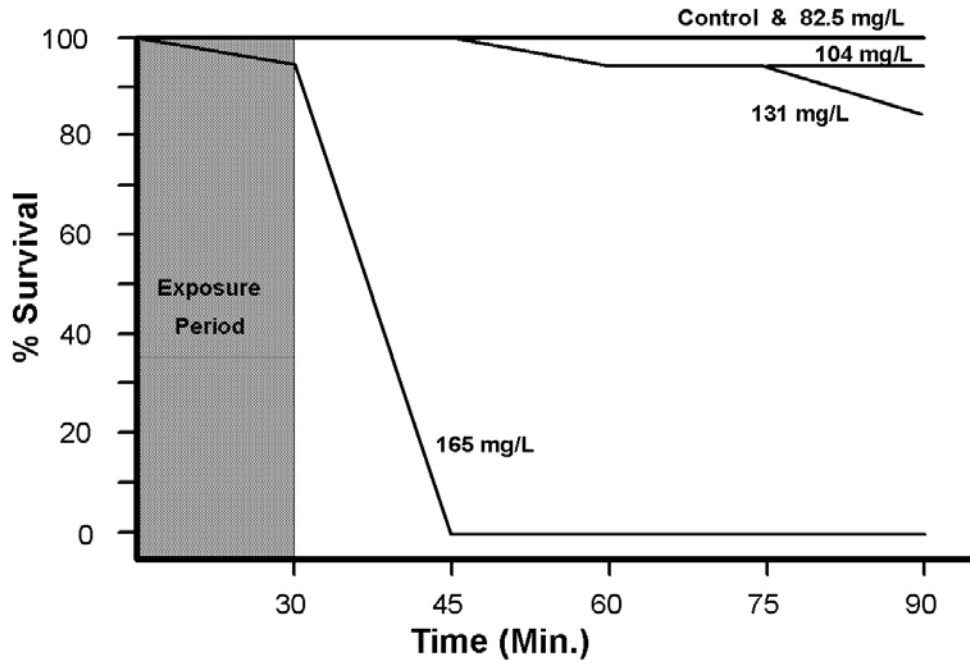


Figure 10. Percent survival of Rainbow Trout (*Oncorhynchus mykiss*) after exposure to 5 concentrations of Silv-Ex (Test #10, Table 1) on the North Fork of the Little Humboldt River, Humboldt County, Nevada, on 7/13/94. Fish were removed from the chemical after 30 min. and allowed to recover in stream water.

Benthic Composition - Phos-Chek D75F Stream Exposure #1

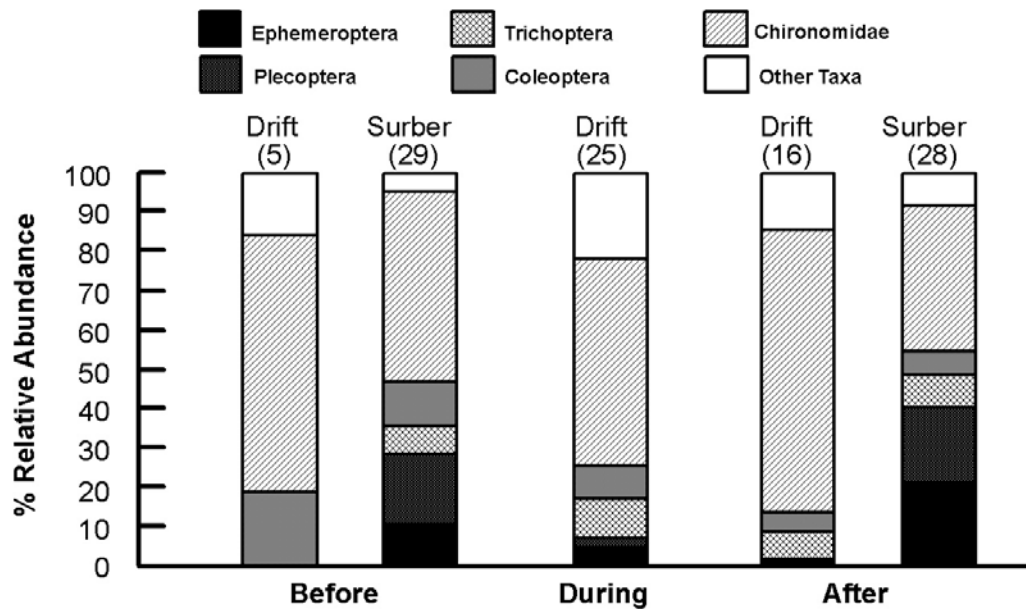


Figure 11. Relative abundance (%) and mean taxa richness (in parentheses) of aquatic macroinvertebrates in the North Fork of the Little Humboldt River, Humboldt County, Nevada, during the first in-stream Phos-Chek D75-F exposure.

Benthic Composition - Phos-Chek D75F Stream Exposure #2

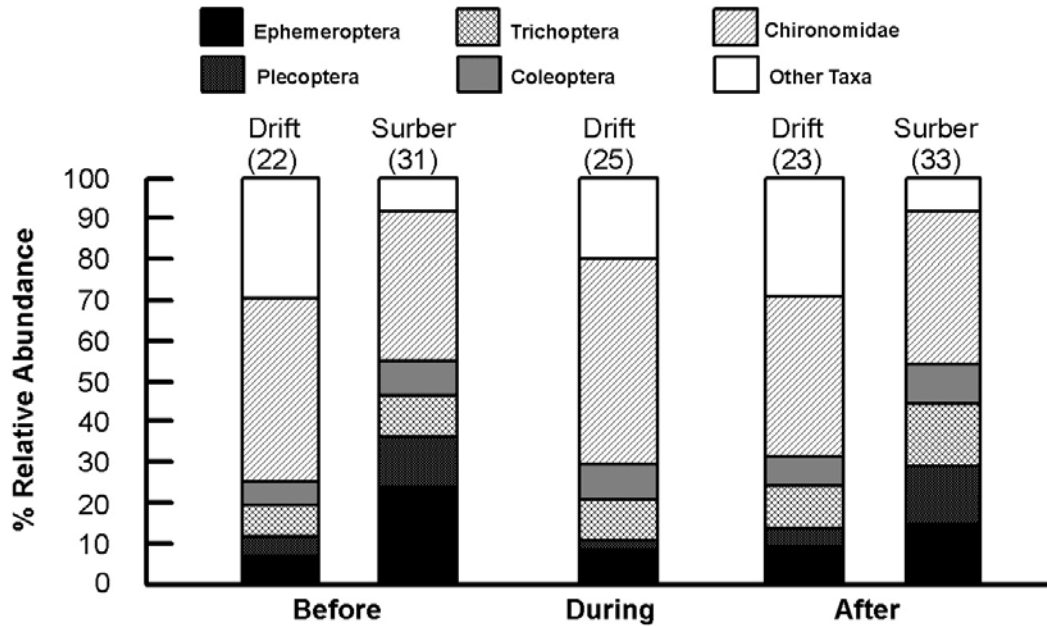


Figure 12. Relative abundance (%) and mean taxa richness (in parentheses) of aquatic macroinvertebrates in the North Fork of the Little Humboldt River, Humboldt County, Nevada, during the second in-stream Phos-Chek D75-F exposure.

Benthic Composition - Silv-Ex Stream Exposure

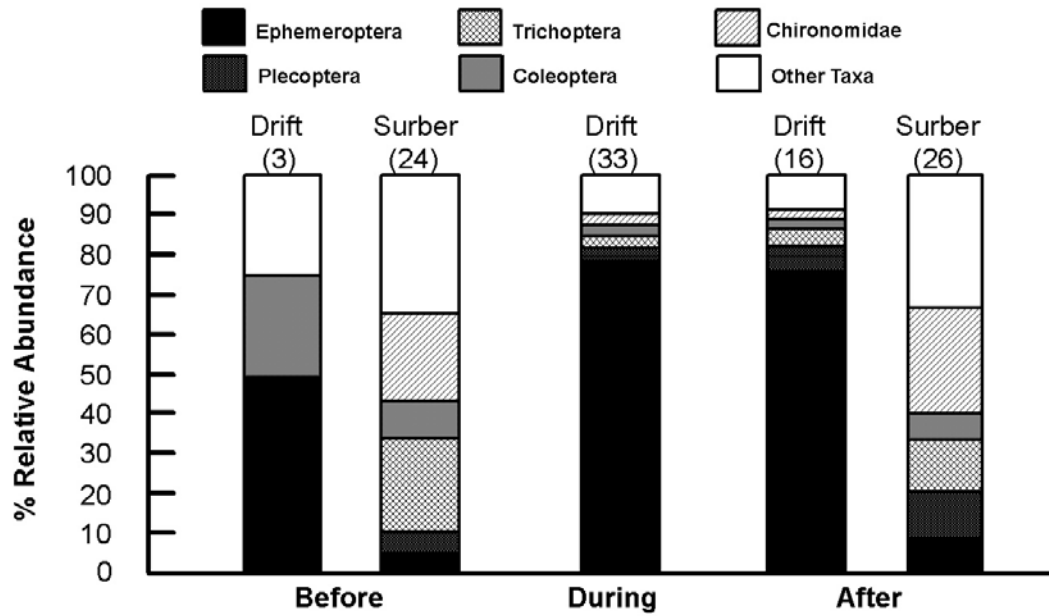


Figure 13. Relative abundance (%) and mean taxa richness (in parentheses) of aquatic macroinvertebrates in the North Fork of the Little Humboldt River, Humboldt County, Nevada, during the in-stream Silv-Ex exposure.

minutes prior to the Silv-Ex dose, a 100-800 fold increase in drift of Ephemeroptera (*Baetis* sp., *Leucrocuta* sp.), Plecoptera (*Skwalla* sp., *Zapada cinctipes*), riffle beetles (*Optioservus* sp., *Zaitzevia* sp.), and water mites (Acarina) was observed during the exposure. Many of these taxa demonstrated a delay in response and were still present in the drift at elevated levels during the 30-minute period after the dose (Figure 16). Continuation of elevated numbers of drifting organisms after the exposure period may be due to a delay in organism response or chemical dilution. Mayflies of the genus Baetis sp. demonstrated the highest drift rate during the Silv-Ex dose (8482/100m³). Drift taxa richness also increased 10-fold during this test, and remained elevated after the 30 minute exposure (Figure 13). During the exposure, 78 % of the drifting organisms were Ephemeroptera, yet mayflies comprised less than 10% of the benthic density in riffles as determined by Surber sampling (Figure 13). This may indicate a higher sensitivity of Ephemeroptera to Silv-Ex as compared to other benthic invertebrate groups. However, no statistically significant decreases in benthic density could be detected in the Surber samples taken after the dose period for any of the in-stream exposure tests.

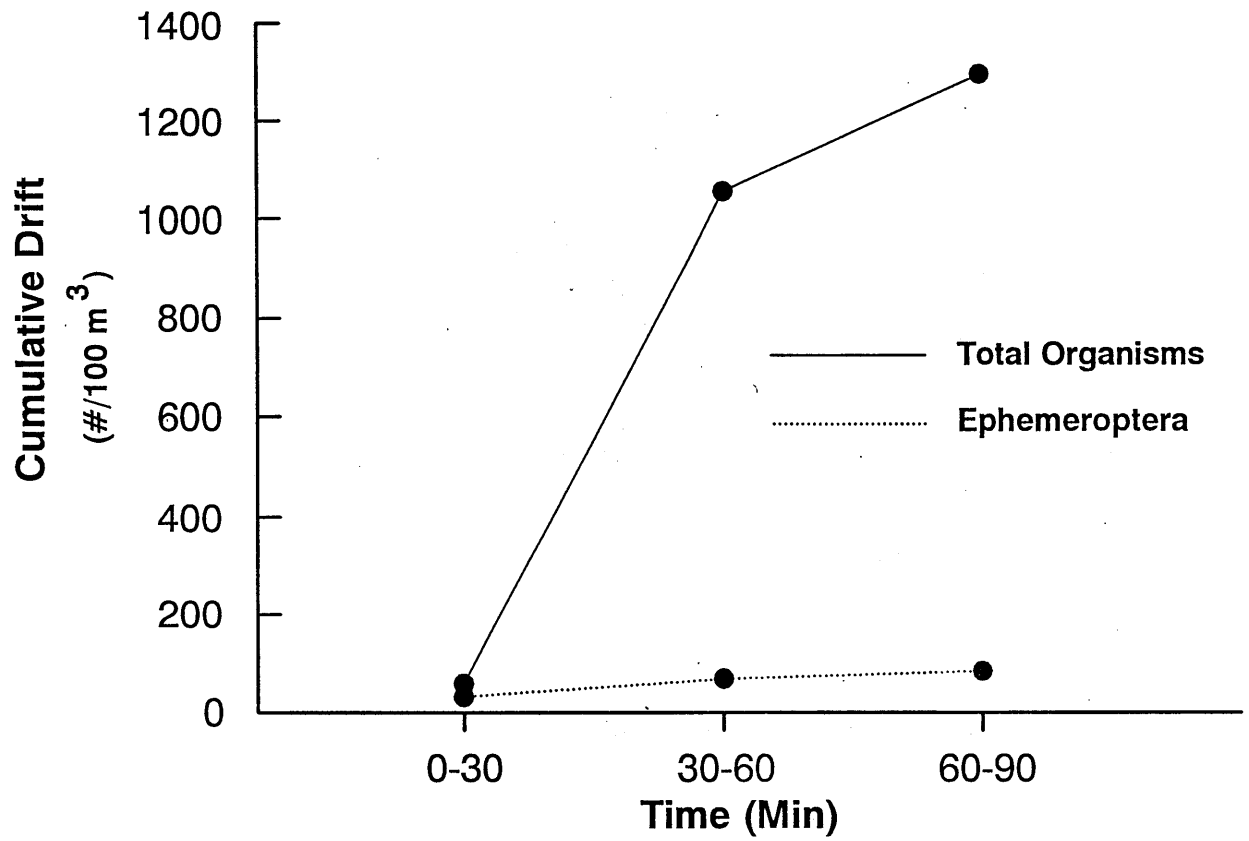


Figure 14. Cumulative drift of aquatic macroinvertebrates before (0-30 min), during (30-60 min), and after (60-90 min) the first in-stream Phos-Chek D75-F exposure on the North Fork of the Little Humboldt River, Humboldt County, Nevada, on 7/14/94.

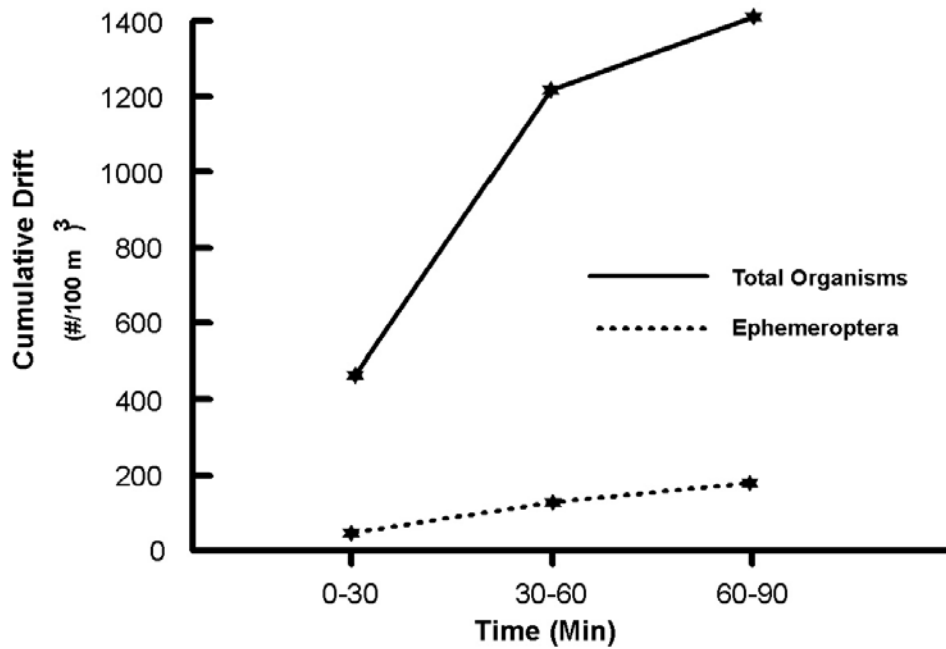


Figure 15. Cumulative drift of aquatic macroinvertebrates before (0-30 min), during (30-60 min), and after (60-90 min) the second in-stream Phos-Chek D75-F exposure on the North Fork of the Little Humboldt River, Humboldt County, Nevada, on 7/15/95.

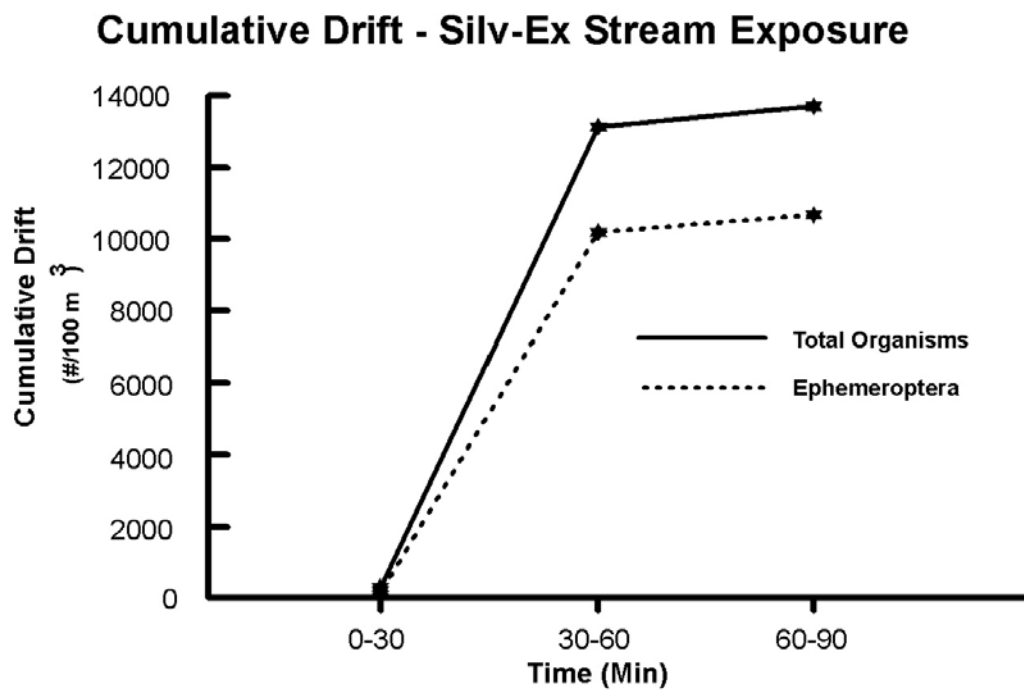


Figure 16. Cumulative drift of aquatic macroinvertebrates before (0-30 min), during (30-60 min), and after (60-90 min) the in-stream Silv-Ex exposure on the North Fork of the Little Humboldt River, Humboldt County, Nevada, on 7/14/95.

In-Stream Effects on Fish

Because of the desire to minimize ecological disturbance in the North Fork of the Little Humbolt River, chemical were applied to the stream at levels that research staff believed would not result in acutely toxic responses based on information derived from the previous field toxicity and artificial channel tests. As predicted, no mortality of rainbow trout, Lahontan trout or fathead minnows occurred as a result of exposure to either Phos-Chek D75-F or Silv-Ex in tests conducted in the North Fork of the Little Humboldt River. After the initial 30 min exposure, both species of trout that had been exposed to Silv-Ex showed signs of stress including increased opercular movements and loss of equilibrium. Similar behavior was not observed in Phos-Chek exposed trout. Trout recovered from this sublethal response within one hour following exposure and observations after two hours indicated no abnormal behavior, suggesting full recovery. In contrast to the artificial stream exposures where mortality was observed after fish were moved to clean water, no mortality occurred when fish were allowed to recover in a natural stream situation. It is possible that handling fish after they had been stressed by Silv-Ex exposure in the artificial streams was sufficient to increase the level of stress and result in mortality. However, in an actual fire situation, stream temperatures and turbidity would likely be higher than those encountered by trout during recovery in the North Fork of the Little Humboldt. If fish could not avoid these conditions, the increased stress could result in mortality similar to that experienced in the artificial stream exposures. Careful observations following these in-stream tests revealed no incidental mortality of fish indigenous to the North Fork of the Little Humbolt River.

Chemical Degradation

Silv-Ex and Phos-Chek WD-881 degraded more rapidly than the three non-foam chemicals. Rates of degradation of the two foams were similar. Degradation of both foam and non-foam chemicals were accelerated in soils with elevated organic content. Also, chemical half-life decreased as clay content of soils increased. Half-life for foams ranged from 24 to 34

hours and for non-foams from 14 to 22 days. All five chemicals degraded most rapidly in a silty clay loam with 75% organic content. In general, degradation rates of fire chemical foams on soils did not differ substantially from those estimated through standard DOC removal and CO₂ evolution methods.

For Foams, no chemical residues were measured in samples of overlying water collected from soil samples 24h after chemical application. For non-foams, levels of nitrate were higher in Fire-Trol GTS-R and Phos-Check D75F than in untreated samples, but did not exceed levels known to be toxic. Ammonia was not elevated in any sample. Although this test differs from an actual rainfall event, it does determine whether the fire chemicals were released from the soils after 24 hours. Overall, chemicals were not highly mobile. Because of their rapid degradation and their low potential for mobilization, foams applied within a watershed have a relatively low chance of entering the aquatic system from a runoff event that occurs within 24 to 48 hours of application. The period of availability for non-foam chemicals applied to the soil is longer than that for foams, but concentrations associated with potential runoff events appear to remain consistently below toxic levels.

Discussion and Management Implications

Prediction of Chemical Concentration and Exposure Time

Future directions in wildfire management and control may require determinations of safe spray distances from aquatic habitats, safe maximum areas of spray coverage, and the prediction of ecological effects of chemical overspray or accidental spillage. Fire has the potential to create long-term damage to small watersheds (Bozek and Young 1994). However, if certain variables concerning the receiving aquatic system are available, simple calculations can be used to predict chemical concentration and time of exposure resulting from spills or spray events where chemicals are inadvertently applied directly to a water body. To augment fire control strategies near small streams, the following parameters are of interest:

A = Area of chemical coverage onto water surface (ft²)

C = Chemical concentration (mg/L)

D = Stream discharge (ft³)

d = mean water depth (Ft)

L = longitudinal length of stream affected by chemical (Ft)

M = Percent spray mixture (%)

R = Spray application coverage rate (Gal/100 ft²)

T = Time of chemical exposure (Min)

W = Mean width of stream length affected by chemical

V = Mean current velocity (ft/sec)

The following equations are commonly used for calculating discharge (D), mean velocity (V), and stream length affected (L):

$$\mathbf{D = WdV \quad V = D / (Wd) \quad \text{or} \quad V = D / ((A/L) \times d) \quad L = A/W}$$

Also, time of exposure (T) is calculated with the following equation:

$$\mathbf{T = (A/W) / (V \times 60)}$$

Mean stream velocity within an affected reach is a required component in the determination of exposure time, but is rarely obtainable except by direct measurement. However, if discharge (D) is known, we can substitute V with D to yield:

$$\mathbf{T = (A \times d) / (D \times 60)}.$$

Combining equations from above, and including coverage rate (R) and percent of spray mixture (M), we can predict a theoretical concentration (C) of the chemical after it enters a known area (A) of stream:

$$\mathbf{C = (A \times M \times R \times 380) / (D \times T \times 1698.6)}.$$

Or, solving for time of exposure (T) yields:

$$T = (A \times M \times R \times 380) / (D \times C \times 1698.6).$$

The equations above are calculated based on wettable perimeter area with the assumption that even chemical coverage occurs and does not consider uneven mixing due to stream turbulence, or fate characteristics such as runoff and percolation through soil. Just prior to the in-stream exposure tests, measurements from the North Fork of the Little Humboldt River determined that $D = 2.17$, $V = 0.385$, $d = 0.867$, and $W = 6.5$. At a coverage rate of 10 gal/100 ft² and spray mixture of 1%, if 4505.2 ft² of stream area was contacted with aerial spray along a 693 ft. section of stream, aquatic organisms would be exposed to a 154.8 mg/L concentration for 30 min. Higher concentrations would result from higher coverage rates or more shallow stream depths (Figure 17). At 10 gal/100 ft², which is the recommended coverage rate for sagebrush areas similar to that found in the Great Basin area, higher discharges and smaller areas of stream coverage would result in shorter exposure times (Figures 18-19). For Silv-Ex, if a 30 min exposure of 82.5 mg/L is considered a targeted maximum for the conditions measured on the North Fork of the Little Humboldt River, coverage rate would have to be reduced to 5.3 gal/100 ft², or percent mixture of the chemical would have to be reduced to 0.53 %. Concentration and time of exposure would be lessened if aerial spray was applied perpendicular to stream flow, rather than parallel to it.

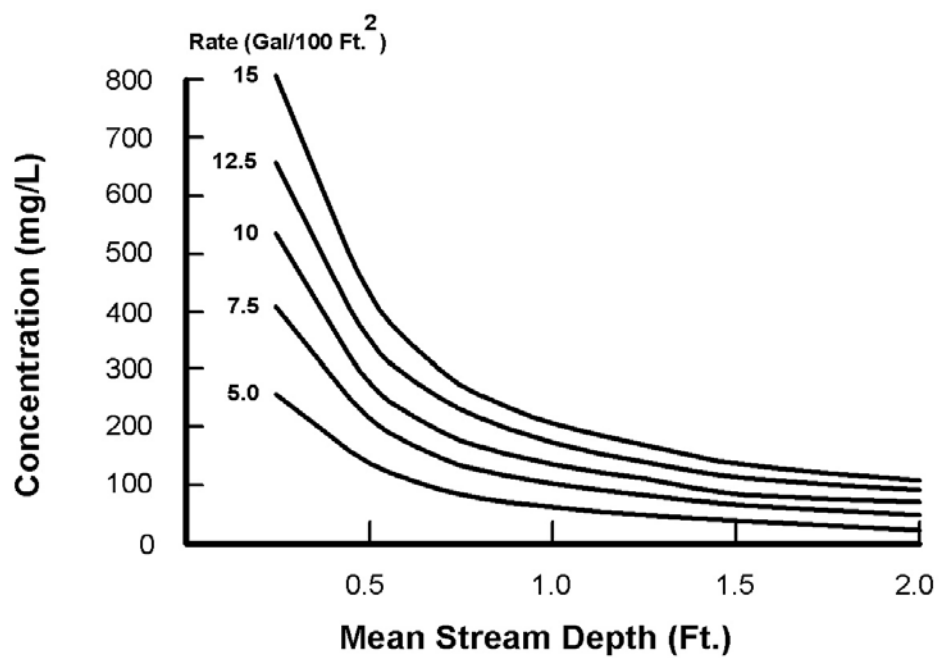


Figure 17. Concentration curves based on mean stream depth and different spray coverage rates for fire retardant and suppressant chemicals.

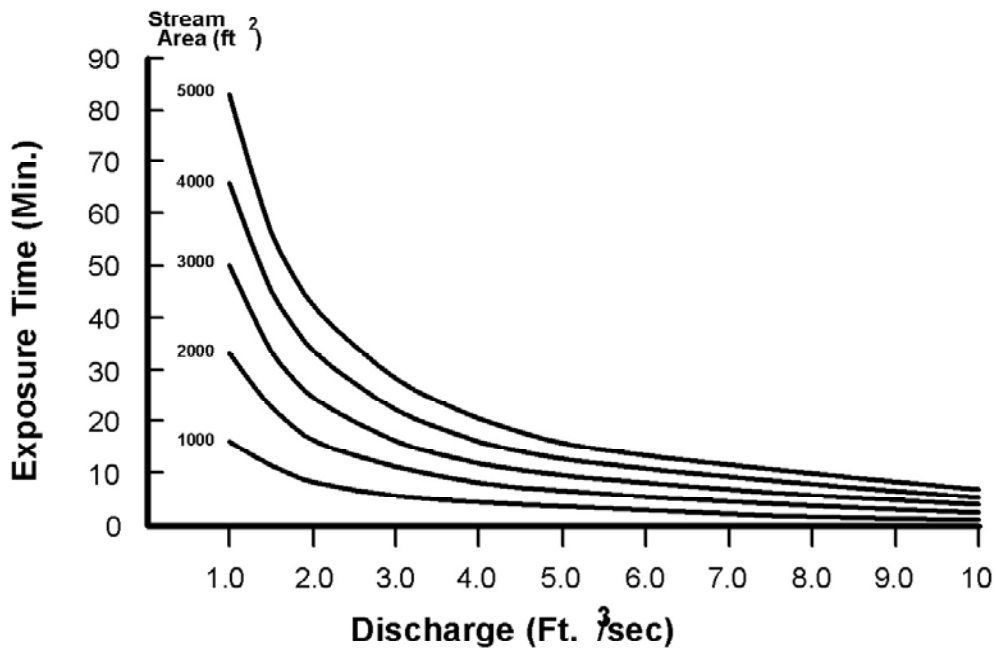


Figure 18. Exposure time curves based on discharge and area of fire chemical spray coverage for small streams with an average depth of 1 ft.

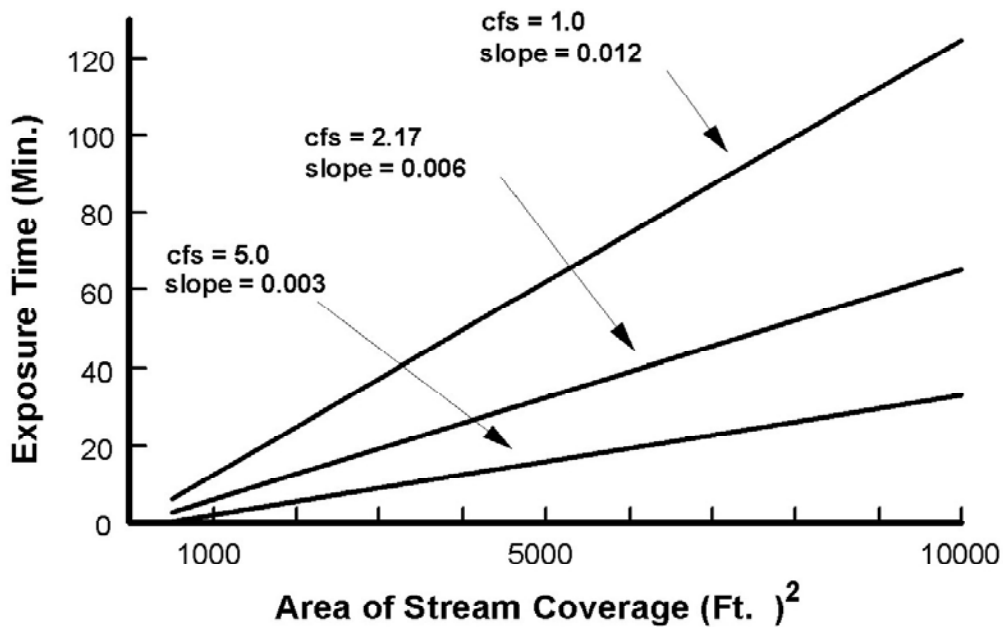


Figure 19. Graph of exposure times vs. area of spray coverage and stream discharge for the North Fork of the Little Humboldt River, Humboldt County, Nevada. Plotted data are based on an average stream width of 6.5 ft.

Literature Cited:

Bozek, M.A. and M.K.Young. 1994. Fish mortality resulting from delayed effects of fire in the Greater Yellowstone ecosystem. *Great Basin Nat.* 54:91-95.

USEPA (U.S. Environmental Protection Agency). 1991. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater organisms and marine organisms. EPA/600/4-90/027. Environmental Monitoring and Support Laboratory, Cincinnati, OH.

Appendix

Survival and behavioral data for on-site artificial stream exposures with *Epeorus albertae* (mayflies) and *Hesperperla pacifica* (stoneflies) exposed to 5 concentrations (mg/L) of Phos-Chek D75-F at the North Fork of the Little Humboldt River, Nevada, on 24 June 1994.

	<i>Epeorus albertae</i>			<i>Hesperperla pacifica</i>		
Treatment / Time	Active	Impaired	Dead	Active	Impaired	Dead
Control	30	0	0	30	0	0
30 min	30	0	0	30	0	0
1 hr	30	0	0	30	0	0
2 hr	30	0	0	30	0	0
3 hr	30	0	0	30	0	0
4 hr	30	0	0	30	0	0
14.65 mg/L						
30 min	30	0	0	30	0	0
1 hr	30	0	0	30	0	0
2 hr	30	0	0	30	0	0
3 hr	28	0	2	29	0	1
4 hr	28	0	2	29	0	1
46.88 mg/L						
30 min	30	0	0	30	0	0
1 hr	30	0	0	30	0	0
2 hr	30	0	0	30	0	0
3 hr	30	0	0	30	0	0
4 hr	29	0	1	30	0	0
150.0 mg/L						
30 min	30	0	0	30	0	0
1 hr	29	0	1	30	0	0
2 hr	29	0	1	30	0	0
3 hr	28	0	2	30	0	0
4 hr	28	0	2	30	0	0
480.0 mg/L						
30 min	30	0	0	30	0	0
1 hr	30	0	0	29	0	1
2 hr	29	0	1	29	0	1
3 hr	29	0	1	28	1	1
4 hr	28	0	2	27	2	1
1536.0 mg/L						
30 min	30	0	0	29	0	1
1 hr	28	0	2	29	0	1
2 hr	16	0	14	29	0	1
3 hr	0	8	22	24	4	1
4 hr	0	5	25	0	21	9

Survival and behavioral data for on-site artificial stream exposure with *Epeorus albertae* (mayflies) and *Hesperperla pacifica* (stoneflies) exposed to 5 concentrations (mg/L) of Silv-Ex at the North Fork of the Little Humboldt River, Nevada, on 26 June 1994.

	<i>Epeorus albertae</i>			<i>Hesperperla pacifica</i>		
Treatment / Time	Active	Impaired	Dead	Active	Impaired	Dead
Control						
30 min	30	0	0	30	0	0
1 hr	30	0	0	30	0	0
2 hr	30	0	0	30	0	0
3 hr	30	0	0	30	0	0
4 hr	30	0	0	30	0	0
14.65 mg/L						
30 min	30	0	0	30	0	0
1 hr	30	0	0	30	0	0
2 hr	30	0	0	30	0	0
3 hr	30	0	0	30	0	0
4 hr	30	0	0	30	0	0
46.88 mg/L						
30 min	30	0	0	30	0	0
1 hr	30	0	0	30	0	0
2 hr	17	13	0	30	0	0
3 hr	0	21	9	30	0	0
4 hr	0	20	10	30	0	0
150.0 mg/L						
30 min	30	0	0	30	0	0
1 hr	30	0	0	30	0	0
2 hr	0	27	3	30	0	0
3 hr	0	9	21	30	0	0
4 hr	0	2	28	30	0	0
480.0 mg/L						
30 min	30	0	0	30	0	0
1 hr	9	14	2	30	0	0
2 hr	0	0	30	30	0	0
3 hr	0	0	30	21	8	1
4 hr	0	0	30	20	5	5
1536.0 mg/L						
30 min	0	22	8	30	0	0
1 hr	0	4	26	30	0	0
2 hr	0	0	30	17	13	0
3 hr	0	0	30	2	23	5
4 hr	0	0	30	4	15	11

Survival and behavioral data for on-site artificial stream exposures with *Epeorus albertae* (mayflies) exposed to 5 concentrations (mg/L) of Silv-Ex at the North Fork of the Little Humboldt River, Nevada, on 28 June 1994.

Treatment / time	<i>Epeorus albertae</i>		
	Active	Impaired	Dead
Control			
30 min	60	0	0
1 hr	60	0	0
2 hr	60	0	0
3 hr	60	0	0
4 hr	60	0	0
20.63 mg/L			
30 min	60	0	0
1 hr	60	0	0
2 hr	54	0	6
3 hr	9	18	3
4 hr	0	43	17
41.25 mg/L			
30 min	60	0	0
1 hr	58	0	2
2 hr	0	13	47
3 hr	0	10	50
4 hr	0	0	60
82.5 mg/L			
30 min	60	0	0
1 hr	30	12	18
2 hr	0	22	38
3 hr	0	11	49
4 hr	0	6	54
165.0 mg/L			
30 min	29	30	1
1 hr	0	41	19
2 hr	0	5	55
3 hr	0	4	56
4 hr	0	0	60
330.0 mg/L			
30 min	0	53	7
1 hr	0	32	28
2 hr	0	3	57
3 hr	0	0	60
4 hr	0	0	60

Survival and behavioral data for on-site artificial stream exposures with *Oncorhynchus mykiss* (Rainbow trout) and *Oncorhynchus clarki henshawi* (Lahontan cutthroat trout) exposed to 5 concentrations (mg/L) of Silv-Ex at the North Fork of the Little Humboldt River, Nevada, on 2 July 1994.

Treatment / Time	<i>Oncorhynchus mykiss</i>			<i>Oncorhynchus clarki henshawi</i>		
	Active	Impaired	Dead	Active	Impaired	Dead
Control						
30 min	40	0	0	40	0	0
1 hr	40	0	0	40	0	0
2 hr	40	0	0	40	0	0
3 hr	40	0	0	40	0	0
4 hr	40	0	0	40	0	0
20.63 mg/L						
30 min	40	0	0	40	0	0
1 hr	40	0	0	40	0	0
2 hr	40	0	0	40	0	0
3 hr	40	0	0	14	26	0
4 hr	40	0	0	0	40	0
41.25 mg/L						
30 min	40	0	0	40	0	0
1 hr	40	0	0	40	0	0
2 hr	0	40	0	0	40	0
3 hr	0	39	1	0	0	40
4 hr	0	33	7	0	0	40
82.5 mg/L						
30 min	40	0	0	40	0	0
1 hr	40	0	0	40	0	0
2 hr	0	40	0	0	0	40
3 hr	0	18	22	0	0	40
4 hr	0	16	24	0	0	40
165.0 mg/L						
30 min	11	29	0	8	30	2
1 hr	0	16	24	0	0	40
2 hr	0	0	40	0	0	40
3 hr	0	0	40	0	0	40
4 hr	0	0	40	0	0	40
330.0 mg/L						
30 min	0	0	40	0	0	40
1 hr	0	0	40	0	0	40
2 hr	0	0	40	0	0	40
3 hr	0	0	40	0	0	40
4 hr	0	0	40	0	0	40

Survival and behavioral data for on-site artificial stream exposures with *Oncorhynchus mykiss* (rainbow trout) and *Oncorhynchus clarki henshawi* (Lahontan cutthroat trout) exposed to 5 concentrations (in mg/L) of Phos-Chek D75-F at the North Fork of the Little Humboldt River, Nevada, on 3 July 1994.

Treatment / time	<i>Oncorhynchus mykiss</i>			<i>Oncorhynchus clarki henshawi</i>		
	Active	Impaired	Dead	Active	Impaired	Dead
Control						
30 min	40	0	0	40	0	0
1 hr	40	0	0	40	0	0
2 hr	40	0	0	40	0	0
3 hr	40	0	0	40	0	0
4 hr	40	0	0	40	0	0
82.5 mg/L						
30 min	40	0	0	40	0	0
1 hr	40	0	0	40	0	0
2 hr	40	0	0	40	0	0
3 hr	40	0	0	40	0	0
4 hr	40	0	0	40	0	0
165.0 mg/L						
30 min	40	0	0	40	0	0
1 hr	40	0	0	40	0	0
2 hr	40	0	0	40	0	0
3 hr	40	0	0	40	0	0
4 hr	40	0	0	40	0	0
330.0 mg/L						
30 min	39	1	0	40	0	0
1 hr	26	14	0	30	10	0
2 hr	0	23	17	0	40	0
3 hr	0	11	29	0	39	1
4 hr	0	1	39	0	36	4
660.0 mg/L						
30 min	0	40	0	0	40	0
1 hr	0	34	6	0	40	0
2 hr	0	0	40	0	15	25
3 hr	0	0	40	0	0	40
4 hr	0	0	40	0	0	40
1320.0 mg/L						
30 min	0	18	22	0	40	0
1 hr	0	3	37	0	20	20
2 hr	0	0	40	0	0	40
3 hr	0	0	40	0	0	40
4 hr	0	0	40	0	0	40

Survival and behavioral data for on-site artificial stream exposures with Oncorynchus mykiss (rainbow trout) and Oncorynchus clarki henshawi (Lahontan cutthroat trout) exposed to 4 concentrations (in mg/L) of Silv-ex at the North Fork of the Little Humboldt River, Nevada, on 13 July 1994. Fish were removed after 30 min and placed in fresh stream water to document recovery after exposure.

Treatment/ time	<i>Oncorynchus mykiss</i>			<i>Oncorynchus clarki henshawi</i>		
	Active	Impaired	Dead	Active	Impaired	Dead
Control						
30 min	20	0	0	20	0	0
45 min	20	0	0	20	0	0
60 min	20	0	0	20	0	0
75 min	20	0	0	20	0	0
90 min	20	0	0	20	0	0
82.5 mg/L						
30 min	15	5	0	20	0	0
45 min	20	0	0	20	0	0
60 min	20	0	0	15	5	0
75 min	20	0	0	11	6	3
90 min	20	0	0	9	8	3
104.0 mg/L						
30 min	14	6	0	15	5	0
45 min	14	6	0	15	5	0
60 min	11	8	1	0	20	0
75 min	12	7	1	0	3	17
90 min	11	8	1	0	2	18
131.0 mg/L						
30 min	14	6	0	15	5	0
45 min	0	20	0	0	14	6
60 min	12	7	1	0	4	16
75 min	4	13	3	0	2	18
90 min	8	9	3	0	0	20
165.0 mg/L						
30 min	0	19	1	0	20	0
45 min	0	2	18	0	0	20
60 min	0	2	18	0	0	20
75 min	0	2	18	0	0	20
90 min	0	2	18	0	0	20

Summary of macrobenthos samples taken to evaluate the ecological effects of fire retardant and suppressant chemicals after addition of chemical doses into the North Fork of the Little Humboldt River on 14 and 15 July 1994.

Exposure Test				
		#1	#2	#3
Chemical Added:		Phoschek D75F	Silv-ex	Phoschek D75F
Nominal Concentration:		330 mg/L	82.5 mg/L	660 mg/L
Discharge within Zone:		1.0 CFS	0.78 CFS	0.63 CFS
Drift Samples-	Before	2 reps	2 reps	2 reps
	During	2 reps	2 reps	2 reps
	After	2 reps	2 reps	2 reps
Surber Samples-	Before	2 reps	3 reps	3 reps
	After	2 reps	3 reps	3 reps