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THE IMPACT OF THE HERBICIDES IMAZAPYR AND TRICLOPYR TRIETHYLAMINE ON BULLFROG TADPOLES

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The imazapyr-based herbicides Stalker® and Habitat® and the triclopyr-based herbicide Garlon® 3A are commonly used to control invasive, exotic plants in wildland settings where non-target amphibian species may be present. Of particular concern is the federally-threatened California red-legged frog, *Rana aurora draytonii,* (CRLF). In order to assess the toxicity risk to amphibians, acute toxicity tests were conducted with the herbicide formulations and their active ingredients, using bullfrog, *Rana catesbeiana*, tadpoles. All of the herbicides, with the exception of Stalker®, were found to be within the U.S. EPA's "practically non-toxic" category for acute lethality to aquatic organisms. Stalker® was found to be in the 'slightly toxic' range. The calculated toxicity values were then compared to herbicide environmental concentrations using the risk quotient (RQ) method. RQ values for all the herbicides were below the U.S. EPA's level of concern for listed aquatic species.

INTRODUCTION

Formulated herbicide products containing either the isopropylamine (IPA) salt of imazapyr or the triethylamine (TEA) salt of triclopyr are commonly used to control invasive, exotic plant species in wildland settings. Common examples include the control of giant cane, *Arundo donax*, in riparian areas in southern and central California, and the removal of the exotic tree species tamarisk, *Tamarix* spp., in riparian and oasis areas in the deserts of southern California. Because these compounds may be used in or near water, concerns have been raised regarding their potential impacts on amphibian species, in particular, the California red-legged frog, *Rana aurora draytonii,* (CRLF). The CRLF is listed as a threatened species by the United States Fish and Wildlife Service under the Federal Endangered Species Act.

The goal of this study was to develop acute toxicity values for technical imazapyr acid and the imazapyr IPA salt products Habitat® and Stalker®, and technical triclopyr TEA salt and the triclopyr TEA salt product Garlon® 3A to larval ranid frogs. Bullfrog,

Rana catesbeiana, tadpoles were used as a surrogate for CRLF tadpoles. Previous research has shown that bullfrog tadpoles have similar sensitivity to pesticides as compared to other ranid species (Relyea 2003, 2004). The toxicity values determined from this study were then compared to known or estimated environmental concentrations (ECs). These comparisons allowed us to assess the acute toxicity hazard for each of these herbicides based on the risk quotient (RQ) method (U.S. EPA 2004). The RQ method estimates risk by comparing an exposure concentration to an effects concentration (exposure/toxicity).

Herbicide Formulations

The imazapyr product Stalker® is an emulsifiable concentrate containing 27.6% of the IPA salt of imazapyr (SERA 2004). It is used exclusively in terrestrial settings, often involving either cut stump or basal bark application methods. Habitat®, another imazapyr formulation, contains 28.7% of the IPA salt of imazapyr. It is water-based and is commonly used in both terrestrial and aquatic settings (Ibid). The triclopyr product Garlon® 3A contains 44.4% of the TEA salt of triclopyr and is typically applied in terrestrial settings via foliar sprays, cut stump treatments, and basal bark sprays (SERA 2003). Garlon® 3A also includes the chelating agent ethylene diamine tetraacetic acid (EDTA) and ethanol (Ibid).

Aquatic Toxicity of the Herbicides

There are no acute toxicity data for larval amphibians for imazapyr or its formulated products Habitat® and Stalker®. Similarly, no acute toxicity data for larval amphibians have been developed for triclopyr TEA and Garlon® 3A. The U.S. EPA uses fish as surrogate species for larval amphibians when larval amphibian toxicity data are not available (U.S. EPA 2004). Previous studies (SERA 2004) have shown that technical imazapyr is practically non-toxic to fish species such as bluegill sunfish, *Lepomis microchirus*; and rainbow trout, *Oncorhynchus mykiss*. The 96-h LC_{50} values determined by these studies all exceeded 100 mg/L. Triclopyr TEA, like imazapyr, is practically non-toxic to both bluegill and rainbow trout with 96-h LC_{50} values of 891 and 552 mg/L, respectively.

Fish toxicity data for formulated products are limited. No data could be found for the imazapyr formulations Habitat® or Stalker®. Previous research had reported a 96-h LC_{50} value of 23,336 mg/L (as imazapyr) for the imazapyr formulation Arsenal® for juvenile rainbow trout[1]. King et al. (2004) found a similar LC_{50} value of 22,305 mg/L (as imazapyr). For Garlon® 3A, Morgan et al. (1991) reported a 96-h LC_{50} value of 400 mg/L (as triclopyr TEA). This value is very similar to the results of previous

[1]Entrix, Inc. 2003. Ecological Risk Assessment of the Proposed Use of the Herbicide Imazapyr to Control Invasive Cordgrass (*Spartina* spp.) in Estuarine Habitat of Washington State, prepared for Washington State Department of Agriculture, October 30, 2003.

studies that found triclopyr TEA LC_{50} values for the formulation between 300 and 450 mg/L (Wan et al. 1987; Barron and Ball 1989[2]; Abdelghani 1997)

METHODS

Adult bull frogs were obtained from a commercial supplier in Twin Falls, Idaho. The adults were bred at the California Department of Fish and Game's Aquatic Toxicology Laboratory in Elk Grove, California (ATL). The resulting tadpoles were maintained at the ATL facility inside constant-temperature environmental chambers set at 22 $\pm$1°C. Tadpoles from 3 to 18 days post-hatch (15 mg, estimated mean) were used for the 96-h bioassay analysis.

Static acute toxicity tests (96-h) with 48-h renewal of test solutions were performed following standard procedures (ASTM 1996, CDFG 2007). Test chambers were 1000-ml Pyrex® measuring cups containing 400 ml of test solution. Tadpoles were exposed to one of five different concentrations of each material based on preliminary range-finding tests. There were four replicates per treatment group. Each replicate had 10 tadpoles per test chamber with a loading ratio of approximately 0.40 (g/L) at a temperature of 22°C.

Test solutions were analyzed for active ingredient concentrations by the DFG Water Pollution Control Laboratory. Samples were analyzed by gas chromatography-mass spectrometry (GC/MS). The LC_{50} values were based on concentrations of active ingredients in the commercial products. Results for the imazapyr IPA salt products Habitat® and Stalker® were converted to acid equivalents in order to provide simplified comparisons to the technical (acid) product[3]. Percent recovery of spikes averaged 98.6% for imazapyr and 103.8% for triclopyr TEA.

RESULTS AND DISCUSSION

Based on 96-h LC_{50} values (Table 1), all of the compounds, with the exception of Stalker®, were within the U.S. EPA's "practically non-toxic" category for aquatic lethality to aquatic organisms (U.S. EPA 2004). Stalker® with an LC_{50} value of 14.7 mg/L was found to be in the "slightly toxic" range. The toxicity values for the technical compounds were similar to those values previously reported for larval fish. This appears to support the appropriateness of using fish as a surrogate test species for larval amphibians.

Stalker® had the highest toxicity of the five compounds. It was more toxic to bullfrog tadpoles than technical imazapyr acid by a factor of 54. Technical triclopyr TEA was similar in toxicity to technical imazapyr. Garlon® 3A was found to be almost five times more toxic than its parent compound triclopyr TEA.

Laboratory-derived toxicity values are of limited use for assessing environmental

[2]Unpublished study prepared by The Dow Chemical Co. 15 p.

[3]The conversion from active ingredient to acid equivalents is [imazapyr a.i.] x (261 amu/320 amu)

Table 1: Bullfrog tadpole 96-h LC_{50} and 95% confidence interval values for technical imazapyr and technical triclopyr TEA and their formulations.

Compound	LC_{50} (mg/L)	95% C.I.
Technical imazapyr acid	799.6	775.8 – 824.6
Stalker®(as imazapyr acid)	14.7	11.2 – 17.9
Habitat® (as imazapyr acid)	1,739	990.6 – 2,256.7
Technical triclopyr TEA salt	814.1	769.6 – 847.1
Garlon®3A (as triclopyr TEA salt)	174.5	174.5 – 174.5

risk when they are not compared to environmental concentrations. In order to more accurately assess the risk, we compared each compound's LC_{50} values to the highest known or estimated environmental concentration of that compound's active ingredient in water. This approach provided a conservative risk estimate for the frog's larval lifestage.

In 2006, the San Francisco Estuary Invasive Spartina Project (ISP) conducted imazapyr monitoring as part of its non-native spartina eradication effort. The imazapyr herbicide Habitat® was used at its maximum label rate (96 oz Habitat®/acre) during the project. Application event samples were collected immediately adjacent to the treatment area approximately 3 hours post-treatment. Sampling points were purposefully selected in areas that might retain herbicide residues longer than sites that would receive a high volume flush of water immediately upon the return of the tide. The maximum imazapyr concentration detected during the project was 0.4 mg a.e./L (mean concentration = 0.23 mg a.e./L). When the maximum concentration is used in an RQ analysis, the resulting RQ value for Habitat® is 0.0002; the Stalker® RQ value is 0.027. The U.S. EPA uses an RQ value of 0.05 as the level of concern (LOC) for listed aquatic species such as the CRLF (U.S. EPA 2004). These results indicate a very low toxicity risk to larval frogs that are present in aquatic sites that receive direct applications of imazapyr IPA salt herbicides (Table 2).

Similar results are obtained with triclopyr TEA. The USDA Forest Service uses 3.5 mg a.i./L[4] triclopyr TEA for estimating environmental exposure in water (SERA 2003). When this concentration value is divided by ranid tadpole LC_{50} values, RQ values less than 0.05 are obtained for both Garlon® 3A and triclopyr TEA (Table 2).

CONCLUSION

The results of this study indicate that the direct application of imazapyr IPA and triclopyr TEA herbicides to water do not pose a significant acute toxicity hazard to bullfrog tadpoles. Calculated RQ values for each of the tested materials were below

[4]The USFS document uses a 2.5 mg a.e./L value which is equal to 3.5 mg a.i./L.

Table 2. Environmental concentrations in water (EC) and risk quotients (RQ) for technical imazapyr and technical triclopyr TEA and their formulations.

Herbicide	**EC (mg/L)**	**RQ (EC/LC_{50})**
Technical imazapyr acid	0.40	0.40/799.6 = 0.0005
Stalker® (27.6% imazapyr IPA salt)	0.40	0.40/14.7 = 0.027
Habitat® (28.7% imazapyr IPA salt)	0.40	0.40/1739 = 0.0002
Technical triclopyr TEA salt	3.50	3.50/814.1= 0.004
Garlon®3A (44.4% triclopyr TEA salt)	3.50	3.50/174.5= 0.020

the U.S. EPA's level of concern of 0.05 for listed aquatic animals. This supports the environmental appropriateness of applying the formulated products assessed in this study to sites that are directly adjacent to ranid tadpole habitat. In the case of the imazapyr herbicide Habitat®, the data indicates a large safety factor when the formulated herbicide is applied directly to these aquatic habitat sites.

A comparison of the LC_{50} values for the technical materials and formulated products reveals how toxicity can be influenced by either the form of the chemical tested or by the presence of non-herbicidal formulation constituents. For example, the two imazapyr IPA salt formulations Stalker® and Habitat® have widely differing toxicity values, presumably because of the presence of non-herbicidal constituents in Stalker®. This difference in toxicity is consistent with the respective label instructions for each of these products. Habitat®, with its low aquatic toxicity, can be used in water. Label directions for the more toxic Stalker® include a prohibition against direct application to water. Garlon® 3A's toxicity also appears to be influenced by non-herbicidal constituents; it's approximately five times more toxic than its parent compound. As with Stalker®, label instructions for Garlon® 3A prohibit the application of the herbicide directly to surface water.

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