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Potential Risks to Freshwater Aquatic Organisms Following a Silvicultural Application of Herbicides in Oregon's Coast Range[††]

Running Head: Aquatic Organism Risk from Silvicultural Herbicides

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ABSTRACT

Glyphosate, aminomethylphosphonic acid (AMPA), imazapyr, sulfometuron methyl (SMM), and metsulfuron methyl (MSM) were measured in streamwater collected during and after a routine application of herbicides to a forestry site in Oregon's Coast Range. Samples were collected at three stations: HIGH at the fish/no-fish interface in the middle of the harvest/spray unit; MID at the bottom of the unit; and LOW downstream of the unit. All herbicides were applied by helicopter in a single tank mix. AMPA, imazapyr, SMM, and MSM were not detected (ND) in any sample at 15, 600, 500, and 1000 ng/L, respectively. A pulse of glyphosate peaking at ≈62 ng/L manifested at HIGH during the application. Glyphosate pulses peaking at 115 ng/L (MID) and 42 ng/L (HIGH) were found during the first two post-application storm events 8 and 10 days after treatment (DAT), respectively: glyphosate was <20 ng/L (ND) at all stations during all subsequent storm events. All glyphosate pulses were short-lived (4 to 12 h). Glyphosate in baseflow was ≈25 ng/L at all stations 3 DAT and was still ≈25 ng/L at HIGH, but ND at the other stations, 8 DAT: subsequently, glyphosate was ND in baseflow at all stations. These results show that aquatic organisms were subjected to multiple short-duration, low-concentration glyphosate pulses corresponding to a cumulative time-weighted average (TWA) exposure of 6634 ng/L*h. Comparisons to TWA exposures associated with a range of toxicological endpoints for sensitive aquatic organisms suggests a margin of safety exceeding 100 at the experimental site, with the only potential exception resulting from the ability of fish to detect glyphosate via olfaction. For imazapyr, SMM, and MSM the NDs were at concentrations low enough to rule out effects on all organisms other than aquatic plants, and the low concentration and (assumed) pulsed nature of any exposure should mitigate this potential.

Keywords: forestry, glyphosate, herbicides, pulsed exposure, time-weighted average exposure

INTRODUCTION

The use of herbicides to control competing vegetation is a crucial component of modern forestry, and reliance on herbicides has increased as historical management practices have come under scrutiny (Kelpsas et al. 2015). For example, prescribed burning for vegetation control has declined during the past two decades, primarily due to concerns about fire escapes and smoke management. In addition, because the use of herbicides reduces the potential for both erosion and nutrient runoff (Neary and Michael 1996), concerns over both factors have led to increased use of herbicides over mechanical site preparation (Beasley 1979; Blackburn et al. 1986; McBroom et al. 2008)

Compared with agriculture, forestry herbicide applications are infrequent (typically two to three applications during the first 5 years of a 30 to 80 year rotation), utilize low application rates (generally less than the maximum allowed rate), and cover a small portion of the overall forest land base in any given year (Michael and Neary 1993; Neary and Michael 1996; Michael 2004; Shepard et al. 2004). In addition, the use of herbicides in forestry is subject to strict label restrictions and state-specific best management practices (BMPs) developed to minimize offsite movement of herbicides. Despite this, the use of herbicides in forestry remains controversial, with potential impacts to terrestrial and aquatic wildlife a primary concern.

When evaluating potential effects on aquatic organisms from use of pesticides in general, the United States Environmental Protection Agency (USEPA) initially uses a screening model (GENEEC2) to estimate expected environmental concentrations (EECs) (USEPA 2004; NRC 2013). The default model scenario is a small farm pond (2 m deep with a surface area of 2.47 ac) in a 24.7 ac farm field assuming application at the maximum allowed rate without any spray buffer. The resulting EEC reflects spray drift and runoff from a 152 mm (6″) rain event lasting 24 hours, with minimal accounting of chemical-specific fate and transport (USEPA 2004; NRC 2013). These assumptions are not a good model for flowing streams in forest lands, where herbicides are generally applied at rates well below the label maximum (Shepard et al. 2004) following modern

BMPs that limit spray drift by controlling drop size, mandating spray and/or riparian buffers, restricting application heights, and specifying meteorological conditions (Felsot et al. 2010). Under these circumstances, delivery of herbicides to streamwater depends on many site-specific factors, including the physicochemical properties of the herbicides, the topography and hydrology of the application site, and soil type (e.g., SERA 2011a, 2011b). Despite this, both the United States Fish and Wildlife Service and the National Marine Fisheries Service (NMFS) have used EECs from the standard farm pond scenario to support findings that specific pesticides pose risks to threatened and/or endangered aquatic species (e.g., NMFS 2010).

The United States Department of Agriculture (USDA) also uses modeling to obtain herbicide EECs. However, USDA treats flowing streams as a unique case and addresses concentrations resulting from spray drift and runoff plus percolation separately (e.g., SERA 2011b). In addition, the model used by USDA (GLEAMS) incorporates chemical-specific fate and transport and allows for consideration of topography (slope) and soil type. Thus, USDA estimates concentrations due to spray drift in a 2 m-wide flowing stream draining a 10 ac area from a default aerial application scenario (454 g/ac) with variable spray buffers. Using the same model, USDA also estimates concentrations due to runoff plus percolation under variable conditions (soil type, precipitation, etc.). In the case of glyphosate and depending on the exact scenario, USDA estimates peak streamwater and annual average concentrations spanning the ranges from 0 to 83,000 ng/L and 0 to 2580 ng/L, respectively (SERA 2011b). Although estimates, these ranges suggest the impact site-specific conditions can have on instream herbicide concentrations resulting from aerial applications.

The approach to developing EECs taken by USDA provides a more realistic assessment than that taken by USEPA (2004) or NMFS (e.g., NMFS 2010). Even so, USDA cautions that refinement to its concentration estimates based on site-specific considerations is warranted whenever aquatic organisms are potentially at risk (e.g., SERA 2011b). Ultimately, streamwater concentrations resulting from silvicultural applications of herbicides will be highly site and application specific, and accurate application-specific assessments of ecological risk require measured application-specific concentrations.

The literature provides a limited number of field studies reporting measured herbicide concentrations in streams during or after silvicultural applications made according to modern BMPs, and results show a wide range of concentrations (e.g., Rashin and Graber 1993; Dent and Robben 2000). More importantly, these studies often show herbicides manifesting in streamwater as pulses (Michael and Boyer 1986; Rashin and Graber 1993; Dent and Robben 2000; Michael 2003; McBroom et al. 2013; Scarbrough et al. 2014). The simplest approach to assessing risk to aquatic organisms from pulsed exposures is to compare the observed maximum (peak) concentration to chemical-specific toxicity metrics developed in laboratories (e.g., Giesy et al. 2000; SERA 2004a, 2004b, 2011a, 2011b). Example metrics include EC50 (concentration causing 50% inhibition of a process), LC50 (concentration causing 50% lethality), NOEC (no observed effect concentration), and NOAEC (no observed adverse effect concentration). However, these metrics generally reflect exposures to nominally constant concentrations for periods ranging from 48 hours to 21 days, so this kind of comparison has the potential to overstate exposure, and thus risk, associated with pulsed exposures. An alternative approach potentially providing a more realistic assessment of exposure, and thus risk, is to base comparisons on time-weighted average (TWA) exposure concentrations (e.g., Reinert et al. 2002; Landrum et al. 2012).

To assess the risks to aquatic organisms resulting from the use of herbicides as part of forestry in Oregon’s Coast Range, we conducted a nominal 70 day study to characterize concentrations of glyphosate, imazapyr, sulfometuron methyl (SMM), and metsulfuron methyl (MSM) in streamwater during and after an aerial site preparation application of herbicides. Maximum (peak) measured concentrations were then compared to traditional toxicity metrics (NOECs and NOAECs) from a range of laboratory and mesocosm studies to assess the potential for effects on the site-specific aquatic community. As an alternative, TWA exposure concentrations were also calculated when results allowed and compared to TWA concentrations from the same laboratory and mesocosm studies.

MATERIALS AND METHODS

Site Description and Herbicide Application

The study site was the Needle Branch watershed in Oregon's Coast Range (Figure 1), about 16 km from Toledo OR. This is one of three watersheds studied as part of the historic (1959-1973) Alsea Watershed Study (Stednick 2008), and is part of the current Alsea Watershed Study Revisited (begun in 2006), which is allowing comparison of watershed responses to logging and reforestation before and after adoption of the Oregon Forest Practices Act (OFPA) and rules. The Needle Branch watershed is a small (175 ac), steep, forested, headwater basin on the Tyee Sandstone formation (Corliss and Dyrness 1965) receiving about 250 cm of precipitation annually, mostly as rain from October through May or June. The forest stand prior to the 2009 harvest was mainly Douglas-fir (*Pseudotsuga menziesii*) with red alder (*Alnus rubra*) in the riparian stands.

Three gauging stations were established in the Needle Branch drainage (Figure 1). The highest elevation station (HIGH) was located in the harvest unit at the fish-no-fish interface, above which no riparian buffer was left (the OFPA does not require RBs around no fish reaches). The mid-elevation station (MID) was located at the bottom of the harvest unit, and a ≈15 m lateral RB on both sides of the stream was left between MID and HIGH. The lowest elevation station (LOW) was near the confluence of Needle Branch and Drift Creek, approximately 1 km downstream of the lower boundary of the harvest (spray) unit. Pressure transducers mounted in stilling wells connected to a compound weir at LOW and a trapezoidal flume at MID were used to monitor stream stage.

The upper portion of the Needle Branch drainage was harvested in August-September 2009. On August 22, 2010, the harvest unit (91 ac) was sprayed (helicopter) with a mixture of Accord® XRT II (glyphosate), Chopper® Gen II (imazapyr), and Sulfomet® Extra (SMM and MSM) corresponding to 681 g/ac glyphosate (acid equivalents or a.e.), 85 g/ac imazapyr (a.e.), 64 g/ac SMM (active ingredient or a.i.), and 17 g/ac MSM (a.i.), rates consistent with recommendations for site preparation prior to replanting of Douglas fir in Oregon's Coast Range (Kelpsas et al. 2015). As required by the OFPA, an 18 m (horizontal distance) spray buffer was respected on each side of the stream between MID and HIGH (fish-bearing reach). Above HIGH, herbicides were applied parallel to the stream with the spray boom on the stream side turned off (half-boom spraying), leaving a spray buffer of at least 3 m.

Sample Collection and Handling

Two automatic samplers (ISCO® 3700, Teledyne Technologies Inc., Lincoln NE) were installed at each of the three gauging stations (Figure 1). Bottles in one sampler contained pH 7 buffer as a means of preserving samples for determinations of imazapyr, SMM, and MSM at collection (NCASI 2007; Fischer et al. 2008). The second sampler collected unpreserved samples for determinations of glyphosate and aminomethylphosphonic acid (AMPA), a by product of the degradation of glyphosate.

The site preparation application was initiated at 11:38 AM and was completed at 1:18 PM. All samplers were programmed to collect a sample every hour starting at 9 AM and ending at 8 AM the next day (August 23). Subsequently, samplers were manually triggered whenever a storm event was predicted. All samplers were programmed to initiate collection at the same time using the same sampling frequency, which varied from one per hour to one every six hours. Manual grab samples were collected nominally once per week during baseflow conditions.

Storm event samples were retrieved as soon as possible after collection (always within 72 h) and delivered to NCASI's laboratory in Corvallis OR (≈1 h drive time). On receipt, ≈800 mL of each pH preserved sample was transferred to a 1 L high density polyethylene (HDPE) bottle and frozen (no filtration). For a subset of these samples, two ≈400 mL splits were generated and one was spiked with imazapyr, SMM, and MSM prior to freezing. Nominally 180 mL of the unbuffered samples was filtered (0.7 µm glass fiber filter) into 250 mL HDPE bottles and frozen. For a subset of these samples, an additional 180 mL volume was spiked with glyphosate and AMPA before filtration and freezing.

Sample Analysis

On thawing, extracts for determination of glyphosate and AMPA were prepared as described by Hanke et al. (2008). Briefly, 80 mL of sample filtrate (filtered prior to freezing) was derivatized using 9-fluorenylmethylchloroformate (FMOC) and then subjected to post-derivatization cleanup on a solid phase extraction (SPE) cartridge. The eluate from the cleanup was brought to an exact 1 mL final volume with 80:20 water:methanol and 25 µL was analyzed by high performance liquid chromatography (HPLC) using an amino column for separation coupled with fluorescence (FLUOR) detection (λ_{ex} = 264 nm, λ_{em} = 315 nm). All quantifications were vs. multipoint external calibrations prepared using purchased pre-derivatized glyphosate-FMOC and AMPA-FMOC (Crescent Chemicals, Islandia NY) spanning the range from 15 to 15,000 ng/L. In addition to analysis of the sample spikes described above, every analytical batch included a calibration standard (calibration verification), method blank, blank spike (ongoing precision and recovery or OPR), and matrix spike (thawed sample spiked immediately prior to analysis). Additional details of this HPLC/FLUOR (henceforth LC/F) analysis as implemented by NCASI are available (NCASI 2013). A small subset of samples was also submitted to AXYS Analytical Services (Sidney BC) for confirmation of LC/F results using nominally the same sample preparation coupled with a liquid chromatography-tandem mass spectrometry (LC/MS-MS) instrumental finish.

Imazapyr, SMM, and MSM were determined in thawed, pH 7 preserved samples using the basic approach described by multiple researchers (Wells and Michael 1987; Powely and deBernard 1998) and previously applied by NCASI (NCASI 2007; McBroom et al. 2013). Briefly, thawed samples were filtered (0.45 µm nylon membrane) and 200 mL of the filtrate was adjusted to pH ≤2.5 and pulled through a conditioned reverse-phase SPE cartridge. After washing and drying, it was eluted through a strong anion exchange SPE cartridge using 50 mL methanol. This volume was reduced to exactly 1 mL and 25 µL was analyzed by HPLC (phenyl-hexyl column) with ultraviolet detection (235 nm). All quantifications were vs. multipoint external calibrations prepared using pure standards (Chem Service, West Chester PA). These calibrations spanned the range of 600 to 50,000 ng/L.

RESULTS

Sample Collection

Figure 2 shows stage data (water height at the flume) for LOW covering the period over which most samples were collected. The figure also shows when storm event and baseflow samples were collected and that sample collection effectively captured all storm events out to 70 days after treatment (DAT).

Quality Assurance

Detection Limits, Background Interference, and Data Censoring

Method detection limits (MDLs) were determined in an unspiked pre-treatment baseflow sample (blank control sample) collected at LOW. The resulting MDLs were 4 ng/L for AMPA, 18 ng/L for glyphosate (a.e.), 200 ng/L for imazapyr (a.e.), 500 ng/L for SMM (a.i.), and 1000 ng/L for MSM (a.i.). In all cases the experimental MDLs reflected the presence of chromatographic interferences equivalent, on average, to 2.4 ng/L AMPA, 13 ng/L glyphosate, 95 ng/L imazapyr, 231 ng/L SMM, and 382 ng/L MSM in the blank control sample.

In all cases, the retention times of the interfering peaks were shifted slightly relative to the respective herbicide, meaning that these peaks were not due to presence of the herbicides in the blank control sample. More importantly, all measured concentrations in post-application field samples for all herbicides except glyphosate were at levels nominally equivalent (i.e., within a factor of 2) to the pre-application background, and were in all cases below the lower calibration levels (LCL). In addition, multiple lines of evidence showed the analyte-specific interferences varying on a sample-specific basis (NCASI 2013; Supplemental Information Table S1). Overall, these factors led to the decision to censor imazapyr and AMPA results at the corresponding LCLs (600 and 15 ng/L, respectively), SMM and MSM results at the corresponding MDLs (500 and 1000 ng/L, respectively), and glyphosate results at the nominal reporting limit of the LC/MS-MS analysis (20 ng/L). Although these censoring levels are somewhat subjective, they were considered a reasonable compromise between reporting false positives vs. false negatives.

Storage Stability, Spike Recovery, and Analytical Bias

Table 1 summarizes herbicide recoveries from unfiltered samples spiked prior to freezing. For imazapyr, SMM, and MSM these recoveries reflect losses incurred during storage, the freeze thaw cycle, filtration of thawed samples, sample preparation, and sample analysis. For these herbicides, recovery was unaffected by storage to 770 days in a freezer (NCASI 2013), and results from Fischer et al. (2008) support little to no loss of imazapyr, SMM, or MSM during the time between sample collection and freezing (≈72 h maximum). The Table 1 results are therefore considered good measures of overall recovery at concentrations greater than ≈5000 ng/L. However, all samples were <1000 ng/L, and overall recovery at these lower concentrations can only be estimated by combining recoveries from low-level (laboratory) matrix spikes (not presented) with the Table 1 results. Thus, considering concentrations <1000 ng/L only, overall recovery was estimated as ≈78%, ≈73%, and ≈68% for imazapyr, SMM, and MSM, respectively.

Glyphosate and AMPA spikes were made to samples prior to filtration and freezing, and Table 1 shows nominally 80% recovery of AMPA and 95% recovery of glyphosate regardless of spike level. Recovery of both analytes was unaffected by storage out to 300 days in a freezer, and a separate study (not presented; NCASI 2013) showed no loss of a 500 ng/L AMPA spike over 7 days at 22°C (in the dark) in Needle Branch water holding 36 mg/L suspended sediment. Corresponding results for glyphosate suggested 5% loss of a 500 ng/L spike over 7 days (NCASI 2013). Based on these results overall recovery of dissolved glyphosate is estimated to be ≈90%. The corresponding estimate for AMPA is ≈80%.

As discussed, bias is approximated as spike recovery and results indicated sample concentrations were biased low by anywhere from 10 to 40% depending on the herbicide. However, this does not account for the impact of background interference, which can add high bias to concentrations measured in unspiked samples using either HPLC analysis. For glyphosate, comparing results from the LC/F and LC/MS-MS analyses (Supplemental Information Table S1) shows bias in the LC/F results ranging from ≈7 to 42 ng/L.

Overall, LC/F results for glyphosate were subject to high bias due to variable background interference and low bias due to losses over sample processing and storage. Thus the absolute bias in the LC/F result for any given sample is unknown. Ultimately, the weight of evidence suggests that most glyphosate concentrations from LC/F analyses were high biased, and for this reason none of the glyphosate results were recovery corrected. Regardless, when available, LC/MS-MS results were taken as the best measures of glyphosate, and these concentrations are considered to be low biased by no more than 10% (and were not recovery corrected).

Measured Herbicide Concentrations

Streamwater Concentrations during Herbicide Application

Results for glyphosate showed a clear pulse at HIGH (Figure 3) peaking at 62 ng/L in the sample collected at 12:00 AM and returning to pre-application background (≈20 ng/L) by 8:00 PM. Although it took approximately 9 hours for the glyphosate signal at HIGH to dissipate, the pulse width-at-half-height was <4 hours (Figure 3). Glyphosate was not detected (<20 ng/L) at LOW during the nominal 20 hour monitoring period following the application (Figure 3), and no samples were collected at MID due to autosampler malfunction. AMPA was not detected (ND) in any samples (<15 ng/L).

The autosampler collecting buffered samples at HIGH malfunctioned during this sampling episode, while all samples collected at MID were ND for imazpayr (<600 ng/L), SMM (<500 ng/L), and MSM (<1000 ng/L). Because of the NDs at MID, samples collected at LOW were not analyzed for imazapyr, SMM, or MSM.

Streamwater Concentrations during Post-Application Storm Events

A distinct pulse of glyphosate was observed at MID during the first post-application storm event 8 DAT (Figure 4). Results from the LC/F analysis show this pulse maximizing at 149 ng/L, while the LC/MS-MS analysis returned 115 ng/L. LC/F results (Figure 4) show this pulse persisting for ≈10 hours, with a width-at-half-height of ≈4 hours.

LC/F results also suggest a glyphosate pulse at LOW maximizing at 58 ng/L during this first storm event (Figure 4). However, the LC/MS-MS confirmation performed on the sample collected 2 hours earlier and showing the second highest concentration at LOW during this storm (51 ng/L from LC/F analysis) returned ND at 19 ng/L (Figure 4), suggesting that this pulse was due in large part to the background interferent known to be present in samples. Based on this, it was concluded that glyphosate was <20 ng/L in all samples collected at LOW during the first storm event.

Results from HIGH show the presence of glyphosate in the first sample during the first storm event (8 DAT), which was collected before the pulse observed at MID manifested (Figure 4). This sample gave 45 ng/L by the LC/F analysis, while the LC/MS-MS analysis returned 25 ng/L. Results from the LC/F analysis (Figure 4) show concentrations at HIGH dropping in all subsequent samples, and the LC/MS-MS analysis of the ninth sample collected at HIGH during this storm showed glyphosate was <19 ng/L vs. 31 ng/L from the LC/F analysis (Figure 4). All this is consistent with glyphosate concentrations decreasing at HIGH during this storm from a maximum of ≈25 ng/L in baseflow immediately prior to onset to <20 ng/L over the first 9 hours of the event.

During the second storm event 10 DAT, a distinct pulse of glyphosate was observed at HIGH (Figure 4). The highest concentration found at HIGH by LC/F during this storm was 84 ng/L. The corresponding LC/MS-MS result was 42 ng/L (Figure 4). Overall, results at HIGH show a nominal 11 to 12 hour pulse maximizing at ≈40 ng/L with a width-at-half-height of approximately 8 to 9 hours. Although LC/F results also suggest lower concentration glyphosate pulses at MID and LOW during this storm, LC/MS-MS results (Figure 4) are consistent with glyphosate being <20 ng/L in all these samples.

Figure 5 shows glyphosate in all three sampling stations during the third post-application storm event, which started 24 DAT and continued through 30 DAT. These results show no evidence of any sustained glyphosate pulse as observed during the first two storm events (Figure 4). In addition, the two samples submitted for confirmation by LC/MS-MS returned <20 ng/L. Considering that one of these samples had the highest concentration found by the LC/F analysis (62 ng/L; Figure 5), these results support concluding that glyphosate was <20 ng/L in all samples collected at all three stations during the third storm event.

AMPA, imazapyr, SMM, and MSM were not detected in any sample collected during this study, and glyphosate was effectively ND in all samples from the third storm event. These factors led to the decision to not analyze samples from later storm events.

Glyphosate in Baseflow

The first set of baseflow grab samples was collected on 8/25/2010 (3 DAT). LC/F analysis of these samples returned glyphosate concentrations of 30, 21, and 33 ng/L at HIGH, MID, and LOW, respectively: results from LC/MS-MS confirmation analyses on the HIGH and LOW samples were 23 and 26 ng/L, respectively (Table S1 in Supplemental Information). The next set of baseflow samples was collected 19 DAT after two storm events, and glyphosate by LC/F was <20 ng/L at all three sampling stations. On 9/14/2010 (23 DAT) baseflow at LOW measured 34 ng/L glyphosate by LC/F but, consistent with LC/F results from HIGH and MID, the LC/MS-MS analysis returned <19 ng/L. LC/F results from the next two baseflow samplings (33 and 40 DAT) were <20 ng/L at all three stations.

Overall, results show that glyphosate was present at ≈25 ng/L in baseflow at all three stations 3 DAT and was still present at ≈25 ng/L at HIGH but <20 ng/L at MID and LOW immediately prior to the first storm event 8 DAT (Figure 4). Subsequently, glyphosate was <20 ng/L in baseflow at all three stations.

DISCUSSION

In-Stream Concentrations

In this study imazapyr, SMM, and MSM were not detected in any sample, including those collected during the aerial application. Using the maximum glyphosate concentration found during application (62 ng/L at HIGH) and an assumption that spray drift for the other herbicides was proportional to application rates suggests the maximum concentrations of imazapyr, SMM, and MSM that might have manifested at HIGH during the application would be <10 ng/L in all cases. This indicates that measurement of these herbicides at Needle Branch would have required an analytical method capable of quantifications to <10 ng/L.

The limited amount of relevant field work in the literature means that there are limited concentration data useful for comparison to the Needle Branch results. In the case of glyphosate, many field studies purposely involved applications directly to streams (e.g., Newton et al. 1984; Kreutzweiser et al. 1989) or ponds (e.g., Goldsborough and Beck 1989; Goldsborough and Brown 1993), and so are not comparable to Needle Branch results. In addition, it is our contention that streamwater concentrations from aerial applications are highly application-specific, meaning that comparisons based on concentrations absent some accounting for site-specific factors (topography, geology, etc.) have limited utility: comparison of the Needle Branch results for all four herbicides to results from relevant field studies and USDA estimates (Tables S2 and S3 in Supplemental Information) are generally supportive of this assessment.

Ecological Significance

Imazapyr, Sulfometuron Methyl, and Metsulfuron Methyl

Imazapyr, SMM, and MSM were <600 ng/L (a.e.), <500 ng/L (a.i.), and <1000 ng/L (a.i.), respectively, in all samples collected during this study. Thus the worst-case exposure scenarios for Needle Branch would be chronic exposure at these concentrations, which are well below levels shown to have adverse effects on fish, amphibians, and aquatic invertebrates based on traditional toxicity testing utilizing nominally continuous (chronic) exposure regimes (SERA 2004 a, 2004b, 2011a). For imazapyr, concentrations <600 ng/L are also below levels shown to impact macroinvertebrate community structure (Fowlkes et al. 2003) or aquatic plants in general (SERA 2011a). Thus, consistent with USEPA's conclusion that the use of imazapyr in forestry will "most likely result in 'no effect'" on endangered anadromous salmonids (Turner 2003), our results suggest no direct or indirect impacts on the Needle Branch aquatic community attributable to imazapyr.

For SMM and MSM, the Needle Branch NDs are high enough that effects on aquatic plants and/or algae cannot be ruled out, meaning that indirect effects on higher level organisms or overall community structure also cannot be ruled out. However, at Needle Branch the glyphosate results indicate that the aquatic community would have been subjected to a series of short-lived (<24 h), low-concentration pulsed exposures to SMM and MSM separated by variable recovery periods spanning a few days to weeks. This type of exposure regimen has been shown to reduce the effects of MSM on aquatic plants relative to continuous exposures at equivalent TWA concentrations (Cedergreen at al. 2005), although contrary results have also been presented (Boxall et al. 2013). Regardless, the potential for direct effects on aquatic plants, and thus indirect effects on higher level organisms or the aquatic community as a whole, depends on the exact exposure regimen, including the length of any recovery period(s). Thus, actual measured concentrations would be required in order to conclude that the aquatic community at Needle Branch was impacted as a result of application of SMM and MSM.

Glyphosate

The maximum confirmed concentration of glyphosate at Needle Branch was 115 ng./L (a.e.) in a storm event sample at MID, and this concentration persisted for only ≈3 to 4 hours. On the other hand, dissolved glyphosate in baseflow at HIGH was nominally 25 ng/L for up to 8 days. Thus, worst-case exposure scenarios for Needle Branch might be an acute (≤96 h) exposure at ≈100 ng/L or a chronic (>96 h) exposure at ≈25 ng/L. However, our results show that exposure at Needle Branch consisted of a series of short-term (acute or pulsed) exposures on top of a longer-term (chronic) background. This kind of exposure regime is not generally modeled by laboratory bioassays from which the various metrics (e.g., NOECs) used to characterize toxicity are obtained, so these metrics are not directly comparable to the glyphosate exposure regime at Needle Branch.

One approach to assessing risk under these conditions would be to compare TWA exposure concentrations associated with the various NOECs and NOAECs to the TWA exposure concentration documented at Needle Branch. Although exposure-response reciprocity between the two (TWA) exposure scenarios will be endpoint- and organism-specific, and will vary depending on multiple factors including the exposure-specific concentration dynamics (e.g., pulse-specific half-life, inter-pulse recovery periods) and scenario-specific toxicokinetics (e.g., Reinert et al. 2002;

Landrum et al. 2012), comparisons based on TWA exposure would provide some first approximation of margin of safety for glyphosate as "margin of exposure." Thus, Table 2 gives calculated TWA exposures to technical glyphosate (i.e., glyphosate absent any of the adjuvants found in commercial formulations) associated with some of the lowest reported NOECs and NOAECs and compares these values to the TWA exposure at Needle Branch calculated by multiplying the highest concentration observed during a storm event by the associated (nominal) pulse-specific width-at-half-height and summing across all events.

Considering "traditional" endpoints only, the lowest reported NOECs and NOAECs for technical glyphosate are all associated with TWA exposures orders of magnitude higher than observed at Needle Branch (Table 2), suggesting that the use of glyphosate had no impact on site-specific aquatic organisms. However, the results listed in Table 2 reflect exposure to technical glyphosate, so do not account for the toxicity of the adjuvants (e.g., surfactants) present in commercial glyphosate formulations (e.g., Accord® XRT II, Roundup®, Vision®) or site-specific tank mixes. Because many of these adjuvants have their own toxicity profiles (e.g., Edington et al. 2004) any margin of safety based on glyphosate alone may be biased high. Thus, Table 3 gives calculated TWA exposures associated with the lowest reported NOECs and NOAECs for Roundup® and/or Vision® formulations.

Again considering traditional endpoints only, the lowest NOEC and NOAECs for Roundup® and Vision® are (again) associated with TWA exposures orders of magnitude higher than observed at Needle Branch (Table 3). In this case, direct comparison of the TWA exposures assumes that all adjuvants are present in all Needle Branch samples at the same proportion (relative to glyphosate a.e.) found in Roundup® and Vision®. Thus, the relative exposures listed in Table 3 suggest no impact on aquatic organisms at Needle Branch even allowing for the presence of adjuvants.

As discussed, assessment of potential impacts on the aquatic community at Needle Branch is based on direct effects acting on single species, and the comparisons summarized in Tables 2 and 3 suggest there was a large margin of safety at Needle Branch even allowing for the potential impact of adjuvants. This, in turn, suggests limited potential for long-term indirect or community level effects. TWA exposures associated with the few studies examining the impact of glyphosate on microbial communities are also listed in Tables 2 and 3, and all exceed the TWA exposures documented at Needle Branch by a minimum factor of 200, suggesting that adverse effects on microbial communities did not occur at Needle Branch.

Another factor to consider is the potential for effects associated with non-traditional or biochemical endpoints, and Tables 2 and 3 list TWA exposures associated with some of these endpoints. The apparent margin of safety at Needle Branch is >100 for most endpoints, and >10 for all endpoints except olfaction by salmon and/or rainbow trout.

Regarding olfaction by salmonids, experimental results (Tierney et al. 2006, 2007) indicate that these fish are orders of magnitude more sensitive to unidentified constituents in commercial formulations (e.g., Roundup®) than to glyphosate itself. Thus, identification of these chemicals followed by measurements in streamwater will be necessary to fully evaluate the potential for olfactory-mediated effects resulting from real world applications. Regardless, at Needle Branch specifically, olfactory-mediated effects would have impacted only those fish present above MID from late August through late September, 2010. Because adult coho are not expected in Needle Branch until early October-November (D. Bateman, personal communication, 8/16/15), 2010 pre-spawn adult coho were subjected to much lower concentrations (<20 ng/L) than documented in September, while juvenile coho from the 2010 spawn were exposed to even lower concentrations.

AMPA and Glyphosate on Suspended Sediment

Glyphosate on solids (suspended sediment, SS) can also affect aquatic organisms, and the study plan called for extraction and analysis of SS for AMPA and glyphosate (see NCASI 2013). However, inspection suggested very low SS concentrations in all samples (most samples had "clarity" equivalent to baseflow) so only a small number of whole (unfiltered) samples, those judged as having the highest SS in a sampling episode, were frozen for analysis. AMPA and glyphosate were not detected at levels exceeding blank levels in any sample SS (8 ng/L AMPA and

13 ng/L glyphosate equivalents: unknown sample mass). This outcome indicates little risk to the Needle Branch aquatic community posed by AMPA or glyphosate on SS, given the generally low SS concentrations during our experiment.

Cumulative Risk

The large margins separating the TWA concentration of glyphosate at Needle Branch and the TWA concentrations associated with the lowest reported NOECs and NOAECs for glyphosate (Tables 2 and 3) combined with the very low concentrations of the other herbicides suggests the potential for cumulative effects at Needle Branch was negligible. This conclusion is supported by work reported by Tatum et al. (2012), which showed the herbicide mixtures used in forestry generally manifesting additive or antagonistic effects, not synergistic effects, on the survival of *Ceriodaphnia dubia* and fathead minnow. Ultimately, however, we have no in-stream concentration data for most components (herbicides and adjuvants) of the site-specific tank mix used at Needle Branch, so our results do not fully address the question of cumulative risk.

CONCLUSIONS

In this study AMPA, imazapyr, SMM, and MSM were not detected in any sample at concentrations exceeding 15 ng/L, 600 ng/L, 500 ng/L, and 1000 ng/L, respectively. However, a clear pulse of glyphosate was observed at the highest elevation station (HIGH, at the fish-no-fish interface in the middle of the spray unit above which there was no riparian buffer) during the application. This pulse maximized at ≈62 ng/L and persisted for approximately 3 to 4 hours, with a lower concentration “tail” persisting for perhaps an additional 9 hours (Figure 3). Glyphosate was not detected (<20 ng/L) at the lowest elevation station (LOW, approximately 1km below the harvest/spray unit) during the application. Glyphosate was present at ≈25 ng/L in baseflow at all three stations 3 DAT and was still ≈25 ng/L at HIGH 8 DAT, but <20 ng/L at the two lower elevation stations. All subsequent baseflow samples were <20 ng/L glyphosate. In addition, discrete pulses of glyphosate were observed at the two sampling stations located within or at the boundary of the harvest (spray) unit during the first two post application storm events. During the first event (8 DAT) a nominal 10 hour pulse maximizing at 115 ng/L was found at the middle elevation site (MID, at the lower boundary of the harvest or spray unit), while a nominal 12 hour pulse maximizing at 42 ng/L was observed at HIGH during the second event (10 DAT). Glyphosate was <20 ng/L in all storm event samples collected at LOW.

The observation that glyphosate manifested as discrete pulses associated with the first few post-application storm events is generally consistent with observations from other field studies reflecting aerial applications according to modern forestry practices (Michael 2004; McBroom et al. 2013; Scarbrough et al. 2014). Thus it is to be anticipated that the other herbicides monitored at Needle Branch also manifested as fairly narrow pulses (<24 h), in this case at concentrations <<1000 ng/L. Based on these results, it appears that analytical tools with detection limits in the low ng/L range (e.g., 1 to 10 ng/L) will be required to fully characterize delivery of imazapyr, SMS, and MSM to streamwater following aerial applications according to modern silvicultural BMPs. In any case, the concentrations documented at Needle Branch are some of the lowest from any field study.

Given that the concentrations of imazapyr, SMM, and MSM in Needle Branch following application of herbicides are unknown, it is difficult to make any statements regarding potential effects on the aquatic community. However, in the case of imazapyr the analytical results are sufficient to suggest that there was no direct or indirect impact, as even effects on aquatic plants are expected to occur only at concentrations >600 ng/L. Likewise, the analytical results are sufficient to document SMM and MSM concentrations well below the levels shown to have adverse effects on fish, amphibians, or invertebrates but leave the potential for direct effects on aquatic plants and algae, and thus indirect effects on higher level organisms. This potential would be mitigated assuming short-term episodic exposures to SMM and MSM as documented for glyphosate.

Although the TWA glyphosate exposure at Needle Branch was of the same magnitude associated with olfaction in salmon and trout, coho salmon were not present in Needle Branch during the study period. Thus, coho salmon specifically were not subjected to any quantifiable insult resulting from this specific application of glyphosate. Beyond this, the TWA glyphosate exposures documented at Needle Branch were well below levels shown to have effects on fish, amphibians, invertebrates, aquatic plants, or the aquatic community as a whole. This outcome supports an overall absence of effects due to exposure to glyphosate, the issue of exposure-response reciprocity when comparing TWA exposures notwithstanding.

Regarding the issue of cumulative risk, the margins of safety documented at Needle Branch combined with an assumption of additivity suggests negligible cumulative risk to aquatic organisms at Needle Branch. However, our results do not fully address cumulative risk, and this may be an issue warranting additional study supported by the ability to measure all components (herbicides and adjuvants) of a site-specific tank mix in streamwater.

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SUPPLEMENTAL INFORMATION

Table S1. Comparison of dissolved AMPA and glyphosate concentrations measured by LC/F and LC/MS-MS in select samples.

Table S2. Streamwater concentrations of imazapyr, sulfometuron methyl, and metsulfuron methyl following nominally routine herbicide applications as part of forestry.

Table S3. Streamwater concentrations of glyphosate following nominally routine herbicide applications as part of forestry.

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Figure Captions

Fig. 1. Herbicide monitoring stations established in the Needle Branch watershed (HIGH = bottom of no-fish stream reach; MID = bottom of harvest and spray unit; LOW = main gauge near mouth of watershed).

Fig. 2. Stage level at LOW from 8/22/2010 through 10/27/2010 with all samples identified.

Fig. 3. Dissolved glyphosate in streamwater (baseflow) during and immediately following application of herbicides (all concentrations plotted regardless of detection limit).

Fig. 4. Dissolved glyphosate in streamwater during first two post-application storm events with results from LC/MS-MS confirmations (all concentrations plotted regardless of detection limit).

Fig. 5. Dissolved glyphosate in streamwaer during third post-application storm event with results from LC/MS-MS confirmations (all concentrations plotted regardless of detection limit).

Table 1. Recovery of Herbicide Spikes Added to Samples Immediately prior to Freezing

	Spike Level (ng/L)	Percent Recovery		
		Mean	Std Dev	n
Imazapyr[a]	4800-11900	94	2.7	8
Sulfometuron methyl (SMM)[a]	4800-11900	73	3.4	8
Metsulfuron methyl (MSM)[a]	4800-11900	92	3.8	8
Glyphosate[b]	2000-10000	97	2.8	9
Glyphosate[b]	500-900	94	5.0	6
AMPA[b]	2000-10000	80	5.9	9
AMPA[b]	500-900	81	5.5	6

[a] whole samples spiked prior to freezing (filtration performed on thawed samples); all samples thawed and analyzed within 770 days of freezing

[b] samples spiked prior to filtration and freezing; all samples thawed and analyzed within 300 days of freezing

Table 2. Comparison of Time-Weighted Average (TWA) Exposures to Technical Glyphosate Associated with Multiple Scenarios[a]

Scenario/Species	Exposure: Conc. (ng/L)[b]	Exposure: Duration (h)	TWA Exposure: Absolute (ng/L*h)	TWA Exposure: Relative[c]	Experimental Endpoint	Reference
Needle Branch:						
application pulse	62	4	248	0.04		this study
storm pulse #1 (8 DAT)	115	10	1,150	0.17		
storm pulse #2 (10 DAT)	42	12	504	0.08		
baseflow (to 8 DAT)	25	192	4,800	0.72		
cumulative exposure			6,702	1		
NOECs and NOAECs for techincal glyphosate based on "traditional" endpoints:						
Myriophyllum sibiricum (watermilfoil)	80,000	336	2.69E+07	4,011	root length	Perkins 1997
Skeletonema costatum (diatom/algae)	2,80,000	168	4.70E+07	7,019	survival, growth	Giesy et al. 2000
Scenedesmus quadricauda (algae)	7,70,000	96	7.39E+07	11,030	survival, growth	Saenz et al. 1997
Scenedesmus acutus (algae)	20,00,000	96	1.92E+08	28,648	survival, growth	Saenz et al. 1997
Navicula pelliculosa (diatom/algae)	17,00,000	120	2.04E+08	30,439	survival, growth	SERA 2011b
Lemna gibba (duckweed sp.)	13,00,000	336	4.37E+08	65,175	survival, growth	SERA 2011b
Selenastrum capricornutum (diatom/algae)	96,00,000	120	1.15E+09	1,71,889	survival, growth	SERA 2011b
Anabaena flos-aquae (cyanobacteria)	1,15,00,000	120	1.38E+09	2,05,909	survival, growth	SERA 2011b
Rana clamitans (green frog)	17,90,000	1008	1.80E+09	2,69,221	survival	Howe et al. 2004
Crinia insignifera (adult sign-	4,50,00,000	96	4.32E+09	6,44,584	survival	Giesy et al. 2000

bearing froglet)						
Daphnia magna (invertebrate)	9,56,00,000	48	4.59E+09	6,84,691	survival	SERA 2011b
Daphnia magna (invertebrate)	5,00,00,000	504	2.52E+10	37,60,072	survival, growth, reproduction	ABC Inc. 1982
Hyalella azteca (invertebrate)	26,50,00,000	240	6.36E+10	94,89,705	survival	Giesy et al. 2000
Chrionomus tentans (invertebrate)	26,50,00,000	240	6.36E+10	94,89,705	survival	Giesy et al. 2000
<u>Effects Concentrations from Studies Examining Commumity Level Effects[d]:</u>						
"microbial community" (microcosm study)	10,000	336	1.44E+6[e]	215	"community composition"	Pesce et al. 2009
<u>NOECs and NOAECs for techincal glyphosate based on biochemical or "nontraditional" endpoints:</u>						
Oncorhynchus kisutch(coho salmon)	1,00,000	0.5	50,000	7	olfaction	Tierney et al. 2006
Oncorhynchus mykiss(rainbow trout)	1,00,00,000	1	1.00E+07	1,492	avoidance	Folmar 1976
Oncorhynchus mykiss(rainbow trout)	1,10,000	168	1.85E+07	2,757	plasma vitellogenin	Xie at al. 2005

[a] results reflecting exposure to various glyphosate formulations (e.g., Roundup® or Vision®) not included
[b] concentrations as ng/L acid equivalent (a.e.)
[c] relative to cumulative exposure at Needle Branch
[d] exposure conditions associated with observed effects
[e] TWA exposure calculated assuming concentration remained stable at 10,000 ng/L for the first 6 days of the 14 day exposure period (per measured concentrations reported by Pesce et al. 2009)

Table 3. Comparison of Time-Weighted Average Exposures to Roundup® or Vision® Formulations Associated with Multiple Scenarios[a]

	Exposure		TWA Exposure			
Scenario/Species	Conc. (ng/L)[b]	Duration (h)	Absolute (ng/L*h)	Relative[c]	Experimental Endpoint	Reference
Needle Branch:						
application pulse	62	4	248	0.04		this study
storm pulse #1 (8 DAT)	115	10	1,150	0.17		
storm pulse #2 (10 DAT)	42	12	504	0.08		
baseflow (to 8 DAT)	25	192	4,800	0.72		
cumulative exposure			6,702	1		
NOECs and NOAECs for Roundup® or Vision® based on "traditional" endpoints:						
Selanastrum capricornutum (algae)	2,26,300	72	1.63E+07	2,431	growth (biomass)	LISEC 1989
Litoria moorei (motorbike frog tadpole)	4,96,000	48	2.38E+07	3,552	survival	Mann and Bidwell 1999
Oncorhynchus mykiss(fingerling rainbow trout)	2,60,000	96	2.50E+07	3,724	survival	Folmar et al. 1979
Daphnia magna (invertebrate)	5,89,000	48	2.83E+07	4,218	survival, growth	Folmar et al. 1979
Chlorella sorokiniana (algae)	6,20,000	48	2.98E+07	4,440	survival, growth	Christy et al. 1981
Oreochromis niloticus (tilapia)	3,10,000	96	2.98E+07	4,440	survival	SERA 2011b
Lepomis macrochirus (bluegill sunfish)	7,00,000	96	6.72E+07	10,027	survival	Forbis et al. 1982
Myriophyllum sibiricum (watermilfoil)	2,42,000	336	8.13E+07	12,132	root length	Perkins 1997
Daphnia magna (invertebrate)	9,92,000	504	5.00E+08	74,600	survival, growth, reproduction	Giesy et al. 2000

Gammarus pseudolimnaeus (invertebrate)	1,40,00,000	48	6.72E+08	1,00,269	survival	ABC Inc. 1982
Lemna minor (duckweed sp.)	1,69,10,000	48	8.12E+08	1,21,110	?	Lockhart et al. 1989
Potamogeton pectinatus (pondweed)	74,40,000	336	2.50E+09	3,72,999	growth	Hartman and Martin 1985
Effects Concentrations from Studies Examining Commumity Level Effects[d]:						
"microbial community" (mesocosm study)	2,10,000	2160	6.51E+6[e]	971	"community composition"	Baker et al. 2014
"microbial community" (mesocosm study)	60,00,000	192	4.57E+8[f]	68,189	"community composition"	Vera et al. 2010
"microbial community" (mesocosm study)	45,00,000	264	4.70E+8[f]	70,128	"community composition"	Perez et al. 2007
NOECs and NOAECs for Roundup® or Vision® based on biochemical or "non-traditional" endpoints:						
Oncorhynchus mykiss(rainbow trout)	7,400	0.0333	246	0.04	neurophysiological olfaction	Tierney et al. 2007
Oncorhynchus mykiss(rainbow trout)	7,400	0.5	3,700	0.6	"behavioral olfaction"	Tierney et al. 2007
Oncorhynchus mykiss(rainbow trout)	7,42,000	0.167	1.24E+05	18	avoidance	Tierney et al. 2007
Ephemeralla walkeri(mayfly)	10,00,000	1	1.00E+06	149	avoidance	Folmar 1978
Oncorhynchus mykiss(rainbow trout)	67,50,000	96	6.48E+08	96,688	"erratic swimming and rapid respiration"	Morgan et al. 1991
Oncorhynchus kisutch(coho salmon)	28,80,000	240	6.91E+08	1,03,133	"several sublethal parameters"	Mitchell et al. 1987
Oncorhynchus mykiss(rainbow trout)	80,00,000	1440	1.15E+10	17,18,890	"aggressive behavior"	Morgan and Kiceniuk 1992

[a] results reflecting exposure to Roundup® or Vision®

formulations only

[b] concentrations as ng/L acid equivalent (a.e.); Roundup® and Vision® concentrations converted to glyphosate a.e. assuming 1 mg of formulation is equivalent to 0.31 mg glyphosate acid (Giesy et al. 2000)
[c] relative to cumulative exposure at Needle Branch
[d] exposure conditions associated with observed effects
[e] two applications at 210,000 ng/L on Day 0 and ≈Day 21 of a nominal 3 month experimental period; concentrations following each application calculated at intervals equivalent to reported half-lives, so TWA exposure is less than the product of initial exposure concentration and time; no effects obseved using two separate applications at 2,880,000 ng/L (TWA exposure 8.91E+7 ppt-h)
[f] concentrations calculated at intervals equivalent to reported half-life, so TWA exposure is less than the product of initial exposure concentration and time

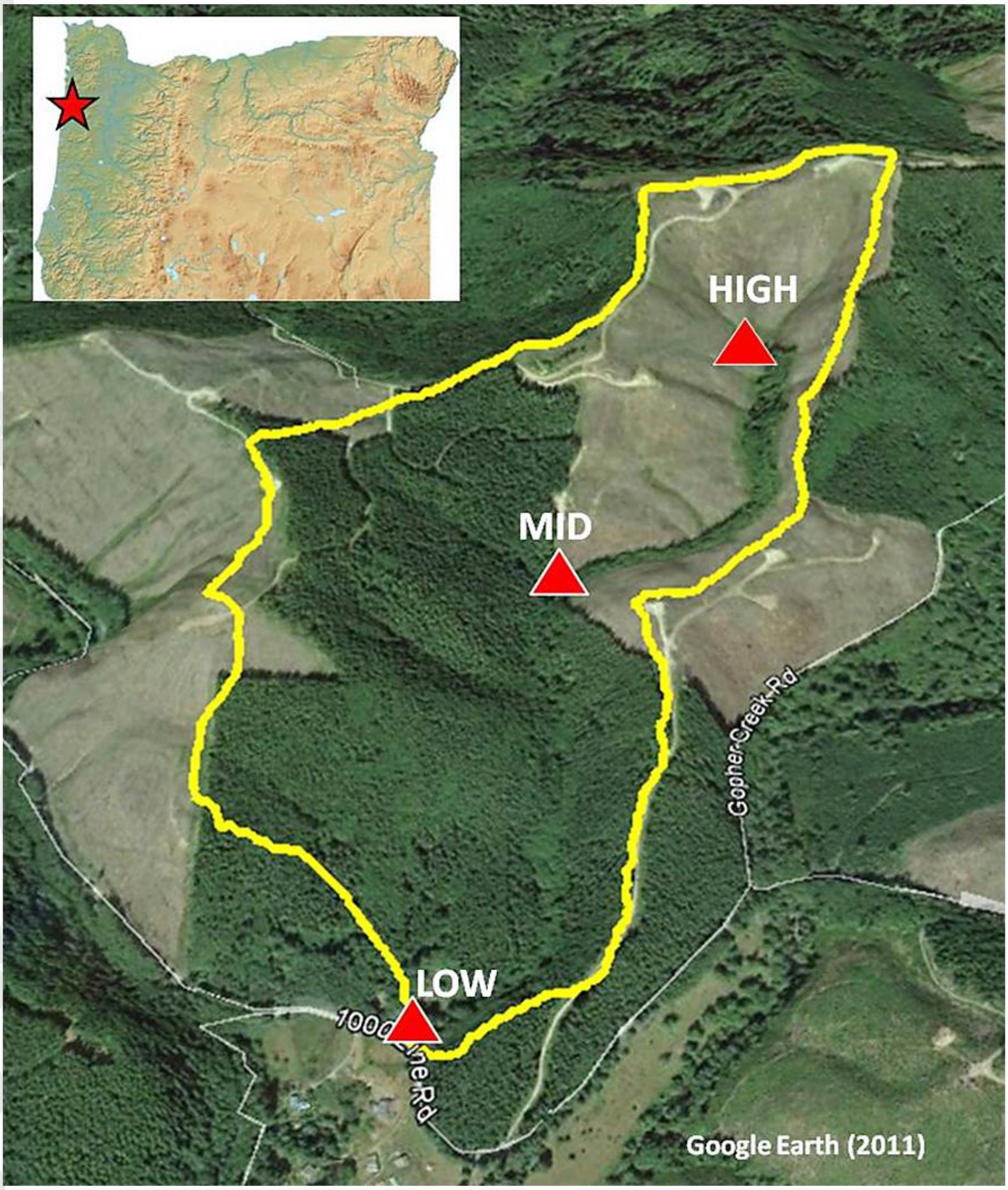
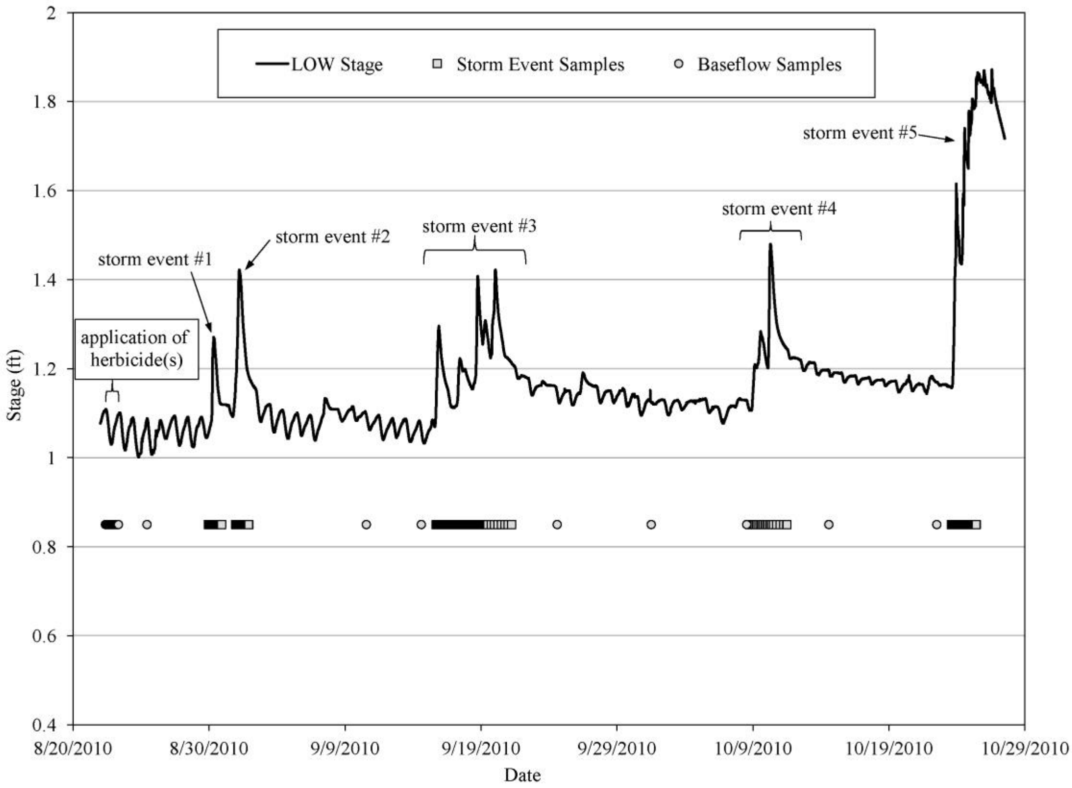
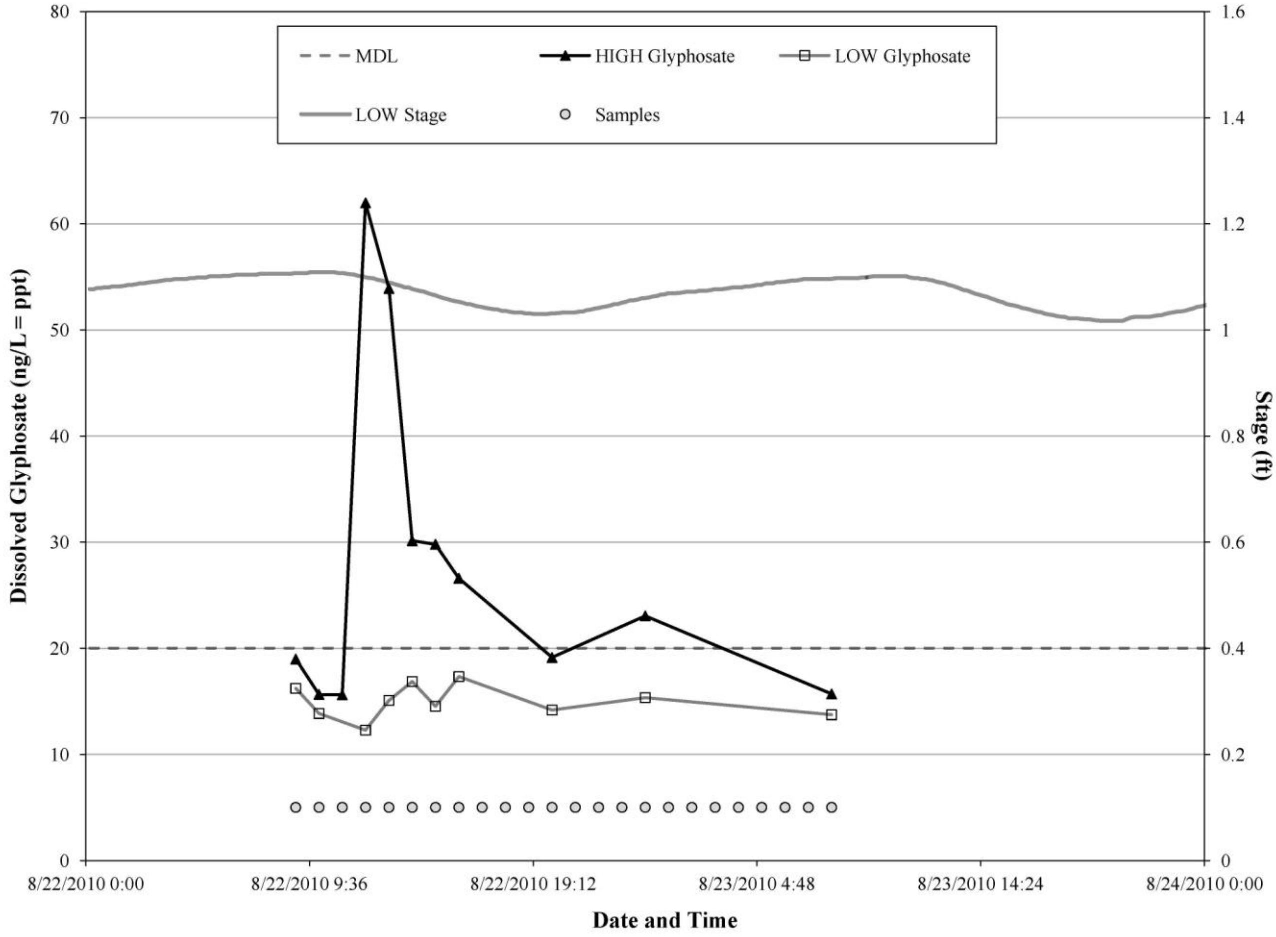


Figure 1



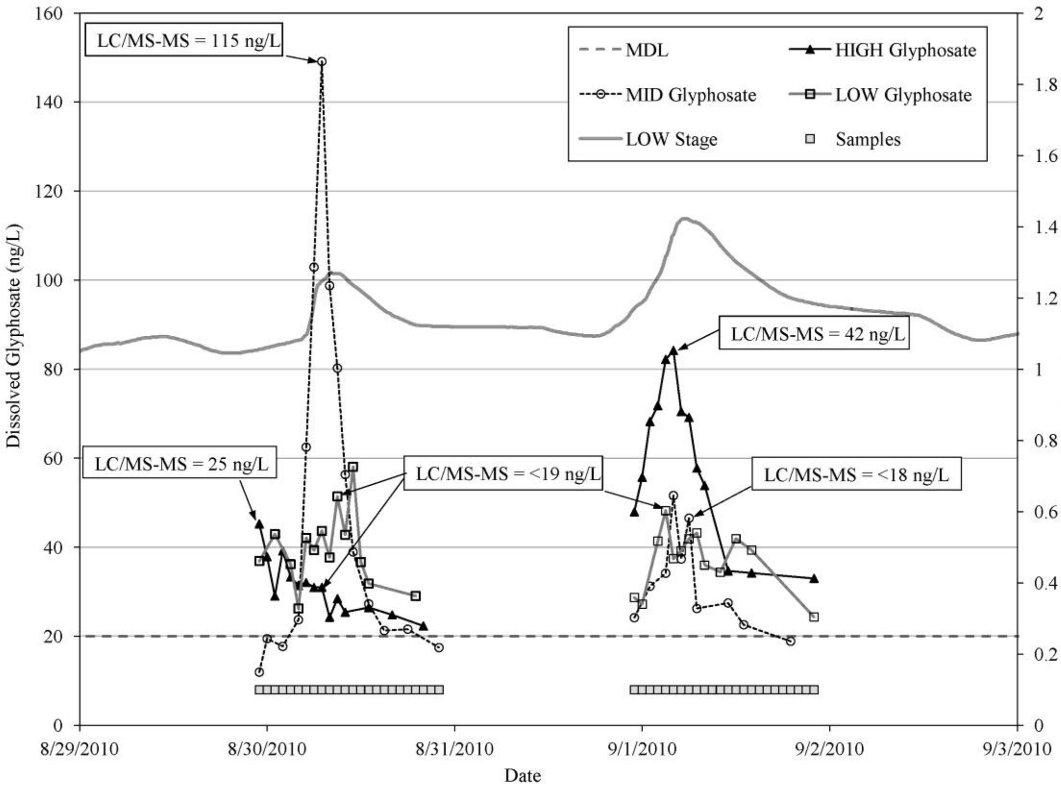
LOW Stage
Storm Event Samples
Baseflow Samples
storm event #1
storm event #2
storm event #3
storm event #4
storm event #5
application of herbicide(s)
Stage (ft)
Date
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
8/20/2010
8/30/2010
9/9/2010
9/19/2010
9/29/2010
10/9/2010
10/19/2010
10/29/2010

Figure 2



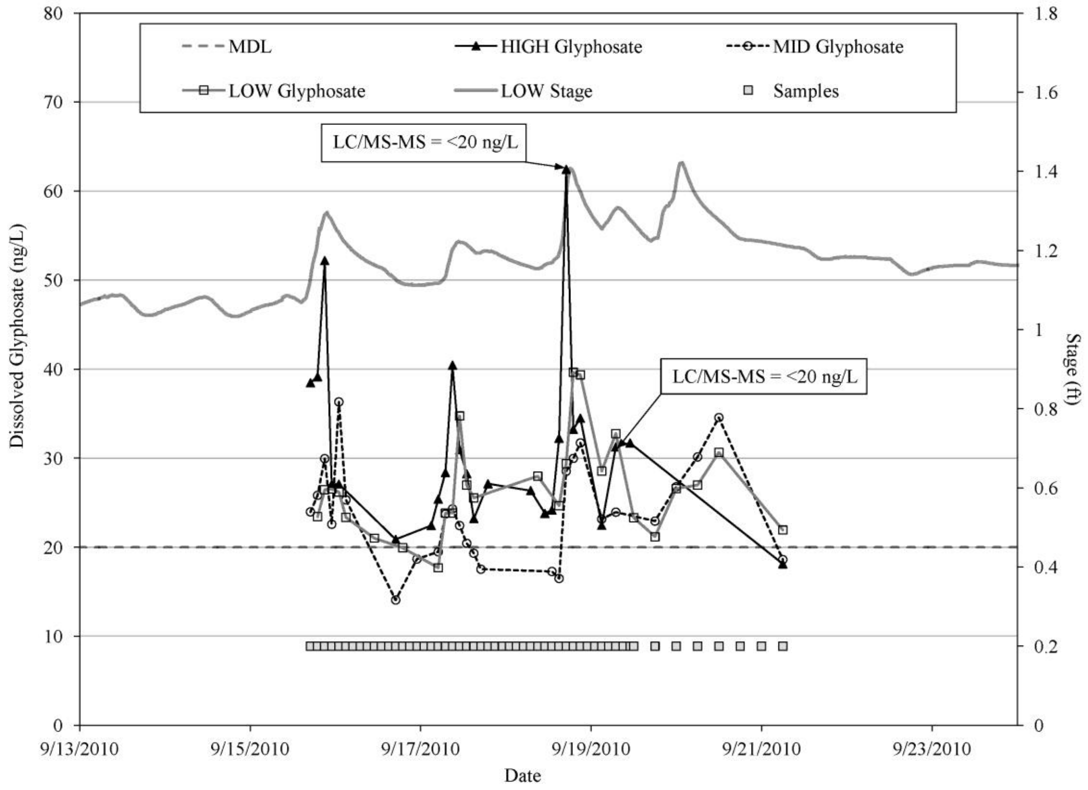
MDL
HIGH Glyphosate
LOW Glyphosate
LOW Stage
Samples
Dissolved Glyphosate (ng/L = ppt)
Stage (ft)
Date and Time
80
70
60
50
40
30
20
10
0
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
8/22/2010 0:00
8/22/2010 9:36
8/22/2010 19:12
8/23/2010 4:48
8/23/2010 14:24
8/24/2010 0:00

Figure 3



LC/MS-MS = 115 ng/L
MDL
HIGH Glyphosate
MID Glyphosate
LOW Glyphosate
LOW Stage
Samples
LC/MS-MS = 42 ng/L
LC/MS-MS = 25 ng/L
LC/MS-MS = <19 ng/L
LC/MS-MS = <18 ng/L
Dissolved Glyphosate (ng/L)
Stage (ft)
Date
8/29/2010
8/30/2010
8/31/2010
9/1/2010
9/2/2010
9/3/2010

Figure 4



80
70
60
50
40
30
20
10
0
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
MDL
HIGH Glyphosate
MID Glyphosate
LOW Glyphosate
LOW Stage
Samples
LC/MS-MS = <20 ng/L
LC/MS-MS = <20 ng/L
Dissolved Glyphosate (ng/L)
Stage (ft)
9/13/2010
9/15/2010
9/17/2010
9/19/2010
9/21/2010
9/23/2010
Date

Figure 5