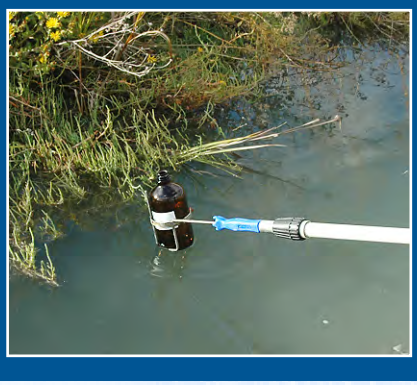
2011

San Francisco Estuary Invasive Spartina Project Water Quality Monitoring Report for 2011



Coastal Conservancy

San Francisco Estuary

Invasive *Spartina* Project

Water Quality Monitoring Report for 2011

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1. Executive Summary

The California State Coastal Conservancy's San Francisco Estuary Invasive *Spartina* Project (ISP) implemented their 2011 Water Quality Monitoring Plan in conjunction with the Bay-wide treatment of non-native *Spartina* (cordgrasses). Water samples and data on conventional water quality parameters were collected pre-treatment, immediately after the herbicide application, and one week after treatment at 13 sites (10% of the infestation sites where herbicide was utilized) in compliance with the Statewide General National Pollutant Discharge Elimination System (NPDES) Permit. This document reports on the results from 2011 and compares them to the overall trends from ISP water quality monitoring from 2007-2010.

Water sampling immediately after *Spartina* treatment has consistently found that any imazapyr concentrations detected in the receiving waters are two to four orders of magnitude below those reported in the toxicology literature as a concern to humans or the animals that inhabit the associated tidal marsh system, including the benthic invertebrates at the foundation of the food web. The mean imazapyr concentration from the 2011 treatment event sampling was 89.63 ppb, which is very consistent with the four-year mean of 99.49 ppb from 2007-2010.

The successful *Spartina* control achieved since 2006 has reduced the baywide infestation from over 800 net acres to 49 net acres. Much less herbicide is now necessary with each passing year, and this success has also enabled ISP partners to shift away from broadcast aerial applications over the historic monocultures to spot applications from airboat and backpack. By 2011, 9 of the 13 sites (69%) monitored for NPDES compliance utilized the backpack sprayer as the only herbicide delivery system needed, and ISP is completing the eradication of virtually all infestations of *S. densiflora* around the Bay with just manual removal after successful reductions with imazapyr in the initial years.

In addition, the one-week post-treatment sampling results are also consistent with the published literature that imazapyr is short-lived in an estuarine environment. In 2011, the mean reduction in the imazapyr concentration measured one week after treatment was 92.2%, no matter what concentration was previously measured from the treatment event, while the four-year mean reduction was 95.8% from 2007-2010. With the rapid degradation of this herbicide in the tidal marsh, as measured by the concentration in the water at the site one week after treatment, it is anticipated that all sites that still had measurable concentrations at that time would likely be below detectable levels within a few more days after the third sample.

The monitoring of conventional water quality parameters (water temperature, dissolved oxygen, pH, conductivity and salinity) verified that there is no indication that the herbicide applications to invasive *Spartina* have had any impact on Estuary surface water quality; this result was entirely anticipated because there is no relevant pathway for the treatment of an emergent plant to alter these parameters in this open system with twice-daily tidal exchange.

2. Introduction

2.1 Invasive Spartina *in the San Francisco Estuary*

The genus *Spartina* refers to cordgrasses, the majority of which are found in tidal salt marshes and sloughs, open mudflats, or brackish channels. Four species of non-native *Spartina* have been introduced to the San Francisco Estuary since the 1970s, and they aggressively spread (both vegetatively from underground rhizomes as well as by seed or vegetative propagules). *Spartina alterniflora* (smooth cordgrass) is native to the Atlantic and Gulf Coasts of the U.S. and was first introduced in 1976 by the U.S. Army Corps of Engineers as part of an experiment in tidal marsh restoration using dredge spoils at the Alameda Flood Control Channel. In the mid-1990s it was discovered that the introduced species had hybridized with our native Pacific cordgrass, *Spartina foliosa*, creating a fertile hybrid swarm with numerous morphologies and phenologies that can be transgressive of the traits of either of the parent species. These hybrids are the most problematic of the invaders, representing 99% of the Estuary-wide infestation of non-native *Spartina* that covered over 800 net acres in 2005-2006 but has been reduced by ISP and its partners to approximately 49 net acres by 2011.The second most prevalent of the invaders is *Spartina densiflora* (Chilean cordgrass), which was mistakenly introduced to Creekside Park along Corte Madera Creek in Marin County as part of a wetland restoration in the 1970s. The species spread throughout the Corte Madera Creek watershed and other eastern Marin wetlands, but it has a very limited distribution elsewhere around the Bay. The other two species of introduced cordgrass, *S. anglica* (English cordgrass) and *S. patens* (salt meadow cordgrass), are each found at only one marsh site in the Estuary and have not spread from these locations after numerous years.

There are many potential impacts from these aggressive non-native *Spartina* species. Over the past 200 years, the development of the marshes of the San Francisco Estuary for homes and commercial interests has reduced the remaining marsh acreage by 85-90%. The resulting habitat loss has contributed to reductions in the populations of several endangered species including the California clapper rail and salt marsh harvest mouse. Invasive *Spartina* further degrades the remnants of habitat by colonizing tidal channels used for foraging by rail, competitively excluding native marsh vegetation such as *Sarcocornia pacifica* (pickleweed) that is essential for harvest mouse habitat and rare plants such as *Chloropyron molle molle* (soft bird's beak, formerly *Cordylanthus mollis mollis*), and by transforming unvegetated mudflats into *Spartina* meadows and thereby eliminating foraging areas for millions of migratory and resident shorebirds. The infestations of non-native *Spartina* also present direct problems to the human population beyond the loss of biodiversity and habitat. Flood control channel capacity can be severely reduced by *Spartina* expansion, and the resulting sediment accretion can significantly raise annual maintenance budgets for regular dredging. Dense stands of *Spartina* can also impound water and create ponded areas in the mid-marsh that become excellent breeding areas for salt marsh mosquitoes. This can also have public health consequences due to the increase in West Nile Virus cases in California in recent years.

2.2 Invasive Spartina *Project*

In response to the expanding infestation of non-native *Spartina*, the California State Coastal Conservancy formed the Invasive *Spartina* Project (ISP) in 2000 to coordinate a regional effort to arrest and reverse the spread of these aggressive invaders and eventually eradicate them from the Estuary. A major impetus for the effort was to protect the $100 million investment in salt ponds acquired from Cargill that will be converted to marsh and other habitat as part of the South Bay Salt Ponds Restoration (SBSP), the largest such effort on the West Coast of the United States. Virtually every tidal restoration project over the past 25 years has been colonized and its marsh development trajectory compromised by hybrid *Spartina*. The ISP is essential in removing this threat from the Estuary so that SBSP can proceed successfully.

After several years of compiling environmental documentation and permits, completing full surveys of the Estuary shoreline, and implementing several pilot projects on control methods, the ISP began widespread control efforts in 2004 and baywide treatment in 2005. Manual methods, such as digging or covering, have proved very effective on small pioneering *Spartina* infestations, and will continue to be utilized where appropriate; however, the Programmatic Environmental Impact Report (PEIR) for ISP found that for most sites the use of aquatic herbicide was the most effective method and caused the lowest environmental impacts, especially when confronted with dense monocultures covering 50-100 contiguous acres of marshland and mudflat at some individual sites. The ISP has developed individual Site-Specific Plans for each of their 170 sub-areas, incorporating Integrated Pest Management (IPM) strategies that evaluate all appropriate control methods to determine the most effective combination to utilize over time.

Treatment is timed to achieve the longest possible tidal exposure of the *Spartina* in order to allow the herbicide to penetrate the leaf cuticle so it is not washed off by the incoming tides (referred to as “dry time”). Therefore, ISP partners usually begin treatment on a low or receding tide just after sunrise during the active growing season of the cordgrass from June through October. Several other key aspects are factored into the timing equation. According to the California Department of Pesticide Regulation (CDPR) and the ISP Programmatic Environmental Impact Report (PEIR), herbicide applications cannot occur when winds exceed 10 mph, a common occurrence on the Bay shoreline by late morning/early afternoon in the summer months even if the winds were calm at sunrise. Hence, ISP partners emphasize the need to begin treatment at dawn on appropriate days because the winds may halt control efforts prematurely. In addition, many of the marshes infested with non-native *Spartina* are home to the endangered California clapper rail. ISP’s 2008-2010 Biological Opinion from the USFWS first permitted earlier entry to treat within clapper rail-occupied marshes in recognition that waiting until their breeding season officially ends on August 31 would allow for continued hybrid *Spartina* seed dispersal.

2.3 Herbicides Utilized for Spartina *Control*

The primary herbicide utilized by ISP partners for invasive *Spartina* treatment is the aquatic formulation of imazapyr (sold under the trade names Habitat® or Polaris™), which is approved for use in estuarine systems. These formulations are essentially identical and contain a solution of 28.7% isopropylamine salt of imazapyr in water, with the remaining inert in-

gredients composed of a small amount of acidifier (probably acetic acid, but this trade secret is not disclosed on the label) as well as blue dye.

Imazapyr is a non-selective herbicide that can be effective on monocots (e.g. grasses such as *Spartina*) as well as broadleaf plants (dicots). It is a systemic herbicide that normally enters through the foliage and is circulated (translocated) throughout the plant and down into the roots causing mortality of the entire plant or clone; this is in contrast to a contact herbicide that would only work on the above-ground portion of the vegetation providing temporary control of a perennial plant like *Spartina* by "burning off" the above-ground biomass but not translocating to the roots where it could achieve full mortality. Imazapyr works by inhibiting the enzyme acetolactate synthase (ALS) needed for the biosynthesis of three amino acids (the branched-chain aliphatic). Animals do not produce these amino acids themselves but rather acquire them by consuming plants, which is one reason for imazapyr's low toxicity to animals because there is no relevant pathway of activity. Although imazapyr does little to alter respiration, photosynthesis or lipid and protein synthesis in the target plant, it does inhibit the rate of DNA synthesis by 63% within 24 hours post-treatment; this inhibition can be used as an indirect measure of cell division which relates directly to growth. To achieve the maximum herbicidal activity, imazapyr should be applied post-emergence when the target plants are growing vigorously and during weather conditions that allow for slow drying of the droplets. ISP has observed that treated *Spartina* plants remain green for a long period of time and can remain in an arrested state of growth for weeks before finally dying; fortunately, seed production is eliminated if the application occurs early enough in the phenology of the *Spartina*, even if full mortality is still weeks away.

The secondary herbicide used by ISP partners is glyphosate, in its aquatic formulation (e.g. Aquamaster®), which is the only other herbicide approved for estuarine use in the U.S. Glyphosate is similar to imazapyr in that it is also a non-selective, systemic herbicide that inhibits amino acid synthesis in plants. Aquamaster® is an aqueous solution containing 53.8% of the isopropylamine salt of glyphosate, with water as the only reported inert ingredient. Prior to the California registration of the aquatic formulation of imazapyr in August 2005, non-native *Spartina* control in San Francisco Bay was attempted exclusively with glyphosate. However, this tool yielded consistently poor results and was falling far short of outpacing the spread of the invader. This failure is probably a result of glyphosate's affinity for adsorbing to sediment, causing it to bind to silt and salt that are deposited on the *Spartina* by the tides, thereby rendering the herbicide inactive. The glyphosate is not able to penetrate the leaf cuticle and enter the plant where it can be systemically circulated.

Within the first treatment season after imazapyr was approved by the State of California in August 2005, 96% of the applications by ISP partners had transitioned away from glyphosate to the new tool, and this rose to 100% utilizing imazapyr by 2006. Over the past several years, glyphosate has only been used by ISP partners on a single site (Southampton Marsh) as part of the IPM strategy for eradication of *S. patens*, but glyphosate was not employed here in 2011 (refer to Section 3.1 below for more details). Glyphosate was added to the tank mix for the *S. densiflora* application at Creekside Park in 2011 in an effort to enhance the efficacy on the small remaining plants by inhibiting synthesis of an additional three amino acids (over the three aliphatic amino acids inhibited by imazapyr).

Neither of these aquatic herbicide formulations contains a surfactant; consequently this is added to the tank mix from a short list of products that are approved for use in aquatic sys-

tems. Since the leaves of the target *Spartina* plants in San Francisco Bay are covered with depositional material, uptake of the herbicide is difficult to achieve and the use of a surfactant plays a vital role in the application process. Surfactants improve efficacy by lowering the surface tension of liquids and thereby improving the spread of the liquid herbicide mixture over the leaf surface, increasing adherence of the formulation to the leaf (wetting) while reducing runoff, and enhancing the penetration of the leaf cuticle. The two surfactants utilized by ISP partners during the 2011 treatment season was Competitor® (Wilbur-Ellis) and Liberate® (Loveland Industries, Inc.). Competitor® is a methylated seed oil (MSO) recommended for use with imazapyr by the original manufacturer (BASF); this product strikes a good balance by combining one of the lowest relative toxicities to aquatic life of the available surfactants while consistently yielding high efficacy results. Liberate® is a natural lecithin-based (soybean) product and consequently is presumed to have rapid biodegradation; this product also acts as a drift retardant which aids in ISP aerial or high-pressure hose applications, also has a relatively low toxicity to aquatic life, and has been highly effective on hybrid *Spartina*. A non-toxic blue marker dye (e.g. Turf Trax or similar) is also included in the tank mix for ground-based treatment to help the applicator get full coverage without retreating, which helps reduce the amount of chemical entering the marsh environment.

Recent studies have raised concern over a group of surfactants containing nonylphenol ethoxylate due to their moderate toxicity and suspected endocrine disruption in fish and aquatic organisms. Consequently, the ISP partners do not use these nonylphenol products, such as R-11® and ProSpreader®, for invasive *Spartina* control, although they are commonly used by other vegetation managers in the Bay Area and are known to perform well by improving herbicide efficacy.

2.4 Regulatory Requirements

On March 12, 2001, the U.S. Ninth Circuit Court of Appeals held that the application of pesticides into waters of the United States, or onto aquatic plants growing in waters of the United States, results in discharges of "pollutants" and requires coverage under a National Pollutant Discharge Elimination System (NPDES) permit pursuant to Section 402 of the Clean Water Act (CWA). Referred to as the Talent decision (from *Headwaters Inc. v. Talent Irrigation District*), this judgment was issued just prior to the major season for applying aquatic pesticides by public agencies in 2001. Because of concern over potentially serious public health, safety, and economic implications of delaying scheduled treatment activities, the State Water Resources Control Board adopted an interim Statewide General NPDES permit (Water Quality Order No. 2001-12-DWQ) on an emergency basis to provide coverage for broad categories of aquatic pesticides.

In a settlement agreement to an August 2001 lawsuit filed by Waterkeepers Northern California, the State Water Board agreed to fund a comprehensive Aquatic Pesticide Monitoring Program (APMP) that would assess pesticide alternatives, receiving water toxicity caused by residual aquatic pesticides, and other monitoring parameters. After two years of assessment, the State issued the Statewide General NPDES Permit for the Discharge of Aquatic Pesticides for Aquatic Weed Control in Waters of the United States (General Permit; Order No. 2004-0009-DWQ) for application of specific herbicides under certain conditions.

On November 20, 2006, USEPA issued its final rule on aquatic pesticides codifying its interpretation of the CWA. The rule upheld EPA's long-standing position that applications

made in accordance with Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) product labels do not require an NPDES permit for the application of pesticides to waters of the United States. Petitions for review of this final rule were filed in 11 Circuit Courts and on January 7, 2009, the Sixth Circuit Court of Appeals ruled in *National Cotton Council, et. al. v. EPA* that NPDES permits are required for all biological and chemical pesticide applications that leave a residue in water when the applications are made in, over, or near a water of the U.S. Their ruling thereby determined that EPA's final rule was not a reasonable interpretation of the CWA and vacated the final rule. On June 8, 2009, the Sixth Circuit granted a two-year stay on their decision to allow EPA and the states to develop and issue the permits and to educate stakeholders on compliance. On March 28, 2011, the Sixth Circuit granted a motion to extend this stay until October 31, 2011 from the original end date of April 9, 2011.

According to the State Water Resources Control Board website (posted on June 27, 2012), a public meeting will be held on August 7, 2012 to review the draft Statewide General National Pollutant Discharge Elimination System Permit for Residual Pesticide Discharges to Waters of the United States from Algae and Aquatic Weed Control Applications. The State Water Board will hold a subsequent meeting to consider adopting the Draft Aquatic Weed Control Permit on November 6, 2012.

The current General Permit allows the use of a small list of U.S. EPA-approved aquatic herbicides, and can be reopened to add coverage for products that have been newly registered by CDPR. The aquatic formulation of imazapyr (Habitat® or Polaris™), the primary herbicide utilized by the ISP, was added to the list shortly after California registration on August 30, 2005. Currently, there are no State or U.S. EPA-based numeric objectives or criteria for imazapyr (or for glyphosate outside of Municipal Use water supplies where the limit for glyphosate is 700 micrograms per liter [µg/L]). Therefore, the General Permit does not have receiving water limitations for the herbicides used on invasive *Spartina* in the San Francisco Estuary. Because ISP partners do not use surfactants containing nonylphenol ethoxylate (e.g. R-11® or ProSpreader®), the ISP did not perform any chemical concentration analyses for these compounds.

3. Water Quality Monitoring Plan

3.1 Aquatic Pesticide Application Plan

The Statewide General Permit requires the discharger to develop and implement an Aquatic Pesticide Application Plan (APAP). The ISP prepared and submitted an extensive programmatic APAP in 2005 and completes an annual update that is submitted to the Water Board. Each ISP partner reviews and adopts the Programmatic APAP, submits a completed Notice of Intent to Comply with the Terms of this General Permit (Notice of Intent, NOI) along with the annual fee to the Regional Water Board. ISP's Programmatic APAP provides an in-depth description of the water body where treatment will occur (the San Francisco Estuary), including background on the ecology, natural processes affecting water quality, and a survey of the general sediment and water quality characteristics currently documented for the system. The four species of non-native cordgrass are described as well as the motivation for the control work and the project's control tolerances. The pros and cons of various treatment methods are evaluated including the reasoning behind the need for herbicide use, and the types of aquatic herbicide and expected application rates. Finally, all ISP sites are described including their location and the status of the *Spartina* infestation, and a baywide map of treatment areas is provided.

The APAP includes a Water Quality Monitoring Plan (WQMP) that ISP implements in the field during the treatment season. ISP contracted with San Francisco Estuary Institute (SFEI) to implement the WQMP at the start of the project but took over the responsibility in 2006. In accordance with the General Permit requirements, ten percent (10%) of the treatment sites must be monitored each year. There were 133 *Spartina* infestation sites treated with herbicide 2011, a reduction from the past few years due to sites that were not permitted for treatment in the Biological Opinion; subsequently 13 sites were monitored for water quality to reflect 10% of the total. The implementation of the annual WQMP will be described in detail later in this report, and the annual list of sites monitored, treatment dates, and treatment methods for 2011 can be found in **Table 2**. Since glyphosate was phased out in favor of the more versatile and effective imazapyr in 2005-2006, this herbicide has been monitored at just one site each year, Southampton Marsh, where it is used around an endangered annual plant to ensure that seed germination of that species cannot be inhibited if there is any unexpected residual action in the tidal marsh from imazapyr. Glyphosate was not used at Southampton Marsh in 2011, but was added to the imazapyr tank mix for Creekside Park and hence was monitored there for the first time.

3.2 Treatment Monitoring Site Selection

The ISP selects treatment sites at locations that are representative of the overall, baywide *Spartina* control effort and uses the following four treatment site types as a guide:

I. Tidal Marsh, Microtidal Marsh, Former Diked Bayland, Backbarrier Marsh

II. Fringing Tidal Marsh, Mudflats, and Estuarine Beaches

III. Major Tidal Slough, Creek or Flood Control Channel

IV. Urbanized Rock, Rip-Rap, Docks, Ramps, etc.

Each year, ISP tries to select a relatively even distribution of these marsh site types to be sampled for water quality, as well as the range of herbicide delivery systems and marsh dynamics present in the work program. However, since Type IV infestation sites are usually very small, sparse, and adjacent to large bodies of water with constant flushing that will serve to quickly dilute any herbicide incidentally entering the water column, this site type is not considered as high a priority for sampling as they were the least likely of the sites to have any water quality issues related to the application. Site Types I and II have been considered to be the sites most likely to develop detectible levels of herbicide in the water column, so the sampling program has normally been weighted in this direction.

3.3 Sampling Design, Procedures & Analysis

Sampling Design

The sampling events were designed to characterize the potential impacts involved with imazapyr (and glyphosate) applications relative to adjacent surface waters. Consistent with permit requirements, the monitoring program included background or pre-treatment sampling within the 24 hour period prior to herbicide application, treatment event sampling immediately following herbicide application, and post-treatment sampling one week after herbicide application. During background sample collection, the location was recorded using GPS and marked with a flagged PVC pipe to aid ISP staff in relocating the point for subsequent sampling events. The treatment event samples were collected immediately adjacent to the treatment area after sufficient time had elapsed such that treated water would have entered the adjacent area on the incoming tide. Since the standard protocol is for the ISP partners to treat *Spartina* on a low or receding tide when possible, application event samples were often taken 2-5 hours post-treatment when the tide had again flooded the site, but some of these samples could be collected within just a few minutes after the treatment crew had left the area if sufficient water was present. Finally, the one-week post-treatment monitoring was conducted when sufficient water was present at the site on the seventh day after the application. To enhance quality assurance, the ISP submitted three duplicates over the course of the season for the approximately 42 total base samples taken. These were normally added to either the treatment event or one-week post-treatment event since the herbicide level in the pre-treatment samples was usually ND (no detection). It is standard for the lab to include blanks as part of their quality control, but the ISP also submitted trip blanks on a regular basis (nine over the course of the season). Trip blanks were prepared by carrying a bottle of distilled water to the sample collection site, and using it to fill a sample collection container at the site. This blank (a known zero-concentration sample) was then transported with the collected samples and submitted to the laboratory.

Field Sampling Procedures

The Invasive *Spartina* Project has conducted its own water quality monitoring program since 2006 modeled after sampling procedures developed for the State Water Resources Control Board (SWRCB) APMP and outlined in the 2004 APMP Quality Assurance Program Plan (QAPP). Water samples were collected using a sampling rod and pre-cleaned amber glass 1-liter bottles. To collect the sample, the bottle was attached to the sampling rod with a clamp, extended out over the water at the application site, and lowered to approximately 50% of the water depth. When the bottle was full it was pulled back out of the water

and the cap was affixed to the mouth of the bottle. The sample was labeled in permanent ink with the sample ID number, date, time, and initials of the sampler.

The sample ID number was determined by the following protocol: a four-letter code unique to the site, followed by the site visit number (e.g., "01" for pre-treatment, "02" for treatment, or "03" for one-week post-treatment), followed by the time since the application (e.g., "pre" for the baseline sample, the number of hours since the application for the treatment sample, or "1w" for the one-week post-treatment event).

Equipment Calibration

Temperature, electrical conductivity, salinity, and dissolved oxygen were measured in the field with a portable YSI Model 85 (Yellow Springs Instruments Inc., Ohio, USA), while pH was measured with an Oakton waterproof pHTestr1 (Oakton Instruments, Illinois, USA). To assure accurate and reliable temperature, electrical conductivity, salinity, and dissolved oxygen measurements, the YSI Model 85 meter was calibrated, operated, and maintained in accordance with the manual specifications found at http://www.ysi.com/media/pdfs/038503-YSI-Model-85-Operations-Manual-RevE.pdf. To assure accurate and reliable pH measurement, the pHTester1 meter was calibrated, operated, and maintained in accordance with the manual http://www.4oakton.com/Manuals/pHORPIon/WPpHTestr1_2mnl.pdf.

Field Data Sheets

At each sampling location, the sample ID number, the time of the sampling, the sample depth, and the water temperature, pH, dissolved oxygen, conductivity, and salinity measurements were entered on a Field Data Collection Form (FDCF, **Appendix 1**). Also recorded on the FDCF was site information including the site ID number, the station location (application point, upstream, downstream), station type (reference, treated), wind conditions, tidal cycle, water color, and the type of herbicide and surfactant utilized. Any other unusual conditions or concerns were noted, and any fish, birds, or other wildlife present at the point were recorded. The FDCFs were dated and numbered consecutively for each site on that date. Upon return to the office, the data were entered into an electronic spreadsheet for processing, and the FDCFs were compiled into a data log and kept permanently in the office.

Sample Shipment

Following collection, water samples were stored on ice packs and shipped for priority overnight delivery to the laboratory. ISP utilized the Pacific Agricultural Laboratory in Portland, OR for 2011 after they satisfactorily fulfilled their contract requirements in 2010. If samples were not shipped until the following day, they were stored in a cooler on ice until they could be transferred to a refrigerator, and subsequently transferred back into a cooler with ice packs for shipping. Samples were not shipped on Fridays because they would not be received until the following Monday and the appropriate temperature could not be maintained.

Field Variances

The ISP usually selects and plans to monitor one or two more sites each season than is necessary for compliance with the NPDES Permit, to allow for failed sampling events or analyses. If a situation arose that precluded the collection of a water quality sample at a designated point at a time suitably close to the specified times (within 24 hours prior to herbicide treatment, within six hours post-treatment, and one week post-treatment), the Water Quality Monitoring Manager (WQMM) determined whether (1) sampling at the site type was need-

ed to complete the sampling events required by the NPDES permit, or (2) sampling at the site type was not needed for permit compliance, and the site/event could be dropped. If the site type was needed, then the WQMM considered whether surrogate sampling of some sort (e.g., sampling at a point reasonably nearby the initial point, or at a later or earlier time) would provide an acceptable substitute. If so, the variation was carefully documented and justified on the Field Data Sheet. If the WQMM determined that surrogate sampling was not suitable, then an alternate, similar site was selected and sampled prior to, within six hours post, and within one week post treatment, as a complete replacement for the initial site.

Sample Analysis

The samples were analyzed within the appropriate holding times for imazapyr (extracted within seven days, analyzed within 21 days of extraction) or glyphosate (within 14 days). Results are reported as parts per billion (ppb), equivalent to µg/L (micrograms per liter). The analytical method used for imazapyr is EPA 8321B in which the extracts are analyzed using liquid chromatography with mass spectroscopy (LC/MS/MS) detection, with a limit of quantitation (LOQ) of 0.02 ppb (the minimum detectable level of the instrument) and reporting limit (RL) of 0.05 ppb. Glyphosate is analyzed using EPA method 547 (High Performance Liquid Chromatography with post column derivatization using orthophthalaldehyde (OPA) and fluorescence), with a reporting limit of 5ppb (10ppb for its primary metabolite AMPA). The lab ran one blank each time it conducted an analysis (minimum of one sample tested per batch, maximum of three). Results are submitted to the San Francisco Bay Regional Water Quality Control Board and placed on the ISP's website for public viewing.

Lab QC & Data Quality Indicators

Each season, the contracted analytical laboratory ("lab") is required to provide a Quality Assurance Plan ("QAP") that meets U.S. EPA standards prior to initiating analysis. The lab plan must specify the method of analysis to be used, and describe any variations from a standard protocol. The WQMM and ISP Director both reviewed the lab QAPs and determined that they were adequate. At a minimum, the following data quality indicators (DQIs)

Table 1. Minimum Data Quality Indicators (DQIs) for an ISP-contracted Laboratory

Criteria	*Method*	*Indicator Goal*
Accuracy of measurement	Analyze matrix spikes and spike duplicates	1 matrix spike per 10 samples (10%) > 65% @ 2.0 µg/L
Agreement between measurements	Analyze lab duplicates and/or matrix spike duplicates	Relative percent difference < 25%
Completeness	Percent of usable data (completed/submitted)	95% return
Comparability of results	Standard reporting units	All data reported in micrograms per liter (µg/L)/parts per billion (ppb)
	Use of standardized analysis methods	Standard method used if possible, any modifications identified, described, and supported.
Detection Limits (imazapyr, EPA 8321B)	Method detection limit	MDL </= 0.02 ppb
	Lab reporting limit	LRL </= 0.05 ppb

have been required for the lab (the QAP submitted for 2011 from Pacific Agricultural Laboratory is attached as **Appendix 2**):

4. 2011 *Spartina* Treatment and Water Quality Monitoring

4.1 Summary of 2011 Herbicide Applications

ISP partners utilized herbicide at 133 sites during the 2011 Treatment Season, with 13 sites monitored for imazapyr in the receiving water and one for glyphosate. Glyphosate was only added to the tank mix for the *S. densiflora* application at Creekside Park in an effort to enhance the efficacy on the small plants remaining at the site by inhibiting synthesis of an additional three amino acids (over the three aliphatic amino acids inhibited by imazapyr). Over the course of the 2011 season, six Type I sites, two Type II sites, four Type III sites and one Type IV site were monitored for water quality by ISP.

ISP partners utilized a variety of herbicide delivery systems for the 2011 treatment season, and **Table 2** lists the methods used at the 13 sites monitored for water quality. This list continues to illustrate an increasing shift away from aerial broadcast to ground-based spot treatment methods that began in 2008. The list also reflects the significant progress ISP partners have made using amphibious tracked vehicles that are no longer needed at most sites around the bay where the eradication can now be completed with backpacks alone . The earlier access to clapper rail habitat first afforded to ISP in the 2008-2010 USFWS Biological Opinion amendment has allowed the partners to maximize the proportion of sites where on-the-ground inventory monitoring and treatment is conducted before September 1. However, the 2011 Biological Opinion was not received until September 23, delaying treatment at many clapper-rail occupied sites for that season.

Low volume aerial herbicide applications were not used at a single site in 2011. High-

Table 2. Summary of Water Quality Monitoring Sites for the 2011 Treatment Season

Sites	*Site Number*	*Marsh Type*	*Treatment Date*	*Application*
Oyster Point Park	19e	IV	7/6/11	Imazapyr – Backpack
San Bruno Creek	18h	III	7/7/11	Imazapyr – Backpack
Sonoma Creek	26c	III	8/3/11	Imazapyr – Backpack
Sanchez Marsh	19k	I	8/4/11	Imazapyr – Airboat
Easton Creek Mouth	19j	II	8/17/11	Imazapyr – Airboat
Blackie's Creek Mouth	3b	III	8/31/11	Imazapyr – Backpack
Beach Drive	23b	II	8/31/11	Imazapyr – Backpack
Creekside Park	4g	I	9/29/11	Imazapyr & *Glyphosate* – Backpack
San Pablo Marsh	22b	I	10/3/11	Imazapyr – Airboat & backpack
Palo Alto Baylands	8	I	10/17/11	Imazapyr – Backpack
Seal Slough	19p	I	10/17/11	Imazapyr – Backpack
Newark Slough	5c	III	10/19/11	Imazapyr – Airboat
Stevens Creek Tidal Marsh	15c	I	10/31/11	Imazapyr – Backpack

pressure hose applications were monitored at four sites around the Bay, conducted entirely in the form of airboat applications. Low-pressure backpack sprayer applications were monitored at 10 (77%) of the sites, which is consistent with the high percentage of sites relying on this method since the 2008 treatment season. This is a reflection of the significant reduction in non-native *Spartina* at most ISP sites after years of successful treatment, as well as a focus on identifying and spot treating each and every plant at an infested site. Nine of the 13 sites (69%) monitored for NPDES compliance in 2011 utilized the backpack sprayer as the only herbicide delivery system needed.

4.2 Herbicide Levels in the Water at Treatment Sites

Imazapyr

ISP contracted with Pacific Agricultural Laboratories (PAL) again in 2011 after the positive experience during the first year working with the lab in 2010. ISP was looking for a lab that was more familiar with imazapyr and had the equipment and expertise to utilize the most rigorous analytical method (LC/MS/MS with a reporting limit of 0.05 ppb). PAL had previously done analytical work for Washington State University researchers working on invasive *Spartina* control with imazapyr in Willapa Bay. No water samples were broken in shipment in 2011 and none were misplaced by the shipping carrier (FedEx).

Figure 1: 2011 ISP NPDES Water Quality Monitoring Locations

Table 3 shows the imazapyr levels at the 13 sites monitored for this herbicide in 2011. The lab reported non-detections of imazapyr from the pre-treatment samples at 10 sites (77%), with three sites (23%) reporting low levels of imazapyr ranging from 0.11 ppb to 0.19 ppb with a mean of 0.15 ppb. While all of these detection levels are extremely low and fall into a very narrow range, they are not down around the 0.05 ppb reporting limit and are therefore less likely to be due to contamination either at the lab or during field collection. These three pre-treatment detections are likely to be related to *Spartina* treatment nearby that occurred a matter of days before the treatment at the monitoring site. All three sites are part of larger complexes that had portions treated for invasive *Spartina* before the associated pre-treatment water sample was collected. Please refer to Section 5.1 of this report for a more detailed discussion of the likely cause of these false positives.

Treatment event monitoring found mean imazapyr levels of 89.63 ppb, with a minimum detection of 0.23 ppb and a maximum of 380 ppb (n=12, SD=129.37 ppb). There was a single non-detection for the treatment event samples in 2011 at Sonoma Creek which may be related to the low volume of imazapyr applied during the backpack application and the high volume of water flowing through this major tidal slough.

The water samples collected at the treated *Spartina* infestations one week post-treatment found mean imazapyr levels of 1.55 ppb in the Bay water for sites with detections, ranging from a minimum of 0.06 ppb to a maximum of 5.90 ppb (SD=2.29 ppb); the imazapyr level had dropped below detectable limits at two of the 13 sites (15%). Herbicide levels in the tidal receiving waters one week post-treatment showed a substantial reduction over the treat-

Table 3. Herbicide Concentrations in Adjacent Surface Water for 2011 *Spartina* Treatment (ND = not detected at 0.05 ppb lab reporting limit)

Sites	*Concentration (ppb = µg/L)*			*Application*
	Pre-Treatment	*Treatment*	*One-Week Post*	
Oyster Point Park	ND	6.50	0.06	Imazapyr – Backpack
San Bruno Creek	ND	0.23	0.08	Imazapyr – Backpack
Sonoma Creek	ND	ND	ND	Imazapyr – Backpack
Sanchez Marsh	ND	130	0.45	Imazapyr – Airboat
Easton Creek Mouth	0.11	330	0.16	Imazapyr – Airboat
Blackie's Creek Mouth	ND	24	ND	Imazapyr – Backpack
Beach Drive	ND	380	0.08	Imazapyr – Backpack
Creekside Park	ND/*ND*	55/*35*	1.4/*ND*	Imazapyr/*Glyphosate* – Backpack
San Pablo Marsh	0.14	44	5.90	Imazapyr – Airboat, backpack
Palo Alto Baylands	ND	20	5.90	Imazapyr – Backpack
Seal Slough	ND	1.8	0.27	Imazapyr – Backpack
Newark Slough	0.19	62	2.60	Imazapyr – Airboat
Stevens Creek Tidal Marsh	ND	22	0.11	Imazapyr – Backpack

ment event as in previous ISP monitoring seasons, with a mean reduction of 92.2% regardless of the concentration level found immediately post-treatment. Two sites had lower reductions of 66% and 71%, but all other samples had no less than an 85% reduction and five sites had greater than 99% reduction (three were 100%). This trajectory indicates a high probability that all sites with trace levels of imazapyr remaining after one week would drop to levels below detection limits within a matter of days beyond that third sample.

Glyphosate

At the one site monitored for glyphosate in 2011, the herbicide was not detected in the pre-treatment sample. The treatment event sample was determined to contain 35.0 ppb by the laboratory, and this dropped below detectable levels one week post-treatment (at a reporting limit of 10 ppb). The lab also tested for AMPA (aminomethyl phosphonic acid), the primary metabolite (i.e. breakdown product) of glyphosate, and all three samples reported no detections of AMPA at a reporting limit of 10 ppb.

4.3 Conventional Water Quality Parameters at Treatment Sites

Table 4 lists the data on the conventional water quality parameters water temperature, dissolved oxygen, pH, conductivity, and salinity collected at the ISP's 13 representative treatment sites over all three monitoring events in 2011. The mean water temperature over all sampling events was 20.7°C with a minimum of 10.8°C and a maximum of 29.2°C. The mean water temperatures for the pre-treatment, treatment, and one-week post treatment events occurred within a narrow range of 20.6, 22.2, and 19.2°C, respectively.

Dissolved oxygen (DO) averaged 5.6 mg/L and varied widely across all sites and sampling events, with a minimum of 2.4 mg/L and a maximum of 8.1 mg/L. The mean DO for the pre-treatment, treatment, and one-week post treatment events were essentially identical at 5.5, 5.5, and 5.9 mg/L, respectively.

The pH of the Estuary water at all treatment sites averaged 8.0 and did not vary much over all samples. The pH ranged from a minimum of 7.6 to a maximum of 8.4; the mean pH for the pre-treatment, treatment, and one-week post treatment events was nearly identical at 7.9,

Table 4. Conventional Water Quality Parameters Measured at 2011 Treatment Sites

Site	Water Temp (C)			DO (mg/L)			pH			Conductivity (mS)			Salinity (ppt)		
	Pre	Treat	Post	Pre	Treat	Post	Pre	Treat	Post	Pre	Treat	Post	Pre	Treat	Post
Oyster Point Park	16.4	29.2	19.6	2.5	6.4	4.8	7.7	8.1	8.0	19.5	19.3	23.9	14.7	10.1	15.4
San Bruno Creek	22.8	26.4	18.3	5.5	3.8	6.3	7.6	7.8	7.8	19.3	21.5	25.4	12.1	12.8	17.1
Sonoma Creek	19.3	19.5	20.5	7.3	3.5	5.9	8.1	8.1	7.7	13.9	22.4	23.5	8.5	14.1	15.6
Sanchez Marsh	18.8	22.1	24.7	5.3	6.4	5.2	7.9	8.0	7.9	36.5	35.7	37.3	23.1	24.0	23.6
Easton Creek Mouth	17.2	28.2	24.2	3.0	4.9	2.4	7.8	7.9	7.9	30.5	41.1	36.3	21.3	24.6	23.3
Blackie's Creek Mouth	23.7	20.3	21.5	6.4	7.2	6.1	7.9	7.9	7.9	33.2	36.3	34.2	21.0	25.6	23.7
Beach Drive	22.6	22.2	21.9	7.0	8.1	6.9	8.1	7.9	8.0	34.1	35.1	36.5	22.4	23.5	22.9
Creekside Park	23.7	22.0	17.9	5.9	6.4	6.7	7.7	7.6	7.8	30.0	33.9	37.2	19.4	22.9	22.8
San Pablo Marsh	23.3	17.7	18.5	7.1	7.7	7.8	8.1	8.2	8.2	34.0	29.9	29.9	22.2	21.9	18.4
Palo Alto Baylands	21.2	22.9	18.1	4.7	3.8	5.8	7.6	7.8	8.1	22.4	27.0	25.6	14.5	17.3	17.0
Seal Slough	21.2	22.2	20.2	7.4	7.2	7.5	8.1	8.1	8.4	34.9	37.5	37.2	24.0	25.2	26.3
Newark Slough	18.5	18.2	13.8	3.9	3.1	4.4	7.9	7.9	8.1	24.2	24.2	22.5	17.0	17.2	17.7
Stevens Creek Tidal Marsh	18.5	17.9	10.8	5.6	3.3	6.4	8.1	8.3	8.3	15.9	19.5	21.6	14.1	18.3	18.3

8.0, and 8.0, respectively.

Conductivity averaged 28.8 mS/cm over all sites and sampling events, with a minimum of 13.9 mS/cm and a maximum of 41.1 mS/cm. The mean conductivity for the pre-treatment, treatment, and one-week post treatment events were essentially equivalent at 26.8, 29.5, and 30.1 mS/cm, respectively.

Salinity averaged 19.3 ppt over all samples, with a minimum of 8.5 ppt and a maximum of 26.3 ppt. The mean salinity for the pre-treatment, treatment, and one-week post treatment events were nearly identical at 18.0, 19.8, and 20.2 ppt, respectively.

As in 2010, Pacific Agricultural Laboratories was not able to test turbidity so this parameter is not reported for 2011.

5. Discussion

5.1 Interpretation of Imazapyr Results

ISP continued to work with Pacific Agricultural Laboratory (PAL) for the 2011 water quality monitoring season. The relationship with this laboratory began in 2010 when they were recommended by researchers in Washington State when ISP sought a laboratory that was more familiar with imazapyr and the apparent challenges this herbicide poses to accurate analyses. PAL built upon the experience they gained in working with ISP over the course of 2010, and the laboratory continued to refine their methodologies to achieve the best possible analytical results.

The most rigorous analytical method for imazapyr, Specific LC/MS/MS (EPA 8321B) with a reporting limit of 0.05 ppb, produced false positives for some of the pre-treatment samples that ISP sent to PAL in 2010. This was not entirely unexpected since a previous lab had so much difficulty implementing this analytical method that *all pre-treatment samples* in 2007 produced false positives within a very narrow range of concentrations, regardless of the treatment history at the site or its position in the Estuary. In working with PAL over the course of the 2010 season, ISP found that imazapyr apparently adheres strongly to glassware, and if the lab tested treatment samples (that normally contain at least some concentration of the herbicide) alongside pre-treatment samples (that normally do not contain any imazapyr) that it routinely got false positives. Consequently, the use of random secret blanks to test the quality of the lab's results is problematic if they are included with treatment samples. Therefore, the WQMM determined that the best way to obtain accurate measurements at the lower end of the scale was to test these samples separately from those we anticipated would have higher levels. Although ISP did continue to implement their quality control procedure of sending secret blanks throughout the 2011 season (nine times overall), the samples were labeled in a manner consistent with the standard identification protocol described above in Section 3.3. This allowed PAL to segregate these samples into the pre-treatment category where the concentration of imazapyr was expected to be either zero or very low (near the reporting limit for the analytical method), and ensured that the blanks would not be tested alongside treatment event samples.

Pre-Treatment Monitoring

Pre-treatment samples provide an important baseline as to the ambient levels of the target chemical present in the water column before any addition from *Spartina* control. However, we expect these background levels to be zero (or non-detection, ND) in most cases because this herbicide is very uncommon. Although the environmental sensitivity and toxicity profile of imazapyr makes it an attractive choice for aquatic vegetation management, there are very few projects of this nature going on around the San Francisco Estuary and they are relatively small, widely scattered, and generally don't overlap with the *Spartina* treatment season. Although imazapyr is used by licensed professionals in forestry, it is not generally available to consumers except as a very small proportion of Ortho® Ground Clear® (0.08%) or similar products. At this low concentration that is available at hardware stores and garden centers, it is unlikely to find significant surface water contamination from urbanized areas that could corrupt one of ISP's pre-treatment samples, especially during treatment season that is con-

ducted in the summer months when there is no rainfall around San Francisco Bay to cause significant runoff.

As listed in Table 3 and described above in Section 4.2, 10 pre-treatment samples from 2011 had no detection of imazapyr, representing 77% of the collections before the herbicide application at the site, while three pre-treatment samples (23%) were reported to have detectable levels of imazapyr. This is consistent with previous results over the three monitoring seasons from 2008-2010, where 81% of the pre-treatment samples were reported as non-detections. While some of these detections probably represent cross-contamination in the lab, they are all very low readings. The mean of the three samples from 2011 was 0.15 ppb, with a minimum of 0.11 ppb and a maximum of 0.19 ppb (SD=0.04 ppb). The eight samples with detections from 2008-2010 showed similar results; the mean was 0.16 ppb, with a minimum of 0.06 ppb and a maximum of 0.28 ppb (SD=0.09 ppb).

Since ISP normally treats invasive *Spartina* at 130-150 sites each year during a relatively narrow window in late summer, it is not uncommon for a pre-treatment sample to be collected adjacent to a site that was treated recently or is being treated even that same day, in which case any pre-treatment imazapyr detection is probably associated with the nearby site. The three pre-treatment detections that occurred in 2011 are likely to be related to *Spartina* treatment nearby that preceded the treatment at these sites by only several days. As described above in Section 4.2, all three sites are part of larger infestation complexes that had portions treated for invasive *Spartina* before the associated pre-treatment water sample was collected. San Mateo County Mosquito and Vector Control District (SMCMVCD) had been working on some small West Bay sites for several weeks before the airboat application to the mudflats of Easton Creek Mouth on 8/17/11, including the upstream infestations in the manmade channels associated with this site along Hwy. 101 and the City of Burlingame. A portion of San Pablo Marsh (the 2nd detection) had been treated several days prior to the treatment of the larger infestation that was monitored for water quality on 10/3/11. Finally Newark Slough (the 3rd detection), is part of the Don Edwards National Wildlife Refuge (DENWR) and is connected hydrologically to several other adjacent infestations that were treated in the days before that sampling event, including Dumbarton and Audubon Marshes. It is likely that residue in the receiving water from these recent applications to contiguous infestation sites is what the lab detected in all three of these cases.

Treatment Event Monitoring

ISP partners normally perform their herbicide applications to invasive *Spartina* on a receding or low tide to allow for full exposure of the target plants and to maximize the amount of time the herbicide has to penetrate the leaf cuticle and enter the plant (referred to as "dry time"). At most sites this means that there is no water in the sloughs or over the mudflats adjacent to the *Spartina* and the treatment event sample must be collected after sufficient water has returned to the site to fill the one-liter bottle without stirring up too much sediment. The Statewide General NPDES Permit states that the sample should be collected at the midpoint of the depth of a given water column (i.e. the sample should be collected at a depth of 0.5 m in a one-meter deep area of water). Over the 2011 ISP Treatment Season, this sample was collected an average of three hours after ISP treatment ended in the adjacent marsh, ranging from a minimum of 1 hour to a maximum of 6.5 hours.

As reported above in Section 4.2, the mean imazapyr concentration reported for the treatment event samples for 2011 was 89.63 ppb, with maximum of 380 ppb and a minimum of 0.23 ppb. Over the four years from 2007-2010 (Kerr 2011), the mean imazapyr level from the treatment event samples was 99.49 ppb, with a maximum of 1310 ppb and a minimum of 0.02 ppb which is actually below the reporting limit (and may have been a result of cross-contamination). The standard deviation for the treatment samples is very high (129.4 ppb for 2011 and 193.5 ppb for the years 2007-2010), reflecting the wide range of lab results and the number of environmental variables involved.

However, if you remove a single outlier sample from 2009, the highest recorded in the seven years of ISP treatment, the mean treatment event lab results are surprisingly similar, with a 4-year average from 2007-2010 of 60.64 ppb and an annual mean ranging from 49.51 ppb to 71.17 ppb. If we use this as the basis of comparison rather than the actual 4-year mean (99.49 ppb) that was skewed by that single high sample, the mean from 2011 of 89.63 ppb is higher than the previous four years. Rather than representing an actual increase in the amount of imazapyr entering surface waters during treatment, this increase is likely to be due to the highly advanced equipment of Pacific Agricultural Laboratory and their level of experience analyzing imazapyr relative to the other labs ISP had used, including the lessons learned from their first season with ISP in 2010. One variable that does not appear to be responsible for much of the variation in mean treatment event concentration is the herbicide delivery system that was utilized. No broadcast aerial applications were part of the water quality monitoring for 2010 or 2011 since ISP has shifted to ground-based spot treatment methods to complete the eradication on most of the sites that were previously managed in this way, but this method was included in previous years. Although the aerial applications have tended to be at or near the maximum measured concentration each year they were sampled, they often share very similar concentrations with a wide variety of other methods including high-pressure hose work from Argos and low-pressure backpack sprayer applications. The highest measured concentration over the seven years of ISP treatment and NPDES water quality monitoring was 1310 ppb and resulted from a standard backpack sprayer application at Elsie Roemer.

In some cases, the target *Spartina* is growing along a channel and any overspray will mix with and become diluted by the incoming tide by the time of the sampling, but in other cases the infestation is situated on the marsh plain and the water may not even reach the base of those plants on the high tide immediately following the application. The flood tide may approach slowly on a given cycle resulting in a low level of mixing with any imazapyr present, resulting in a higher sample concentration, or the tide could come roaring in with a high volume and quickly dilute the receiving water. A large, heavily-infested site that received comprehensive treatment could have a higher result depending on the location of the accessible sample collection. Wind or the incoming tide could magnify the concentration in the receiving water by pushing it along and allowing it to collect overspray along the length of a channel or bayfront, or the sampling location could be in a cove where only the imazapyr that ran off the adjacent plants contributed to the result.

Toxicology

Although there are currently no State or U.S. EPA-based numeric objectives or criteria for imazapyr, one can compare the post-treatment levels to the LC_{50} (defined as the lowest tested concentration of a chemical that was lethal to 50% of test organisms in a laboratory ex-

periment) for various species of wildlife to determine whether these should be a cause of concern for a given species. ISP partners normally apply imazapyr at the FIFRA label rate of 96 oz/A, equivalent to 680,389 mg 'acid equivalent' per acre (a tank mix of 2,998 mg/L in a 60 gal/A application up to 17,905 mg/L in a 10 gal/A application). A sample was actually submitted to Pacific Agricultural Laboratory by ISP in 2010 straight from a hand-mixed tank that was to be applied at 25 gal/A and the lab reported it contained 3,600 mg/L.

The highest ISP imazapyr sample from the receiving waters from 2011 was 380 ppb, equivalent to just 0.38 mg/L. Grue (cited in Entrix 2003) reported a 96-hr LC_{50} for juvenile rainbow trout of 23,336 mg/L and King *et. al*. (2004) affirmed that level with their results of 22,305 mg/L. These lethal levels are more than four orders of magnitude greater than the ISP's maximum sample from the 2011 treatment season, well below any level of concern; they are also far greater than even the undiluted tank mix being applied to the target *Spartina*, and are obviously even further below lethal levels at the ecologically relevant dose that may be found in adjacent surface water.

A survey of the available literature on imazapyr by Leson & Associates (2005) includes studies with various fish species [bluegill sunfish (*Lepomis macrochirus*), rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictaluras punctatis*), fathead minnow (*Pimephales promelas*), Atlantic silverside (*Menidia menidia*), Nile tilapia (*Tilapia nilotica*), and silver barb (*Barbus genionotus*)] exposed to both the technical grade imazapyr as well as tank mixes with surfactants (Hasten® and Agri-Dex®). Hasten® is the pre-cursor to the Competitor® product that many of the ISP partners use, and is therefore a very similar formulation that can serve well for comparison. As expected, the 96-hour LC_{50} was lower when surfactants are included, and some fish species are more sensitive than the previously reported rainbow trout that are the standard for EPA fish toxicology evaluation. However, the lowest lethal concentrations were in the range of 100 mg/L, as compared with the ISP's 2011 maximum measured environmental concentration of 0.38 mg/L, representing more than two orders of magnitude difference. In addition, with this relatively low observed environmental concentration, salt marsh birds and mammals are also at very low risk because even the lowest no-observable-effect-level (NOEL) reported from an 18-week dietary study on mallards was ≈ 200 mg/kg of body weight (b.w.); levels for rats were actually in the 2,000-10,000 mg/kg b.w. range. Either of these representative wildlife species would have to ingest many liters of treated water to reach the reported NOEL, and many more than that to reach the lethal level. This is obviously a very unlikely scenario.

These imazapyr levels may also be compared to published toxicity data for aquatic invertebrates; these organisms can be more sensitive in general than the fish species reported above. Mangels & Ritter (2000) reported the no-observable-effect concentration (NOEC) for imazapyr for the Eastern oyster (*Crassostrea virginica*) was >132 mg/L (the highest dose tested). Manning (1989) found the 21-day NOEC for the freshwater flea (*Daphnia magna*) was 97.1 mg/L. Again, comparing these values to the ISP's 2011 maximum measured post-treatment concentration of 0.38 mg/L shows more than two orders of magnitude difference, well below any level of concern, and four orders of magnitude separation when you consider the 2011 mean across all sites of 89.63 ppb (0.009 mg/L) for the treatment event.

In addition, imazapyr is reported to have a low potential for bioaccumulation, and is therefore not expected to adversely impact predators that feed on exposed aquatic invertebrates. Finally, the applications to invasive *Spartina* occur just once annually, with rare exceptions

when late season surveys find plants that were missed during the initial application which then receive a follow-up treatment before they senesce. With the herbicide applications occurring just once each year, the inhabitants of the tidal marsh ecosystem are only subjected to a single acute exposure and are not receiving chronic, prolonged exposure; this fact enhances the buffer of safety already described above in the survey of the toxicology literature.

One-Week Post-Treatment Monitoring

Laboratory analysis of the one-week post-treatment samples for 2011 found mean imazapyr levels of 1.55 ppb in the San Francisco Bay water for sites with detections, ranging from a minimum of 0.06 ppb to a maximum of 5.90 ppb (SD=2.29 ppb); the imazapyr level had dropped below detectable limits at two of the 13 sites (15%). These levels are remarkably consistent with the water quality monitoring results ISP has collected over the history of this regionally-coordinated invasive *Spartina* treatment project around the Estuary; in fact, the mean, minimum and maximum for 2011 are almost identical to the results for 2007-2010. The mean reported imazapyr concentration from the one-week post-treatment samples over the four year period from 2007-2010 was 1.16 ppb (SD=1.30 ppb), ranging from 0.06 ppb (near the reporting limit) up to 6.50 ppb. Previously in this report, the toxicology of imazapyr was briefly discussed relative to the highest level found immediately post-treatment. The maximum concentration one-week post treatment in 2011 was equivalent to .0059 mg/L, an additional several orders of magnitude below the concentrations reported as lethal to aquatic animals, providing a substantial buffer of safety against the risk of impacts to the ecosystem.

Laboratory results from ISP water quality monitoring for the 2011 treatment season continues to demonstrate that imazapyr breaks down quickly in the estuarine environment, as expected from what is reported in the literature. The mean reduction in reported imazapyr concentration from the treatment events in 2011 to the one-week post-treatment samples was 92.2%, and three sites (23%) showed a 100% reduction over the first week. Only two samples had reductions less than 85%; one of these already had a very low treatment event concentration of 0.23 ppb and dropped 66% to 0.08 ppb down near the reporting limit for the analytical method. Over 2007-2010, the average reduction in imazapyr concentration from the treatment event samples to the one-week post-treatment samples was 95.8%, ranging from a low of 89.0% in 2007 to a high of 99.1% for 2009. With this consistently high degree of imazapyr reduction as measured by the concentration in the water at the site one-week after treatment, it is anticipated that all sites that still had measurable concentrations at that time would likely be below detectable levels within a few days after this third sample.

The rapid photolysis of imazapyr in the aquatic environment along with the twice-daily tidal exchange of the San Francisco Estuary contributes to the number of one-week post-treatment samples that are reported as non-detections. But proper implementation of the analytical method also plays a critical role in the results (e.g. the laboratory utilized in 2007 not only produced false positives for *all pre-treatment samples* but also reported only one sample as non-detection for the one-week post-treatment event). In 2008 there were five non-detections (36%) , in 2009 there were nine (64%), and in 2010 there was just one (6%). As reported previously, in 2011 there were two samples (15%) that had dropped below detectable levels after one week. The number of very low concentrations of imazapyr detected in one-week post-treatment samples may be related to the highly advanced equipment of Pacific Agricultural Laboratory (PAL) and their level of experience analyzing imazapyr relative

to the other labs ISP had used. Although there were certainly problems in the past with false positives, especially at the extreme lower end of the spectrum, the switch to PAL in 2010 has allowed for much more sensitive detection thresholds.

5.2 Interpretation of Glyphosate Results

ISP monitored glyphosate at the only site in 2011 at which this herbicide was used for *Spartina* control, Creekside Park in Marin County. Glyphosate was added to the imazapyr in the tank mix for the *S. densiflora* application at this site in an effort to enhance the efficacy on the small plants remaining at the site by inhibiting synthesis of an additional three amino acids (over the three aliphatic amino acids inhibited by imazapyr). Creekside Park was the original introduction site of *S. densiflora* in San Francisco Bay and was by far the most extensive infestation of this species that ISP was tasked with eradicating. ISP and Friends of Corte Madera Creek have been implementing a complex IPM strategy at Creekside since 2005-2006 that has adapted to the changing face of the infestation and responded with shifts in methodology in recognition that dependence on a single treatment method would not complete the eradication.

The herbicide imazapyr was found to be far less effective on the long-established meadows at Creekside, partially because *S. densiflora* doesn't senesce fully each year and completely replace its above-ground biomass like *S. foliosa* or hybrid *S. alterniflora. S. densiflora* maintains significant amounts of dead leaf material from the previous season's growth mixed in with the current year's new growth. This thatch can shield green, growing leaves from proper coverage during applications, and can complicate the assessment of the eradication status of a given plant. As a consequence of this growth habit as well as *S. densiflora* leaf morphology, plants previously treated with imazapyr in these meadows were compromised but did not die completely. Field observations showed sub-lethal herbicide effects like chlorosis or leaf browning, rendering target plants unable to effectively absorb a subsequent herbicide application.

Although the herbicide application was useful in stopping seed production for the year, the original plants remained alive, albeit compromised. It was reasoned that if the above-ground biomass was removed, still-living plants would respond with vigorous green growth and create additional options for follow-up treatment. ISP began to mow these persistent *S. densiflora* meadows and any adjacent large stands in autumn. This was followed by the annual summer herbicide application to inhibit seed production and dispersal until the end of the clapper rail breeding season when ISP could return for more intensive manual removal work in the marsh. However, the thick leaf cuticle, inrolled leaves, and low leaf surface area to root ratio on the *S. densiflora* regrowth and seedlings is apparently insufficient for good systemic herbicide control, hence the experimental addition of glyphosate in an attempt to achieve greater mortality of the target invader.

The treatment event sample at Creekside in 2011 was determined to contain 35.0 ppb by the laboratory, and this dropped below detectable levels one week after treatment (at a reporting limit of 10 ppb). The lab also tested for AMPA (aminomethyl phosphonic acid), the primary metabolite (i.e. breakdown product) of glyphosate, and all three samples reported no detections of AMPA at a reporting limit of 10 ppb. As discussed previously in this report, there is no total maximum daily load (TMDL) or other numeric criteria for glyphosate outside of municipal use water supplies where the limit is 700 μg/L (ppb). The lone detection in the

ISP monitoring data set is more than an order of magnitude below this standard, and is obviously in a tidal system that has no contact with a freshwater municipal supply.

The fact that detections of this herbicide are rare over the years ISP has monitored (largely at Southampton Marsh) is not entirely unexpected for several reasons. Glyphosate adsorbs tightly to the substrate upon contact and is deactivated, and this lack of mobility means that only overspray directly into a tidal channel at low tide or the receiving waters at an intermediate tide would probably be detected during treatment event monitoring. This also may explain the absence from pre-treatment samples since any excess herbicide from nearby homeowner use or from commercial property management would also bind to the substrate or suspended solids in the tidal water. The lack of mobility of glyphosate coupled with the small amount applied means it would be unusual to find the herbicide in adjacent surface waters one week after treatment after the repeated pulses of tidal inundation, and that result is consistent with the results ISP received from the laboratory in 2011.

5.3 Interpretation of Conventional Water Quality Parameters

The data on conventional water quality parameters collected during the three sampling events at each site in 2011 were reported above in Section 4.3. In addition to the summary statistics generated for 2011, each parameter was also analyzed against the other sampling events (pre, treatment, and one week), and there was no indication that the mean values varied by sampling event. In fact most of the means were essentially equivalent across all three events for all parameters.

The four-year averages from 2007-2010 for the various water quality parameters show this same, consistent pattern; while there is a good degree of variation from site to site and event to event, the overall mean values are essentially equivalent. They indicate the stability of this enormous estuary and, as expected, do not reflect any discernable changes resulting from *Spartina* treatment. For example, water temperature varies considerably over the course of the treatment season, reflecting differences in temperature due to cloud cover, morning vs. afternoon sampling, and summer vs. autumn solar exposure. However, the four-year mean water temperature was 22.4°C and the 2011 mean was 20.7°C. Similarly, pH had a four-year average of 8.1 and the 2011 mean was 8.0, while individual monitoring events showed the natural, expected variation based on the many variables at work around San Francisco Bay on any given day and tidal cycle.

Although there was little variation in certain parameters, the replacement of an old monitoring instrument apparently influenced the mean values for some other parameters over the past two seasons. In 2010, ISP replaced their YSI 85 that was quite old after many years of use for other projects and may have had some minor issues that we were not aware of because we were not comparing the values to those collected by a second YSI. When values for conductivity and salinity from 2010 were compared to the means from 2007-2009 they were consistently lower with the new piece of equipment, while dissolved oxygen (DO) was quite a bit higher. A second year of data has now been collected in 2011 with the new instrument, and these values are closer to the 2010 means than the prior years. While the 2007-2010 mean DO was 3.9 mg/L, the 2010 three-event mean was 6.2 mg/L, and in 2011 the mean was very similar to the previous year at 5.6 mg/L. Based on the literature, the measured DO levels in 2010 & 2011 are more consistent with historical averages. During the late summer months, the height of the *Spartina* treatment season, DO in the Bay tends to

average 6-9 mg/L, but the levels in semi-enclosed embayments can be much lower than in the main water body (SFEI 1994). Some ISP samples are taken in very shallow water over muddy channel bottoms in the hot summer sun, and we would expect these DO values to be amongst the lowest.

While the 2007-2009 mean conductivity was 42.7 mS/cm, the 2010 three-event mean was 35.1 mS/cm, and in 2011 it was 28.8 mS/cm. The story is similar for salinity; the three-event means from 2007-2009 were almost identical, yielding a mean for that time period of 28.9 ppt. In 2010 the mean salinity was 23.4 ppt, and in 2011 it was even lower at 19.3 ppt. In addition to the influence of a brand new instrument still set at factory calibration with more sensitive sensors, the salinity (and possibly conductivity) could also have been influenced by the characteristics of the infestation sites that were monitored. The sites monitored in 2011 include two brackish marshes (Sonoma Creek and Stevens Creek Tidal Marsh) due to their position in the Estuary, two very urban marshes (Oyster Point and San Bruno) that are receiving freshwater input from upstream discharges throughout the summer, and Palo Alto Baylands that continues to see changes in its native plant community distribution caused by the vast quantities of wastewater discharged into San Francisquito Creek.

The Statewide General Permit requires these conventional water quality parameters to be measured and documented for each sampling event. As with many regulations, they are necessarily applied to a broad range of scenarios and are not designed to be a perfect fit for any single scenario. The results of ISP's water quality monitoring for 2011 do not indicate any substantial alteration or degradation of the surface water quality of the Estuary as a result of the herbicide applications to non-native *Spartina*. While all parameters had some outliers, none showed any trends related to the herbicide applications; some outliers were from the pre-treatment event and others occurred during either the treatment or one-week post treatment events, with no discernable pattern for any of the parameters.

The ISP treatment sites are not part of a closed system; they all receive twice daily tidal exchange with the immense volume of San Francisco Bay. Any fluctuations in the water quality parameters measured for compliance with the Statewide General Permit appear to be normal perturbations unrelated to the application of a U.S. EPA-approved aquatic herbicide to emergent vegetation in the adjacent marsh, slough or mudflat. The sheer volume of the Estuary, along with a variety of abiotic factors and the complex dynamics between them is what determines the levels of these parameters on a given day, not the input of the relatively small amount of herbicide associated with *Spartina* control. There is no mention on the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) labels of these products regarding negative impacts to water quality from proper use in the estuarine system, and no measures are recommended to avoid or reduce the chance of such problems.

It is conceivable that two of the parameters, turbidity and dissolved oxygen, could be altered indirectly by an herbicide application intended to kill *submersed* vegetation, particularly in a closed freshwater system such as a pond or small lake with a restricted outlet. After some period of time, the vegetation would begin to die and the decomposition of the plant matter by microorganisms would utilize dissolved oxygen in the water column, potentially lowering the value for this parameter, while simultaneously increasing the turbidity and suspended solids. This would be particularly true if the infestation of the submersed plant was dense and extensive and the herbicide treatment was very effective. However, dissolved oxygen and turbidity should not be influenced by the application of an aquatic herbicide formulation

to *emergent* vegetation with a small degree of overspray (the scenario for *Spartina* control), especially with the massive exchange of water in the Estuary each day. Imazapyr is also a very slow-acting herbicide and takes several weeks to show any signs of impending mortality. In addition, neither imazapyr nor glyphosate effectively controls submersed vegetation, so despite any presence in the water post-treatment, neither herbicide would damage submersed vegetation (either invasive or native) and contribute to potential water quality impacts.

6. Conclusion

The State Coastal Conservancy's Invasive *Spartina* Project successfully conducted water quality monitoring at the required representative sample of their aquatic herbicide application sites around the San Francisco Estuary during the 2011 *Spartina* treatment season, in compliance with the Statewide General Permit and National Pollutant Discharge Elimination System (NPDES). The successful *Spartina* control achieved since 2006 has reduced the baywide infestation from over 800 net acres to 49 net acres. Much less herbicide is now necessary with each passing year, and the success has also enabled ISP partners to shift away from broadcast aerial applications over the historic monocultures to spot applications from airboat and backpack. By 2011, 9 of the 13 sites (69%) monitored for NPDES compliance utilized the backpack sprayer as the only herbicide delivery system needed, and ISP is completing the eradication of virtually all infestations of *S. densiflora* around the Bay with just manual removal after successful reductions with imazapyr in the initial years.

The imazapyr sampling conducted immediately after *Spartina* treatment has consistently found that any concentrations detected in the receiving waters are up to four orders of magnitude below those reported in the toxicology literature as a concern to humans or the animals that inhabit the associated tidal marsh system, including the benthic invertebrates at the foundation of the food web. The mean imazapyr concentration from the 2011 treatment event sampling was 89.63 ppb, which is very consistent with the four-year mean of 99.49 ppb from 2007-2010.

This report discussed the difficulties that the laboratory encounters with accurately measuring imazapyr in the field-collected samples, especially at the lower end of the spectrum down near the reporting limit. ISP was fortunate to work with Pacific Agricultural Laboratory (PAL) again in 2011 and to benefit from their experience with imazapyr and their advanced instrumentation capable of the most rigorous analytical method for this herbicide. ISP plans to work with PAL in 2012 to identify and eliminate any other potential sources of cross-contamination in either the lab extractions and analysis, or during sample collection and handling in the field, and thereby eliminate any false positives from the data set that aren't actually related to the applications for invasive *Spartina* eradication.

The one-week post-treatment sampling results are also consistent with the published literature that imazapyr is short-lived in an estuarine environment. In 2011, the mean reduction in the imazapyr concentration measured one week after treatment was 92.2%, no matter what concentration was previously measured from the treatment event, while the four-year mean reduction from 2007-2010 was 95.8%. With the rapid degradation of this herbicide in the tidal marsh, as measured by the concentration in the water at the site one week after treatment, it is anticipated that all sites that still had measurable concentrations at that time would likely be below detectable levels within a matter of days after this third sample.

As expected, the monitoring of conventional water quality parameters (water temperature, dissolved oxygen, pH, conductivity, turbidity and salinity) showed no indication that the herbicide applications to invasive *Spartina* have had any impact on Estuary surface water quality. There is no relevant pathway for the application of an approved herbicide to emergent vegetation to negatively affect these conventional water quality parameters in the massive open system of San Francisco Bay with twice-daily tidal exchange. In addition, most of

these parameters can vary widely on a given day or tidal cycle, even within a given site, and a change could be attributed to any number of natural variables, without any means of showing causality.

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Appendix I

ISP Field Data Collection Form
for the Water Quality Monitoring Program

Invasive *Spartina* Project
2011 Water Quality Monitoring Report

Page ___ of ___ on this date

Field Data Collection Form

San Francisco Estuary Invasive *Spartina* Project, Aquatic Pesticide Application Plan, 2612-A 8th St, Berkeley, CA, 94710

Site ID (XXXX) (eg. DEAD): _____ Date: _____ Collected By: _____

Station Location (circle): at application point upstream downstream Station Type (circle): Reference Treated

Wind (circle): low high Tidal Cycle (circle): high low slack Water Color (circle): green green-brown brown blue (dye)

Herbicide (circle): imazapyr glyphosate Surfactant:_____ Application Time (Start/Finish): _____/_____

Field Measurements

Air Temp	Water Depth	pH	Dissolved Oxygen	Water Temp	Conductivity	Salinity	Meter Used
^{O}C	Meters		mg/L	^{O}C	mS	ppt	
							YSI 85

Samples Collected

Sample ID (XXXX-YY-Ab)*	Time	Sample Depth (m)	Notes

*** XXXX-YY-Ab** (eg. DEAD-01-pre, ZIPY-02-0.5h) = **XXXX:** Site, **YY:** site visit number (01-1st, 02-2nd, 03-3rd, etc.), **A:** time to application (either pre, increments thereafter in half hours – 0.5), **b:** time increment (h=hour, w=week (for 1 week post-treatment))

Additional Notes or Comments: _____

Wildlife presence: _____

Appendix II

2010 Quality Assurance Plan (QAP)
Pacific Agricultural Laboratory (PAL)

Invasive *Spartina* Project
2011 Water Quality Monitoring Report

Extraction of Imidazolinone Herbicides in Water

1.0 Scope and Application

1.1 This procedure describes the extraction of imidazolinone herbicides from aqueous samples. This method is applicable to all types of water including, but not limited to, drinking water, storm water, surface water, and groundwater.

2.0 Summary of Method

2.1 A 500mL aliquot of sample is acidified to pH 2 and 12.5g sodium chloride is added. Sample is shaken in a separatory funnel with three 50mL portions of dichloromethane. Organic layers are drained **[through acidified sodium sulfate]** into a round bottom flask, and concentrated by rotary evaporation (SOP-AM-027).

3.0 Interferences

3.1 Potential interferences may include contamination from glassware and solvents, and co-extracted materials from the sample matrix. Care must be taken to avoid and/or minimize these potential interferences.

4.0 Sample Handling and Preservation

4.1 Samples should be taken in 1-L amber glass bottles with a PTFE lined cap.

4.2 Samples are taken at neutral pH, and stored at 4°C prior to extraction.

4.3 All water samples shall be extracted within seven (7) days of sampling.

5.0 Apparatus and Instrumentation

5.1 1000 mL glass separatory funnel
5.2 500 mL graduated cylinder
5.3 600 mL beaker
5.4 250 mL round bottom flask
5.5 Large glass funnel
5.6 pH meter
5.7 Top-loading balance, accurate to ±0.01g
5.8 Magnetic stir bar
5.9 Magnetic stir plate
5.10 Rotary evaporator, Rotavap; Yamato RE50

6.0 Reagents and Supplies

6.1 Organic-free water, DI H_2O
6.2 Methanol (MeOH) w/0.5% Formic Acid
6.3 Pesticide-grade Dichloromethane, DCM
6.4 6 N Hydrochloric Acid, HCl
6.5 Sodium chloride, ACS grade
6.6 Glass beads
6.7 **[Glass wool]**
6.8 **[Acidified sodium sulfate, Na_2SO_4]**

7.0 Procedure

7.1 For each sample, the necessary glassware items (separatory funnel, 600 mL beaker, and flat-bottom flask) are obtained, rinsed with Dichloromethane if necessary, and labeled with sample number. Beakers contain a magnetic stir bar, and two glass beads are added to each flat-bottom flask. Using a graduated cylinder, measure 500 mL of organic-

free water for QC and transfer to a beaker with a stir bar. Likewise, measure and transfer 500 mL of sample into a beaker with a stir bar.

7.2 **[Sodium sulfate funnels are prepared by placing a small plug of glass wool into a glass powder funnel, to which ~25g *acidified* Sodium Sulfate is added. Funnels are rinsed with ~10mL DCM, and solvent is drained into waste. A funnel is placed on each labeled collection flask.]**

7.3 Using a 500 mL graduated cylinder, a 500 mL aliquot of sample is measured and transferred to the labeled 600 mL beaker.

7.4 Method Blank (BLK) consists of 500 mL deionized water in a 600mL beaker. This sample will be the negative control (QC) for the analysis.

7.5 Lab Control Sample/Lab Control Sample Duplicate (LCS/LCSD) each consist of 500 mL DI water in a 600mL beaker. Project specific spike compounds are added to each, and the standard log number and spike volume are recorded on extraction bench sheet. These samples will be the positive control (QC) for the analysis.

7.6 The pH of each sample and QC is adjusted to 2.0 by dropwise addition of 6N hydrochloric acid.

7.7 12.5 g of sodium chloride is added to each beaker, stirring until salt is completely dissolved.

7.8 The contents of each beaker are transferred into the appropriately labeled separatory funnel. Samples and QC are extracted by shaking three times with 50mL DCM. The lower (DCM) layers are drained **[through the acidified sodium sulfate funnel]** into the corresponding flat-bottom round flask.

7.9 **[After all solvent is collected, Na_2SO_4 funnels are rinsed with ~20mL Dichloromethane, to optimize recovery of analytes.]**

7.10 Extracts are concentrated to ~0.5 mL using rotary evaporation (SOP-AM-027), and remaining solvent is evaporated to dryness under a steady stream of nitrogen gas.

7.11 Extract is transferred to labeled culture tubes as per SOP-AM-XXX (Rotavap) using MeOH w/0.5% Formic acid as final solvent. Final volume is 2mL for most Imidazolinone extractions.

7.12 Extracts should be stored in refrigerator until analysis.

8.0 Calculations

8.1 N/A

9.0 Quality Control

9.1 At a minimum, batch QC will include a method blank (MB), and a Laboratory Control Sample/Laboratory Control Sample Duplicate (LCS/LCSD). Additional QC will be performed if there are project and/or method specific requirements. An extraction batch consists of a batch of 20 consecutive samples extracted within 7 days.

9.2 Spike recoveries are calculated after analysis to evaluate extraction efficiency.

10.0 Reporting

10.1 N/A

11.0 References

11.1 American Cyanamid Method 2261

11.2 American Cyanamid Method M1900

Imidazolinone Herbicides in Water by EPA 8321B

1.0 Scope and Application

1.1 This procedure is used to determine the concentrations of Imidazolinone herbicides in liquid matrices.

2.0 Summary of Method

2.1 A measured volume of sample is extracted using AM-033, Extraction of Imidazolinone Herbicides in Water.

2.2 Extracts are analyzed using liquid chromatography with mass spectroscopy (LC/MS) detection.

3.0 Interferences

3.1 Potential interferences may include contaminated solvents and extraction glassware, dirty chromatographic equipment, and co-extracted materials from the sample matrix. Care must be taken to avoid and/or minimize these interferences.

4.0 Sample Handling and Preservation

4.1 Store samples at 4^{o}C out of direct sunlight. Water samples should be extracted within 7 days of sampling and analyzed within 40 days of extraction

4.2 Personal protection measures should be taken while handling solvents and samples.

5.0 Apparatus and Instrumentation

5.1 Analytical balance, Sartorius model CP124S, accurate to 0.0001g. Calibration of balance shall be checked daily (SOP EQ-001).

5.2 N-EVAP evaporation manifold with heated water bath

5.3 HPLC System

5.3.1 Agilent 1100 HPLC system equipped with binary pump, autosampler, solvent degasser, and single quadrapole mass spectrometer.

5.3.2 Agilent Chemstation software

5.3.3 Analytical Column – C18 reverse phase column, 100mm x 3.0mm ID, 2.5 µm particle size, Agilent Zorbax SB-C18 or equivalent.

6.0 Reagents and Supplies

6.1 Organic-free reagent water

6.2 Methanol, Chemsolve, HPLC Grade

6.3 Acetonitrile (ACN), Chemsolve, HPLC Grade

6.4 Formic Acid, EMD, ACS Grade

6.5 Luer lock tipped syringe

6.6 Screw capped tubes with Teflon lined lids

6.7 13mm 45 µm nylon syringe filters

6.8 Auto sampler vials with PTFE lined caps

6.9 Volumetric flasks, class A

6.10 Gas tight syringes with PTFE tipped plungers

6.11 HPLC/MS Tuning Standard – Aglient ES Tuning Mix G2421A

7.0 Procedures

7.1 Sample Extraction:

7.1.1 Extract waters via the procedure outlined in Pacific Agricultural Laboratory SOP AM-033 “Extraction of Imidazolinone Herbicides in Water”.

7.1.2 Store extracts in refrigerator until analysis.

7.2 Solvent exchange of water extracts:

7.2.1 Transfer a 1 ml aliquot of the sample extract to a culture tube. Mark the meniscus of the liquid in the tube.

7.2.2 Evaporate the solvent under a steady stream of nitrogen using the N-Evap evaporation manifold.

7.2.3 Reconstitute the extract as follows: add 500 uL methanol, then 500 uL Mobile Phase A (95% organic free water, 5% ACN, 0.05% formic acid). .

7.2.4 Filter the sample extract into an autosampler vial through a 45 µm 13 mm syringe filter using a luer tipped syringe.

7.2.5 Cap the vial and label with appropriate moniker.

7.3 Preparation of HPLC mobile phase:

7.3.1 The mobile phase is contained in two reservoirs, one containing the aqueous portion (Mobile Phase A) and one containing the organic(Mobile Phase B) portion.

7.3.2 Prepare Mobile Phase A by combining 950 mL of organic free water, 50 mL ACN, and 0.5 mL formic acid.

7.3.3 Prepare Mobile Phase B by combining 950 mL of ACN, 50 mL organic free water, and 0.5 mL formic acid.

7.4 Chromatographic conditions:

7.4.1 Flow rate: 0.40 mL/minute

7.4.2 Injection volume: 10 ul

7.4.3 Column Temperature: 45 ^{o}C

7.4.4 Solvent Gradient:

Time	%A	%B
0.0	80	20
1.5	80	20
8.0	30	70
10	30	70

7.4.5 Re-equilibration time: 3 minutes, 80% A/20% B

7.5 Mass Spectrometer Conditions:

7.5.1 Ionization Mode: API-Electrospray

7.5.2 Drying Gas: N_2, 11.0 L/min, 250 ^oC

7.5.3 Nebulizer Pressure: 30 psig

7.5.4 Capillary Voltage: 1500 V

7.6 Mass Spectrometer Detector settings:

7.6.1 Settings for use in MS data acquisition (SIM ions and fragmentor voltages) vary by analyte and are displayed in Table 2 of the Appendix (12.2).

7.7 If the peak areas of the sample signals exceed the calibration range of the system, dilute the extract as necessary and reanalyze the diluted extract.

7.8 Calibration:

7.8.1 Electrospray MS System: The MS system is calibrated for accurate mass assignment, sensitivity, and resolution using the Agilent ES Tuning Mix G2421A. The following masses are calibrated in positive and negative ionization modes:

MASS	POSITIVE	NEGATIVE
1	118.09	112.99
2	322.05	431.98
3	622.03	601.98
4	922.01	1033.99
5	1521.97	1633.95

Tune parameters are adjusted to ensure ions are present at each of the masses with counts >50000 and peak widths within the range of 0.60 – 0.70 amu.

7.8.2 Stock Standards: Individual analyte stock standards are made at concentrations between 500-1000 μg/ml by transferring 25-50 mg neat standard to a 50 mL class A volumetric flask, dissolving the neat standard in acetonitrile or methanol, and diluting to the mark with acetonitrile or methanol. Stock standards prepared from neat standards may be used for a maximum of two years. Alternatively, a solution containing 1000 μg/ml of analyte may be obtained from ChemService or other reputable manufacturer and used as a stock standard. In this case, the stock standard may be used until the expiration date provided by the manufacturer.

7.9.3 Working Standards: A 10 μg/ml working standard is made by transferring appropriate amounts, depending on initial concentrations, of stock standards to a 10 mL class A volumetric flask and diluting to the mark with methanol or acetonitrile. The

amount of stock standard to transfer will range between 100-200 µL and is calculated using the formula:

$$\text{Amt. Stock Std.}(\mu L) = \frac{[\text{Final Conc. } (10\mu g/ml)] \times [\text{Final Vol. } (10ml)}{\text{Initial Stock Conc. } (\mu g/\mu L)}$$

The working standard solution is transferred to an appropriately labeled screw cap tube and may be used for a maximum of one year.

7.9.4 Preparation of external standard calibration curve: an appropriate aliquot of the working standards are added to an autosampler vial and diluted to 1 ml with Mobile Phase A. A minimum of 5 standards are prepared at the following suggested levels: 0.005 ug/ml, 0.010 ug/ml, 0.020 ug/ml, 0.05 ug/ml, and 0.10 ug/ml. The calibration range can be adjusted to meet expected levels in the samples. The calibration standards are prepared as follows:

Calibration level	Aliquot volume	Concentration of aliquot(s)	Volume of buffer	Final volume
100 ng/ml	100 µl	1000 ng/ml	900 µl	1.0 ml
50 ng/ml	50 µl	1000 ng/ml	950 µl	1.0 ml
20 ng/ml	200 µl	100 ng/ml	800 µl	1.0 ml
10 ng/ml	100 µl	100 ng/ml	900 µl	1.0 ml
5 ng/ml	50 µl	100 ng/ml	950 µl	1.0 ml

7.9.5 The system is calibrated prior to the injection of a set of sample extracts. After injecting a set of standards, a linear calibration curve is prepared. Exclude the origin as a point. The R value of the generated curve should be 0.99 of better. If the calibration fails to meet these criteria, the cause of the deviation should be rectified and the system recalibrated.

7.9.6 The calibration is verified by injecting a CCV at the mid point concentration of the calibration curve after no more than twenty

samples. If the response deviates by more than +/- 15% from the initial calibration, the system should be recalibrated and the samples bracketed by the either the initial calibration or the prior passing CCV and the failing CCV should be reanalyzed. If the CCV is >15% of initial calibration, the samples bracketed by the either the initial calibration or the prior passing CCV and the failing CCV can be used if the sample contains no detectable residues.

8.0 Calculations

8.1 Water Samples:

$$\frac{\text{amount f/curve (ng/ml) x final volume (ml) x dilution factor}}{\text{sample volume (ml)}} = \text{result (ug/liter, ppb)}$$

9.0 Quality Control

9.1 Initial Demonstration of Proficiency – the laboratory shall demonstrate initial proficiency with each sample preparation technique, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the demonstration whenever new staff is trained or significant changes in instrumentation are made.

9.1.1 Calculate the average recovery and the standard deviation of the recoveries of the four QC reference samples. Refer to Section 8.0 of EPA Method 8000 for procedures in evaluating method performance.

9.2 Method Reporting Limits (MDLs)

9.2.1 The MDL is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix type containing the analyte.

9.2.2 The extraction and analysis of seven replicates of a spiked sample determine the MDL.

9.2.3 Multiply the standard deviation of the seven replicate results by the one sided 99%t-statistic (3.14) to obtain the MDL for each analyte.

9.2.4 These results are kept on file and should be re-evaluated annually, when significant changes in instrumentation are made, or when new staff are added.

9.3 Sample Quality Control for Preparation and Analysis

9.3.1 The laboratory will have procedures for documenting the effect of matrix on method performance.

9.3.2 Water matrix – minimum QC samples shall include a method blank (MB), Laboratory Control Sample (LCS), and a Laboratory Control Sample Duplicate (LCSD). A matrix spike may be prepared and analyzed provided there is adequate sample.

9.4 QC Frequency – an analytical batch is defined as a set of no more than 20 samples extracted within 14 days. The QC frequency for each analytical batch is as follows:

Method blank – 5%

Matrix Spike/Matrix Spike Duplicate – 5%

Laboratory Control Sample/Laboratory Control Sample Duplicate – 5%

9.4.1 In house method performance criteria for spike and surrogate compounds should be developed using guidance found in Section 8.0 of EPA Method 8000.

9.4.2 If the recovery data is outside acceptance limits, the samples should be re-extracted and/or the data flagged as necessary.

10.0 Reporting

10.1 If all QC criteria have been met, the data is then compiled and a report is generated, including sample raw analytical results and QC data, and submitted to the client.

11.0 References

11.1 EPA Method 8321B, SW-846 Revision 2, December 2007.

11.2 Pacific Agricultural Laboratory Quality System Manual.

11.3 EPA Method 8000B, SW-846 Revision 2, December 1996.

11.4 SW-846, Chapter One, Revision 1, 1992.

12 Figures and Appendices

12.1 Table 1 - Analyte list and reporting limits

12.2 Table 2 – Mass Spectrometer Data Acquisition Settings

Approved: _____

Date: _____

TABLE 1	
ANALYTE LIST AND LIMIT OF QUANTITATION (LOQ)	
Analyte	**LOQ, ug/L**
Imazamox	0.02
Imazapic	0.02
Imazapyr	0.02
Imazethapyr	0.02

TABLE 2 – MASS SPECTROMETER DATA ACQUISITION SETTINGS

Time	**SIM Ions**	**Fragmentor Voltage**	**Capillary Voltage**
0.00	220,222,234, 248,262,277, 278,290,293, 306,307	200	2000 V

TABLE 3 – SIM IONS FOR IDENTIFICATION/QUANTIFICATION

Analyte	Quantification Ion	Qualifier Ions	Ionization Mode	Fragmentor Voltage
Imazamox	306	307,278	positive	200
Imazapic	293	277,220	positive	200
Imazapyr	262	234,222	positive	200
Imazethapyr	290	262,248	positive	200