Appendix A

Review of Potash Toxicology to Zebra Mussels and Other Organisms

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DATE: March 4, 2011

Background and Purpose

Zebra mussels (*Dreissena polymorpha*) are a non-native, freshwater, invasive species that have caused ecosystem disruptions and infrastructure fouling throughout eastern North America since their invasion from Europe in the late 1980s. San Justo Reservoir in San Benito County is the first record of their invasion in California waters. The relative hydrologic isolation of the reservoir and operational ability to isolate it as a virtually closed system presents the opportunity for eradication of the mussels in the reservoir before they are able to spread to other locations.

Zebra mussel control strategies have been well researched since the mussels' North American invasion and the history of control attempts have resulted in useful literature that can be applied to the San Justo project. In 2009, San Benito County Water District (SBCWD) and the Bureau of Reclamation (Reclamation), in concert with independent technical experts, evaluated alternatives for killing zebra mussels at San Justo Reservoir. After analysis of existing treatments, potash (potassium chloride, KCl) was selected as the primary molluscicide for the reservoir. Potential technologies and strategies for zebra mussel control in the Hollister Conduit and SBCWD Distribution System were evaluated, including potash (CH2M HILL 2009). Reclamation and SBCWD selected potash as the preferred chemical to concurrently dose the reservoir, conduit, and distribution system. As freshwater organisms, zebra mussels are particularly susceptible to potassium toxicity; they are known to be intolerant of elevated ion concentrations (Horoshov et al., 1992).

The purpose of this memorandum is to review and summarize toxicological information of KCl, as appropriate for the San Justo Reservoir mussel eradication program. The design of an effective KCl dosing strategy for the reservoir requires a documentation of the toxicology of KCl both to zebra mussels as well as to the co-located aquatic organisms and others (birds, mammals) that may use the reservoir. The goal is to be completely effective at eradication of the mussels but with the least damage to the associated biotic community. In addition, a pond immediately adjacent to the reservoir contains the listed California red-legged frog (*Rana draytonii*) and potentially, the California tiger salamander (*Ambystoma californiense*). Toxicity to these amphibian species is of particular concern because of their protected status.

Potash Toxicity for Zebra Mussel Control

The potential toxicity of KCl applications was evaluated to zebra mussels and representative members of the associated aquatic community at San Justo reservoir. Toxicity was evaluated both as documented in the example of a successful zebra mussel eradication resulting from KCl application and, more generally, from the toxicology literature.

Successful Eradication Example

The Millbrook Quarry, Virginia zebra mussel eradication example supports the use of potash at a target concentration of 100 ppm KCl (as potassium). Millbrook Quarry is the only example of a successful lake or reservoir eradication in the U.S except for the Offutt Air Force Base, Nebraska eradication that used copper sulfate. In the Millbrook case, an estimated whole-lake concentration of 100 ppm KCl was applied in order to ensure that at least 50 ppm KCl was achieved in the lake margin or deep areas or arms of the reservoir that may have experienced incomplete mixing. Incomplete mixing is of most concern in a reservoir application; the 50 ppm value was considered a minimum concentration necessary to kill all life stages of the mussel, which includes planktonic, water-column larvae (veligers) as well as substrate-attached juveniles and adults (Aquatic Sciences 2005). Monitoring results revealed that final measured concentrations in the quarry ranged from 98 to 115 ppm KCl (Virginia DGIF, 2006).

The Millbrook data indicated extreme toxicity to zebra mussels but much less toxicity to other organisms. There was a general lack of significant toxicity to reservoir fish or other invertebrates at target concentrations of 100 ppm KCl. Turtles, fish, aquatic insects, and snails were all observed to survive the Millbrook Quarry treatment (Virginia DGIF, 2006). The Millbrook eradication was judged 100% effective at both killing resident mussels and those in bioassay enclosures scattered around the lake while allowing the survival of the quarry's other aquatic life (Virginia DGIF, 2006; Watson and Fernald, 2007).

The Millbrook eradication example serves as a useful model for future attempts at whole-reservoir zebra mussel control, as in San Justo Reservoir. The target KCl concentrations of 100 ppm KCl proved effective at both eliminating the mussels and causing minimal harm to the rest of the aquatic community. In addition, in both the Millbrook and Offutt examples, the possibility of success of eradication using chemical treatments was enhanced by the small size and hydrologic isolation of the lakes. San Justo Reservoir is an offline water supply reservoir with a distribution system that allows for flow control; treated water will not flow to a natural stream system and allow the spread of mussels.

Toxicity Literature

In addition to zebra mussels, the toxicity of KCl concentrations in freshwater were examined for a range of organisms. The objective of this review was to characterize the toxicity levels of KCl to members of a typical reservoir biological community, as would be expected at San Justo Reservoir, as well as for amphibians as may be found in nearby ponds. The reservoir is known to have been stocked with non-native populations of trout, crappie, bass, bluegill, and catfish (San Benito County, 2010). Toxicity results were derived from a search of EPA's online toxicology database, ECOTOX (USEPA, 2009) as well as selected, additional toxicology articles.

Results are summarized in Table 1. Note that the primary sources available from the ECOTOX database are not presented here because they were not examined. Instead, the summary results from ECOTOX were screened by toxicant, organism, and type of toxicology endpoint to provide the results shown here.

Table 1. Toxicology endpoints and KCl concentrations for typical, reservoir organisms. Zebra mussels in bold, italics. No-effect concentrations are shaded. LC50 = Concentration showing 50% mortality over the test period.

Taxonomic Group	Species	Endpoint	KCl (mg/L)	Source
Crustaceans	*Ceriodaphnia dubia* (water flea)	LC50	630	ECOTOX
		Lethal	299-596	ECOTOX
		No-effect	193	Aquatic Sciences, 1997
	Hyallela azteca (scud)	LC50 (4 day)	134-630	ECOTOX
	Orconectes limosus (crayfish)	LC50 (30 day)	330 – 450	ECOTOX
Aquatic insect	*Chironomus tentans* (midge)	LC50 (4 day)	1,250 – 6,830	ECOTOX
Annelid Worms	*Tubifex tubifex*	LC50 (4 day)	813*	ECOTOX
	Nais variabilis	LC50 (2 day)	67 – 75*	ECOTOX
Snails	*Physa hertostropha*	LC50	940	Daum, et al., 1977
	Bimophalaria alexandrina	Lethal	1,000 – 2,600	ECOTOX
Bivalve molluscs	*Corbicula fluminea* (clam)	LC50	225	Anderson, et al., 1976
	Dreissena polymorpha (zebra mussel)	***95% mortality/56 hrs at 20^{o}C (approximate temperature for treatment)***	***100***	***Aquatic Sciences, 1996***
		LC50 (1 day)	***138***	***Fisher, et al., 1991***
Fish	*Lepomis macrochirus* (bluegill sunfish)	LC50 (4 day)	951 – 2,010	ECOTOX
		LC50	2,010	Daum, et al., 1977
	Gambusia affinis (mosquitofish)	LC50 (4 day)	435 - 485	ECOTOX

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Taxonomic Group	**Species**	**Endpoint**	**KCl (mg/L)**	**Source**
	Pimephales promelas (fathead minnow)	LC50 (4 day)	880	ECOTOX
		Lethal	1,191	ECOTOX
		No-effect	302	Aquatic Sciences, 1997
		Near zero	299	ECOTOX
	Cyprinus carpio (carp)	Lethal	5,910 – 6,590	ECOTOX
	Ictalurus punctatus (catfish)	LC50 (2 day)	720	ECOTOX
	Oncorhynchus mykiss (rainbow trout)	No-effect (7 day)	500 – 1,000	ECOTOX
Amphibians	*Microphyla ornata* (frog)	LC50 (4 day)	1,414 – 2,539	ECOTOX
		Lethal	2,000	ECOTOX
	Rana breviceps (frog)	Mortality	1,000 – 10,000	Kegley et al., 2010

[*test conditions for worms did not allow their normal burial in substrate and may produce unnaturally low toxicity values]

Conclusions

A review of the toxicology literature for KCl, as summarized in Table 1, is generally supportive of the findings from the Millbrook Quarry zebra mussel eradication. Most toxicity information is available as LC50 values, and longer exposures were chosen to more closely reflect the field eradication plan (4 days or longer, if results were available). Also, lethal and no-effect concentrations were shown wherever available since the objective was to confirm the lethality of KCl to zebra mussels and safe concentrations for other organisms. As is shown in Table 1, zebra mussels are among the most sensitive aquatic organisms to KCl toxicity, with expected mortality in the 100 ppm range (as is being recommended for treatment dosage at San Justo Reservoir). However, the time of year for application is important because KCL toxicity is temperature dependent. Bivalve sensitivity to KCl increased 10-fold from 10 to 20 degrees centigrade water temperature (Aquatic Sciences, 1996). Although informative and comparative, laboratory toxicity tests that are typically 4 to 7 days are more limited in duration than the planned eradication for San Justo of 2 or more months. However, as seen in the Millbrook example, the target KCl concentrations produced a 100% zebra mussel kill after a month's exposure (Virginia DGIF, 2006). In addition, the Millbrook results suggest that the other aquatic invertebrates and fish in San Justo should survive the long-term duration of what, for them, is a sub-lethal dosage. Should any unexpected degradation of the San Justo fishery occur as a result of the mussel treatment, the affected non-native target fish could easily be re-stocked.

A variety of aquatic species of fish and invertebrates appear to be less susceptible than zebra mussels to the effects of KCl. In contrast to zebra mussels, no mortality is expected for fish in the 300 – 1,000 ppm KCl range or for planktonic crustaceans at approximately 200 ppm (Table 1). Most invertebrates and fish show LC50 endpoints far higher than those for zebra mussels. Note that worms show relatively low toxicity values, but that the values in Table 1 are probably unnaturally low due to the lack of natural sediment burial of the animals under test conditions. Although no-effect concentrations were unavailable for amphibians and most invertebrate groups, the toxicity values in Table 1 indicate that the potential for harmful effects to non-target species should be minimized with the planned dosage of 100 ppm KCl.

There is a particular concern for listed species of amphibians in the downstream pond. Note that the red-legged frog is known to be a pond resident while this general area is only known to be in the range of tiger salamanders (currently there is no actual record of their occurrence at the reservoir or pond). The amphibian data in Table 1 provides a direct surrogate toxicity estimate for red-legged frog and a close surrogate for tiger salamander. These types of literature surrogates are typical of what is used in assessing toxicity as part of Ecological Risk Assessments when exact species information is unavailable; the results shown in Table 1 indicate no likely impacts to amphibians as part of the implementation of this project.

The planned whole-reservoir target of 100 ppm KCl should be fatal to zebra mussels and potentially may affect some sensitive members or life stages of various members of the invertebrate community of San Justo Reservoir but should not adversely affect the reservoir fish or amphibian communities in the adjacent pond. These effects are comparable to the observations following the successful treatment of Millbrook Quarry, Virginia.

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